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BAS 500 06 F

DOCUMENT I

Other data on the formulants

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All data available for the formulants contained in the product

BAS 500 06 F

are listed in the safety data sheets which are included
in Document H.

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BAS 500 06 F

DOCUMENT L-CP, Section 1

IDENTITY OF THE PLANT PROTECTION PRODUCT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

There are no references submitted with this section.

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BAS 500 06 F

DOCUMENT L-CP, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE PLANT PROTECTION PRODUCT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162291 (version 1)
27-Feb-2017	Addition of two references in 2.5.	BASF DocID 2017/1032195 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 2.1/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.2/1	Loeffler U.	2004 a	Evaluation of physical and chemical properties according to Directive 92/69/EC, Annex A9-A17 2004/1004104 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.3/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.3/2	Loeffler U.	2004 a	Evaluation of physical and chemical properties according to Directive 92/69/EC, Annex A9-A17 2004/1004104 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 2.4/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.5/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.5/2	Siebecker M.	2016 a	BAS 500 06 F - Additional information on the rotational viscosity determination which was described in the accelerated study 181369_1 2016/1306845 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 2.5/3	Siebecker M.	2016 b	BAS 500 06 F - Additional information on the rotational viscosity determination which was described in the accelerated study 181369_1 2016/1306847 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.6/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.7/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 2.7/2	Kroehl T.	2006 a	Shelf life at 23°C in original container of the formulation BAS 500 06 F, 24 month storage, analytical results and physical properties 2006/1019717 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.8.2/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.8.2/2	Kroehl T.	2008 a	Emulsion stability, persistent foaming and pH value of Pyraclostrobin 200 g/L EC (BAS 500 06 F) 2008/1086283 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 2.8.6/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.8.6/2	Kroehl T.	2008 a	Emulsion stability, persistent foaming and pH value of Pyraclostrobin 200 g/L EC (BAS 500 06 F) 2008/1086283 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.9/1	Rudoll B., Schneider K.-H.	2004 a	Physical and chemical compatibility in aqueous tank mixtures of BAS 500 06 F 2004/1008462 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

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BAS 500 06 F

DOCUMENT L-CP, Section 3

DATA ON APPLICATION

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

There are no references submitted with this section.

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BAS 500 06 F

DOCUMENT L-CP, Section 4

FURTHER INFORMATION ON THE PLANT PROTECTION PRODUCT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162293 (version 1)
27-Feb-2017	Addition of a reference in 4.2 and 4.3	BASF DocID 2017/1032196 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 4.2/1	Anonymous	2015 a	Safety data sheet - Retengo 2015/1280947 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 4.2/2	Nolte M.	2010 a	BAS 500 06 F: Effectiveness of procedures for cleaning application equipment and protective clothing 2010/1048392 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 4.3/1	Anonymous	2015 a	Safety data sheet - Retengo 2015/1280947 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 4.4/1	Schreiner B.	2005 a	EU performance tests of BAS 500 06 F (Standard-Coex-Bottle, 1 L, Spec.-No. 775 5108) 2005/1025057 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 4.4/2	Kroehl T.	2006 a	Shelf life at 23°C in original container of the formulation BAS 500 06 F, 24 month storage, analytical results and physical properties 2006/1019717 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

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BAS 500 06 F

DOCUMENT L-CP, Section 5

ANALYTICAL METHODS

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18-Jul-2014		BASF DocID 2014/1162294 (version 1)
27-Feb-2017	Addition of a reference in 5.1.1.	BASF DocID 2017/1032197 (version 2)

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Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 5.1.1/1	Ziegler H., Machauer B.	2003 a	Analytical method CF-A 669: Quantitative determination of the active ingredient Pyraclostrobin in BAS 500 06 F by HPLC 2003/1022228 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 5.1.1/2	Ziegler H.	2004 a	Validation of the analytical method CF-A 669: Determination of the active ingredient Pyraclostrobin in BAS 500 06 F by HPLC 2004/1004041 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 5.1.1/3	Siebecker M.	2016 a	BAS 500 06 F - additional document to the validation of the analytical method AFL0669/01 (formerly CF-A 669) 2016/1299807 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 5.1.1/4	Stegmaier W.	2011 a	Gas chromatographic determination of dimethyl sulfate in BAS 500 06 F 2011/1009063 BASF SE - GMC Competence Center Analytics, Ludwigshafen, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 5.1.1/5	Stegmaier W.	2011 b	Validation of an analytical method for the determination of dimethyl sulfate in BAS 500 06 F 2011/1009064 BASF SE - GMC Competence Center Analytics, Ludwigshafen, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

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BAS 500 06 F

DOCUMENT L-CP, Section 7

TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 7.1.1/1	[redacted]	2008 a	BAS 500 06 F: Acute oral toxicity study in rats 2007/1053390 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 7.1.2/1	[redacted]	2009 a	BAS 500 06 F: Acute dermal toxicity study in rats 2009/1084157 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 7.1.3/1	[redacted]	2010 a	BAS 500 06 F - Acute inhalation toxicity study in Wistar rats - 4-Hour liquid aerosol (head-nose only) 2009/1122167 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 7.1.4/1	[redacted]	2010 a	BAS 500 06 F - Acute dermal irritation / corrosion in rabbits 2009/1100358 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 7.1.5/1	[redacted]	2010 b	BAS 500 06 F - Acute eye irritation in rabbits 2009/1100359 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 7.1.6/1	[redacted]	2008 b	BAS 500 06 F: Murine local lymph node assay (LLNA) 2007/1053391 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 7.1.6/2	[redacted]	2009 a	BAS 500 06 F - Maximization test in guinea pigs 2009/1018498 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 7.2.1.2/1	Stadler R.	2007 a	Determination of dermal and inhalation operator exposure for mixing/loading and application of BAS 601 KD F in cereals 2007/1033389 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 7.2.1.2/2	Blaschke U.	2010 a	Determination of operator exposure (passive dosimetry) during typical activities associated with a ground boom application of BAS 480 31 F to cereal crops at farm locations in the United Kingdom and Germany, 2008 2010/1089364 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 7.3/1	Fabian E., Landsiedel R.	2010 a	14C-BAS 500 F in BAS 500 06 F - Study of penetration through human skin in vitro 2010/1059865 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 7.3/2	Fabian E., Landsiedel R.	2014 a	14C-BAS 500 F in BAS 500 06 F - Study of penetration through human skin in vitro 2014/1001501 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

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BAS 500 06 F

DOCUMENT L-CP, Section 9

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 9.1.3/1	Kallweit W.	2014 a	Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes 2014/1000685 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 9.2.4.1/1	Kallweit W.	2014 a	Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes 2014/1000685 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 9.2.5/1	Kallweit W.	2014 a	Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes 2014/1000685 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

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BAS 500 06 F

DOCUMENT L-CP, Section 10

ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162299 (version 1)
09-Jun-2015	The document was amended in order to reflect that the 2 studies in M-CP 10.7 had been moved M-CP 10.4. New or changed text is marked in yellow.	BASF DocID 2015/1117544 (version 2)
27-Feb-2017	KCP 10.1.3/2: new reference KCP 10.1.3/3: revised numbering KCP 10.1.3/4: new reference Changes are marked in blue.	BASF DocID 2017/1032194 (version 3)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.1/1	Schroeer A., Grimm T.	2011 b	Field monitoring of hares and rabbits in cereal fields 2011/1112612 RIFCon GmbH, Heidelberg, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.1/2	Schroeer A., Grimm T.	2012 b	First amendment to final report - Field monitoring of hares and rabbits in cereals fields 2012/1105899 RIFCon GmbH, Heidelberg, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.1/3	Pedall I., Riffel M.	2004 b	Population dynamics of common voles in winter cereal fields - Field monitoring in South Western Germany 2004/1025715 Riffel BioConsult, Hirschberg, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 10.1.1.2/1	[redacted]	2008 b	BAS 500 06 F - Acute toxicity in the bobwhite quail (Colinus virginianus) after single oral administration (LD50) 2008/1078602 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.1.2.2/1	Anonymous	2011 a	Generic statement - Relevance of the common vole scenario in arable crops 2011/1248329 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 10.1.2.2/2	Anonymous	2014 a	Pyraclostrobin - Ecologically relevant reproductive toxicity endpoint for the wild mammal risk assessment 2014/1010736 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 10.1.2.2/3	Anonymous	2014 b	BAS 500 06 F: Acute risk to mammals of the representative formulation - Supplementary information to Chapter 10.01 of document MC-P of the renewal dossier for Pyraclostrobin 2014/1010735 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.1.3/1	[redacted]	2014 a	Effects of BAS 500 06 F to juvenile Rana temporaria under worst case laboratory conditions 2013/1375098 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 10.1.3/2	[redacted]	2015 a	Effects of residues of BAS 500 06 F on soil to juvenile bufo bufo under laboratory conditions 2014/1221859 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 10.1.3/3	[redacted]	2014 b	Effects of BAS 500 06 F to juvenile Rana temporaria in winter wheat (semi-field trial) 2013/1375099 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 10.1.3/4	[redacted]	2016 a	Effects of BAS 500 06 F to juvenile Bufo bufo in winter wheat (semi-field trial) 2015/1240163 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.2.1/1	[redacted]	2008 a	BAS 500 06 F - Acute toxicity study with the rainbow trout (Oncorhynchus mykiss) 2008/1018046 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 10.2.1/2	[redacted]	2012 a	BAS 500 06 F - Acute toxicity study in the common carp (Cyprinus carpio) 2012/1250190 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 10.2.1/3	Funk M.	2004 a	Effect of BAS 500 06 F on the immobility of Daphnia magna STRAUS in a 48 hours static, acute toxicity test 2004/1004393 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.2.1/4	Hoffmann F.	2008 a	Effect of BAS 500 06 F on the growth of the green alga Pseudokirchneriella subcapitata 2008/1009325 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.3.1.1.1/1	Bocksch S.	2004 a	Assessment of side effects of BAS 500 06 F to the honey bee, Apis mellifera L. in the laboratory 2004/1015008 GAB Biotechnologie GmbH & GAB Analytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.1.1.2/1	Bocksch S.	2004 a	Assessment of side effects of BAS 500 06 F to the honey bee, Apis mellifera L. in the laboratory 2004/1015008 GAB Biotechnologie GmbH & GAB Analytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.1.5/1	Barth M.	2012 a	Effects of BAS 500 06 F on the honeybee Apis mellifera L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development 2011/1112669 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.3.2.1/1	Sipos K.	2007 a	Effect of BAS 500 06 F on the predatory mite (Typhlodromus pyri) in a laboratory trial 2007/1035599 LAB International Research Centre Hungary Ltd., Veszprem, Hungary yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.2.1/2	Sipos K.	2007 b	Effect of BAS 500 06 F on the parasitic wasp (Aphidius rhopalosiphi) in a laboratory trial 2007/1035600 LAB International Research Centre Hungary Ltd., Veszprem, Hungary yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.2.1/3	Sipos K.	2007 c	Amendment to the final report: Effect of BAS 500 06 F on the parasitic wasp (Aphidius rhopalosiphi) in a laboratory trial 2007/1050841 LAB International Research Centre Hungary Ltd., Veszprem, Hungary yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.2.2/1	Vaughan R.	2008 a	A rate-response extended laboratory test to determine the effects of BAS 500 06 F on the predatory mite, Typhlodromus pyri (Acari: Phytoseiidae) 2008/1010712 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.3.2.2/2	Stevens J.	2008 a	A rate-response extended laboratory test to determine the effects of BAS 500 06 F on the parasitic wasp, Aphidius rhopalosiphi (Hymenoptera, Braconidae) 2008/1010713 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.2.2/3	Roehlig U.	2008 a	Effects of BAS 500 06 F on the green lacewing Chrysoperla carnea STEPH. under extended laboratory conditions - Rate-response test 2008/1032666 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.2.2/4	Roehlig U.	2008 b	Effects of BAS 500 06 F on the green lacewing Chrysoperla carnea STEPH. in an extended laboratory test (under semi-field conditions aged residues on bean plants) 2008/1042190 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.3.2.2/5	Schmitzer S.	2008 a	Effects of BAS 500 06 F on the reproduction of rove beetles (Aleochara bilineata) - Extended laboratory study 2008/1010700 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.4.1/1	Fleischer G.	2004 a	Effect of BAS 500 06 F on the mortality of the earthworm Eisena fetida 2004/1004367 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.4.1.1/1	Luehrs U.	2008 a	Effects of BAS 500 06 F on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5% peat 2008/1036409 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.4.1.2/1	Luehrs U.	2010 a	Field study to evaluate the effects of BAS 500 06 F on earthworms 2010/1000056 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.4.2.1/1	Friedrich S.	2008 b	Effects of BAS 500 06 F on the reproduction of the collembolans Folsomia candida in artificial soil with 5% peat 2008/1037495 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.4.2.1/2	Schulz L.	2012 c	BAS 500 06 F - Effects of BAS 500 06 F on the reproduction of the predatory mite Hypoaspis aculeifer 2012/1129444 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.4.2.2/1	Luehrs U., Schabio S.	2010 a	Effects of BAS 500 06 F on the breakdown of organic matter in litter bags in the field 2010/1000081 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.5/1	Schulz L.	2012 b	Effects of BAS 500 06 F on the activity of soil microflora (Nitrogen transformation test) 2012/1129443 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.6.2/1	Stroemel C. et al.	2013 a	Effect of BAS 500 06 F on vegetative vigour of ten species of terrestial plants under greenhouse conditions 2012/1115894 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.6.2/2	Stroemel C. et al.	2012 a	Effect of BAS 500 06 F on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions 2012/1115895 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

BASF
The Chemical Company

BAS 500 06 F

DOCUMENT L-CP, Section 11

LITERATURE DATA

Reference List

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E-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

There are no references submitted with this section.

BASF
The Chemical Company

BAS 500 06 F

DOCUMENT M-CP, Section 1

IDENTITY OF THE PLANT PROTECTION PRODUCT

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E-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 1 IDENTITY OF THE PLANT PROTECTION PRODUCT

BAS 500 06 F is a new representative formulation, which has not been evaluated during the previous Annex I inclusion process.

CP 1.1 Applicant

BASF SE
Crop Protection Division
P.O. Box 120
67114 Limburgerhof
Germany

Contact person:

(a) Contact: [redacted]
BASF SE
Agricultural Center
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Germany
Telephone: [redacted]
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E-mail:

(b) Alternative: [redacted]
BASF SE
Agricultural Center
P.O. Box 120
67114 Limburgerhof
Germany
Telephone: [redacted]
Telefax:
E-mail:

CP 1.2 Producer of the plant protection product and the active substances

Manufacturer of BAS 500 06 F (legal entity):

BASF SE
67056 Ludwigshafen
Germany
Crop Protection Division:
P.O. Box 120
67114 Limburgerhof
Germany

Contact person: Please refer to CP 1.1 Applicant.

Location of the manufacturing site of BAS 500 06 F:

Confidential information - data provided in Document J.

Manufacturer of pyraclostrobin (legal entity):

BASF SE
67056 Ludwigshafen
Germany
Crop Protection Division:
P.O. Box 120
67114 Limburgerhof
Germany

Contact person: Please refer to CP 1.1 Applicant.

Location of the manufacturing site of pyraclostrobin:

Confidential information - data provided in Document J.

CP 1.3 Trade names or proposed trade name and producer's development code number of the plant protection product if appropriate

Code number: BAS 500 06 F

Trade Names: BAS 500 06 F, Comet 20 EC, Comet 200, Comet Pro, Modem 200, Platoon, Retengo, Retengo New, Solaram 200

CP 1.4 Detailed quantitative and qualitative information on the composition of the plant protection product

Pyraclostrobin pure active substance:

Minimum purity: 975 g/kg

CP 1.4.1 Composition of the plant protection product

Pure active substance

content of pure active substance:	**200.0 g/L**	**(19.16% w/w)**
limits:	188.00 – 212.00 g/L	(18.01 – 20.31% w/w)

Technical active substance [1)]

content of technical active substance:	**205.13 g/L**	**(19.65% w/w)**
limits:	192.82 – 217.44 g/L	(18.47 – 20.83% w/w)

[1)] at a minimum purity of technical pyraclostrobin of 97.5%

Safeners, synergists and co-formulants

Confidential information - data provided in Document J.

CP 1.4.2 Information on the active substances

Type	Name/Code Number
ISO common name	pyraclostrobin
CAS No	175013-18-0
EC No	n.a.
CIPAC No	657
salt, ester anion or cation present	none

CP 1.4.3 Information on safeners, synergists and co-formulants

Confidential information - data provided in Document J.

CP 1.5 Type and code of plant protection product

Nature: Emulsifiable concentrate [Code: EC]

CP 1.6 Function

Fungicide

BASF
The Chemical Company

BAS 500 06 F

DOCUMENT M-CP, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE PLANT PROTECTION PRODUCT

Compiled by:

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E-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162271 (version 1)
27-Feb-2017	Inclusion of additional information on viscosity in chapter 2.5.	BASF DocID 2017/1032191 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 2 PHYSICAL AND CHEMICAL PROPERTIES OF THE PLANT PROTECTION PRODUCT

BAS 500 06 F is a new representative formulation, which has not been evaluated during the previous Annex I inclusion process.

All tests have been performed in GLP-certified laboratories.

Test or study & Data point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CP 2.1 Appearance	Visual assessment and organoleptic determination	8241 205.04 g/L	BAS 500 06 F is a dark yellow liquid with a moderate naphthalene-like odour (remains unchanged after accelerated storage test).	Y	[see 2004/1010381 Kaestel R. 2004 a]
CP 2.2 Explosive and oxidising properties	UN test O.2 OECD 113	8241 200 g/L	The test substance is not considered to be an oxidising substance according to the UN recommendations on the Transport Of Dangerous Goods (Division 5.1). The test EEC A 14 has been omitted, because the exothermic decomposition energy determined by a DSC is less than 500 J/g.	Y	[see 2004/1004104 Loeffler U. 2004 a]
CP 2.3 Flammability and self-heating	EEC A9.1.6.3.2 EEC A15	8241 205.04 g/L 8241 200 g/L	The flash point of BAS 500 06 F is 104°C. Auto-flammability: 450°C (self-ignition temperature)	Y Y	[see 2004/1010381 Kaestel R. 2004 a] [see 2004/1004104 Loeffler U. 2004 a]
CP 2.4 Acidity/alkalinity and pH value	CIPAC MT 75	8241 205.04 g/L	The pH of BAS 500 06 F was determined to be 6.5 (1% in CIPAC water D) and 6.1 (1% in pure water). The values do not change throughout the storage period.	Y	[see 2004/1010381 Kaestel R. 2004 a]

<table>
<tr><th>Test or study & Data point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td>CP 2.5 Viscosity and surface tension</td><td>OECD 114
OECD 115 / EEC A5 1.6.1</td><td>8241
205.04 g/L</td><td>The kinematic viscosity (at 40°C) was determined to be 12.9*10^{-6} m²/s.
The analysis of the surface tension yielded 36.6 mN/m at 0.06%, 33.4 mN/m at 0.8% and 36.3 mN/m undiluted (all at 20°C).
Dynamic Viscosity:
measurement at 20°C and three shear rates:
<table>
<tr><td colspan="2">Measurement temp.: 20 C</td><td colspan="3">Dynamic viscosity [mPa s]</td></tr>
<tr><td colspan="2"></td><td colspan="3">Shear rates</td></tr>
<tr><td>Storage temp. [C]</td><td>Storage time [weeks]</td><td>D = 10s^{-1}</td><td>D = 100s^{-1}</td><td>D = 400s^{-1}</td></tr>
<tr><td>-</td><td>initial</td><td>31.4</td><td>31.4</td><td>31.4</td></tr>
<tr><td colspan="2">Flow behavior:</td><td colspan="3">Newtonian</td></tr>
</table>
measurement at 40°C and three shear rates:
<table>
<tr><td colspan="2">Measurement temp.: 40 C</td><td colspan="3">Dynamic viscosity [mPa s]</td></tr>
<tr><td colspan="2"></td><td colspan="3">Shear rates</td></tr>
<tr><td>Storage temp. [C]</td><td>Storage time [weeks]</td><td>D = 10s^{-1}</td><td>D = 100s^{-1}</td><td>D = 400s^{-1}</td></tr>
<tr><td>-</td><td>initial</td><td>13.3</td><td>13.3</td><td>13.3</td></tr>
<tr><td colspan="2">Flow behavior:</td><td colspan="3">Newtonian</td></tr>
</table></td><td>Y</td><td>[see 2004/1010381 Kaestel R. 2004 a]
[see 2016/1306845 Siebecker M. 2016 a]
[see 2016/1306847 Siebecker M. 2016 b]</td></tr>
<tr><td>CP 2.6 Relative density and bulk density</td><td>EEC A.3.1.4.1.1</td><td>8241
205.04 g/L</td><td>The relative density of BAS 500 06 F D$^{20}_{4}$ is 1.044 g/cm³.</td><td>Y</td><td>[see 2004/1010381 Kaestel R. 2004 a]</td></tr>
</table>

<table>
<tr><th>Test or study & Data point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr>
<td>CP 2.7 Storage Stability and shelf-life: effects of temperature on technical characteristics of the plant protection product</td>
<td>CIPAC MT 46.3
CIPAC MT 39.3
GIFAP Technical Monograph No. 17 (May 1993), EEC 91/414 Annex III 2.7.3</td>
<td>8241
205.04 g/L

8241
203.9 g/L</td>
<td>
<table>
<tr><th>Storage interval</th><th>Active substance content [g/L (% of initial)]</th></tr>
<tr><td>2 weeks 54°C (initial value: 205.04 g/L)</td><td>204.04 (99.51)</td></tr>
<tr><td>24 months 23°C (initial value: 203.9 g/L)</td><td>200.9 (98.53)</td></tr>
</table>
After 7 days at 0°C, no separated material could be observed. The sample remained homogenous.
The product is stable for all tested storage intervals and temperatures.

<u>Content of dimethyl sulfate:</u>
Dimethyl sulfate could not be detected in BAS 500 06 F (see M-CP 5.1.1). Furthermore, it is not formed during the production process of the formulation and its content in formulation does not increase during storage, because it is a molecule with a high energy content.
No significant changes of the physical properties: appearance, odour, pH-value, density and emulsion stability (see corresponding Annex points) were found after the accelerated storage stability test in glass bottles.
</td>
<td>Y

Y</td>
<td>[see 2004/1010381 Kaestel R. 2004 a]

[see 2006/1019717 Kroehl T. 2006 a]</td>
</tr>
</table>

<table>
<tr><th>Test or study & Data point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
After storage for 104 weeks at 23°C, the formulation showed good chemical and physical stability:

Content of pyraclostrobin (CF-A 669):
203.9 g/L before and 200.9 g/L after storage for 2 years at 23°C

Relative density (OECD 109/EEC A3):
D^{20}_4 =1.043 before and 1.043 after storage for 2 years at 23°C

Appearance (visual):
dark yellow clear liquid with moderate naphtaline-like odour before and after storage for 2 years at 23°C

pH (CIPAC MT 75):
Concentration of 0.8%:
<table>
<tr><th>Storage period / Dilution medium</th><th>Initial</th><th>After storage for 2 years at 23°C</th></tr>
<tr><td>CIPACwater D</td><td>6.5</td><td>6.8</td></tr>
<tr><td>Pure water</td><td>6.0</td><td>5.7</td></tr>
</table>
Concentration of 0.06%:
<table>
<tr><th>Storage period / Dilution medium</th><th>Initial</th><th>After storage for 2 years at 23°C</th></tr>
<tr><td>CIPACwater D</td><td>6.3</td><td>6.8</td></tr>
<tr><td>Pure water</td><td>6.0</td><td>5.9</td></tr>
</table>
Persistent Foaming (CIPAC MT 47.2):
Concentration of 0.8% in CIPAC water D: 14 mL foam after 1 min before and 18 mL foam after 1 min after
</td><td></td><td></td></tr>
</table>

Test or study & Data point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
			storage for 2 years at 23°C Concentration of 0.06% in CIPAC water D: 8 mL foam after 1 min before and 4 mL foam after 1 min after storage for 2 years at 23°C Viscosity (OECD 114): D=100s^{-1}: 31.7 mPa s before and 31.3 mPa s after storage for 2 years at 23°C, Newtonian flow behavior Emulsion stability (CIPAC MT 36.3): Values before and after storage for 2 years at 23°C remain unchanged. Max. sediment: <1ml; no top cream/oil, no bottom cream/oil at 0.8% and 0.06% concentration at all standing times (for CIPAC water A and D). Spontaneous emulsion: Yes, little froth; re-emulsification: Yes, homogenous The condition of the containers remained unchanged; no adverse effect of the product to its packaging could be observed.		

<table>
<tr><th>Test or study & Data point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td>CP 2.8 Technical characteristics of the plant protection product</td><td colspan="5"></td></tr>
<tr><td>CP 2.8.1 Wettability</td><td></td><td></td><td>Not applicable to EC formulations.</td><td></td><td></td></tr>
<tr><td>CP 2.8.2 Persistence of foaming</td><td>CIPAC MT 47</td><td>8241
205.04 g/L

8265</td><td>
<table>
<tr><td rowspan="2">storage interval</td><td colspan="4">0.06% / 0.8%, Volume of foam [ml]</td></tr>
<tr><td>10sec</td><td>1min</td><td>3min</td><td>12min</td></tr>
<tr><td>Initial</td><td>10/20</td><td>8/14</td><td>8/10</td><td>0/4</td></tr>
</table>
<table>
<tr><td rowspan="2">storage interval</td><td colspan="4">0.06% / 1.25%, Volume of foam [ml]</td></tr>
<tr><td>10sec</td><td>1min</td><td>3min</td><td>12min</td></tr>
<tr><td>Initial</td><td>6/16</td><td><1/16</td><td><1/16</td><td><1/16</td></tr>
<tr><td>2 weeks, 54°C</td><td>10/22</td><td>8/20</td><td>8/20</td><td>8/20</td></tr>
</table>
</td><td>Y

Y</td><td>[see 2004/1010381 Kaestel R. 2004 a]

[see 2008/1086283 Kroehl T. 2008 a]</td></tr>
<tr><td>CP 2.8.3 Suspensibility, spontaneity and dispersion stability</td><td></td><td></td><td>Not applicable to EC formulations</td><td></td><td></td></tr>
<tr><td>CP 2.8.4 Degree of dissolution and dilution stability</td><td></td><td></td><td>Not applicable to EC formulations</td><td></td><td></td></tr>
</table>

Test or study & Data point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CP 2.8.5 Particle size distribution, dust content, attrition and mechanical stability			Not applicable to EC formulations		
CP 2.8.5.1 Particle size distribution			Not applicable to EC formulations		
CP 2.8.5.2 Dust content			Not applicable to EC formulations		
CP 2.8.5.3 Attrition			Not applicable to EC formulations		
CP 2.8.5.4 Hardness and integrity			Not applicable to EC formulations		
CP 2.8.6 Emulsifiability, re-emulsifiability, emulsion stability	CIPAC MT 36.3	8241 205.04 g/L 8265	Before accel. storage, after low temperature storage and after accel. storage, at concentrations of 0.06% and 1.25% both in CIPAC water A and D, results are the same: <u>Initial emulsification:</u> After 1 inversion the mixture has emulsified spontaneously and appears homogeneous, little froth. <u>Emulsion stability:</u> At a conc. of 0.06% BAS 500 06 F in CIPAC water D: after 0.5h/2h/24h standing time: 0/0/0 mL sediment 0/0/0 mL cream 0/0/0 mL oil	Y Y	[see 2004/1010381 Kaestel R. 2004 a] [see 2008/1086283 Kroehl T. 2008 a]

Test or study & Data point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
			At a conc. of 1.25% BAS 500 06 F in CIPAC water D: 0/0/0 mL sediment 0/<1/<1 mL cream 0/0/0 mL oil At a conc. of 0.06% BAS 500 06 F in CIPAC water A: 0/0/<1 mL sediment 0/0/0 mL cream 0/0/0 mL oil At a conc. of 1.25% BAS 500 06 F in CIPAC water A: 0/0/0 mL sediment <1/<1/1 mL cream 0/0/0 mL oil <u>Re-emulsifiability after a standing time of 24h and 10 inversions:</u> At a conc. of 0.06% BAS 500 06 F in CIPAC water D/CIPAC water A: homogeneous emulsion Final emulsion stability after addit. 0.5h: 0/0 mL sediment 0/0 mL cream 0/0 mL oil		

Test or study & Data point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
			At a conc. of 1.25% BAS 500 06 F in CIPAC water D/CIPAC water A: homogeneous emulsion Final emulsion stability after addit. 0.5h: 0/0 mL sediment <1/<1 mL cream 0/0 mL oil		
CP 2.8.7 Flowability, pourability and dustability			Not applicable to EC formulations		
CP 2.9 Physical and chemical compatibility with other products including other plant protection products with which its use is to be authorised	ASTM E 1518-93; Mixing by hand, evaluation at different standing times, additional characterizations	8241	Nine mixtures of BAS 500 06 F with other plant protection products were tested (tank mix partners: Opus (SC), Opus Top (SE), Evidan/Flamenco FS (SE), Corbel (EC), Cerone (SL), Medax Top (SC), Topik 80 Plus (EC), Rustica Oel (OL), Atlantis (WG), Actirob (EC), Starane XL (SE) and Sumicidin Alpha EC (EC)). All mixtures appeared to be homogeneous and can be used in spray applications, but a running agitator should be used in 8 of them. No indication of any chemical reaction between the mixed products was observed.	N	[see 2004/1008462 Rudoll B., Schneider K.-H. 2004 a]
CP 2.10 Adherence and distribution to seeds			Not applicable to EC formulations		
CP 2.11 Other studies			Not required		

BASF
The Chemical Company

BAS 500 06 F

DOCUMENT M-CP, Section 3

DATA ON APPLICATION

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-July-2014		BASF DocID 2014/1162272 (version 1)
09-June-2015	The document according to SANCO/2012/11251 (following data point 3.8) was revised in order to a) better reflect the applicant's intention to achieve the renewal of approval for both, the use as fungicide and the use as plant growth regulator b) have the complete information for the representative uses in this document instead of referring to M-CA, section 3 c) shorten table 2 (critical uses only instead of all registered uses). New or changed text is marked in yellow.	BASF DocID 2015/1106131 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 3 DATA ON APPLICATION

BAS 500 06 F is a new representative formulation, which has not been evaluated during the previous Annex I inclusion process.

CP 3.1 Field of use envisaged

Please see below efficacy information according to SANCO/2012/11251 (chapter 3).

CP 3.2 Effects on harmful organisms

Please see below efficacy information according to SANCO/2012/11251 (chapter 8).

CP 3.3 Details of intended use

Please see below Table 2 in the efficacy information according to SANCO/2012/11251 (following chapter 8).

CP 3.4 Application rate and concentration of active substance

Please see below Table 2 in the efficacy information according to SANCO/2012/11251 (following chapter 8).

CP 3.5 Method of application

Please see below efficacy information according to SANCO/2012/11251 (chapter 7).

CP 3.6 Number and timing of applications and duration of protection

Please see below for general information M-CP 3.8 and for detailed information Table 2 (following chapter 8) of the efficacy information given according to SANCO/2012/11251.

BAS 500 06 F can provide control for up to seven weeks after application when applied before the start of foliar disease attack.

CP 3.7 Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops

Pyraclostrobin has been applied since many years with a several different formulations across a wide range of crops without any reports of a phytotoxic effects on succeeding crops. Due to the broad range of crops in which the product has been used, most rotational crop possibilities have been appeared in practise. Therefore no negative impact on succeeding crops is to be expected. Consequently, there is no necessity for restrictions in the choice of following crops, even in the event of crop failure on a field which has been treated with BAS 500 06 F.

CP 3.8 Proposed instructions for use

BAS 500 06 F is a fungicide with protectant and curative properties for disease control in winter and spring wheat, winter and spring barley, oats, rye and triticale and with protectant properties for disease control in maize (forage and grain). Details are shown in the efficacy information according to SANCO/2012/11251 (chapter 8).

Time of application

Cereals
Apply BAS 500 06 F at the start of foliar disease attack. A maximum of two applications can be made, starting from mid tillering (BBCH 25) until end of flowering (latest application at BBCH 69). The described application window can be more limited in specific countries or for specific uses. The recommended spray interval is 21 days depending on disease pressure and the general spray program strategy.

Maize (forage and grain)
Best results are achieved by an application of BAS 500 06 F before the start of foliar disease attack or at very early signs of infection. Once disease is established in the crop, BAS 500 06 F can stop or reduce the development of the disease. Application can be made after beginning of stem elongation (earliest application at BBCH 30) and up to and including mid-flowering (BBCH 65).

Number and rate of application

Cereals
Apply maximum 2 times per season up to 1.25 L BAS 500 06 F in 100 – 400 L of water per ha.

Maize (forage and grain)
Apply maximum once per season up to 1.0 L BAS 500 06 F in 100 – 400 L of water per ha.

On the specific country labels additional detailed guidance for the use of the product e.g. with regard to filling and application, tank cleaning and field use are given considering the local requirements. These instructions will be covered by the dRRs to be submitted for the re-registration of the plant protection product BAS 500 06 F following the renewal of approval for the active substance pyraclostrobin.

Further information can be found in Document C.

Efficacy Information

Active Substance: Pyraclostrobin

Product Code: BAS 500 06 F
200 g/L EC

Applicant: BASF SE
Date: 09/June/2015

Statement

Pyraclostrobin, the active substance contained in the plant protection product BAS 500 06 F, has been tested in field development trials which demonstrated efficacious activity against a broad range of fungal diseases. It was included in Annex I of Directive 91/414/EEC on 1 June 2004 (entry into force) under Inclusion Directive 2004/30/EC for the use as fungicide. This use was amended on 22 April 2009 (entry into force) by Inclusion Directive 2009/25/EC to the use as fungicide or plant growth regulator.

BAS 500 06 F is registered in many EU countries (for details please see Table 1) based on detailed national assessments of the efficacy data package in compliance with Regulation (EC) No 545/2011 and according to the Uniform Principles as given in Regulation (EC) No 546/2011.

1. INTRODUCTION

This document summarises the information related to the efficacy of the representative formulation BAS 500 06 F containing pyraclostrobin as active substance for which a dossier has been submitted for the renewal of approval.

2. FUNCTION

BAS 500 06 F is used as a fungicide to control harmful diseases in cereals, maize and other crops. It shows local systemic and translaminar activity and can be used for preventative and curative treatments. In addition to the fungicidal effects, plant physiology can be also affected by the application of BAS 500 06 F, e.g. reduction of nonparasitic leaf spots in cereals or yield increase in the absence of fungal diseases. Pyraclostrobin is registered as fungicide and as plant growth regulator.

3. FIELD OF USE

BAS 500 06 F is used in agriculture in several crops for the control of a broad range of harmful and economically important pathogens. In addition it is applied due to its physiological effects.

4. SUPPORTED USES

BAS 500 06 F in cereals and maize had been identified as representative uses to support the renewal of approval for the active substance pyraclostrobin (for details see Tables 1 and 2).

These uses are representative because of

- their wide geographical distribution covering the Southern, Central and Northern Zone
- their broad range of controlled diseases
- their different application timings
- their agricultural importance in the EU
- their importance in the human diet (including their huge importance as feedstuff being the basis for food of animal origin)

BAS 516 07 F in potatoes had been identified as an additional representative use to support the renewal of approval for the active substance pyraclostrobin (for details see separate dossier for BAS 516 07 F).

5. OVERVIEW OF CURRENT REGISTRATIONS

Current registrations of BAS 500 06 F in the EU for the representative uses (cereals and maize) are shown in Table 1.
Besides the use of BAS 500 06 F in cereals and maize, many other uses of pyraclostrobin containing plant protection products are registered covering all EU member states and most of the crops professionally cultivated (further information on these additional uses can be found in M-CA 3.5). In addition to various solo formulations other active substances such as boscalid, dimethomorph, dithianon, epoxiconazole, fenpropimorph, fluxapyroxad, folpet and metiram are used in pyraclostrobin containing mixture formulations (further information on these uses can be found in document D2).

6. HARMFUL ORGANISMS CONTROLLED AND CROPS TREATED

Harmful organisms controlled by BAS 500 06 F in cereals and maize are given in chapter 8 below.
An overview on additional harmful organisms controlled by pyraclostrobin and on additional crops treated in the EU with pyraclostrobin-containing formulations can be found in M-CA 3.5.

7. METHOD OF APPLICATION

BAS 500 06 F is applied in cereals and maize by broadcast foliar spraying using water as carrier. The water volume varies between 100 – 400 L/ha.

8. MODE OF ACTION - EFFECTS ON HARMFUL ORGANISMS

The **mode of action** for the active substance pyraclostrobin is described below for the use as fungicide and the use as plant growth regulator.

Use as fungicide

Pyraclostrobin belongs to the QoI group of fungicides. The mode of action is the inhibition of mitochondrial respiration resulting from a blockage of the electron transport from ubihydroquinone to cytochrome c by means of a binding to the ubihydroquinone oxidation centre (Qo) of the cytochrome bc1 complex (Complex III). This disrupts the mitochondrial electron transport chain, thus blocking phosphorylation further down in the respiratory chain. In consequence, this leads to a reduction of energy-rich ATP which is required to support a range of essential processes in the fungal cell such as maintenance of membrane potentials and concentration gradients up to DNA, RNA and protein biosynthesis. In the end, the various fungal development processes of spore germination, formation of infection structures, mycelium growth and sporulation are permanently disrupted.

Use as plant growth regulator

In addition to the effects on yield through inhibition of fungal pathogens, pyraclostrobin also delivers a positive effect on yield through influence on the plant metabolism and physiology.
A decrease in ethylene levels has been demonstrated in several lab studies after treatment with pyraclostrobin. This can explain the observed delay in senescence. The reduction in ethylene levels can result in a variety of cellular changes being involved in yield gain (e.g. by an increased and longer photosynthetic performance during the vegetation period of a treated crop):

- enhancement of cytokinin levels resulting in increased chlorophyll concentrations in the leaves
- higher catalase and superoxide dismutase (SOD) activities reducing reactive $O_2^{\cdot}$-levels producing e.g. H_2O_2, resulting in reduced chlorophyll degradation

Furthermore, studies demonstrated that pyraclostrobin increases nitrate reductase, improving nitrogen assimilation and nitrogen use efficiency.

In the following, information on the **foliar uptake** of pyraclostrobin and a description of its **general effects on harmful organisms** are given.

After application to the plant, the active ingredient is taken up via the leaf and then translocated at low rates via the transpiration flow. Due its relatively low mobility, it shows local systemic and translaminar activity. Because of its very high intrinsic activity, pyraclostrobin has been observed to have systemic effects in a number of authorized uses. By that, it can control fungal stages which have already become established in deeper tissue layers. Pyraclostrobin is thus suitable for preventative and curative treatments. Since the vapour pressure of pyraclostrobin is very low, a marked gas phase activity was not observed.

Pyraclostrobin is active against different fungal stages on and in the plant. When applied protectively, pyraclostrobin prevents not only the germination of fungal spores landing on the plant surface but also re-infection, since during these extremely energy-consuming phases fungi react very sensitively to disturbances of their mitochondrial respiratory chain. Due to its ability to penetrate into the leaf and its further translocation as well as its high intrinsic activity, it can also control fungal stages which have already become established in deeper tissue layers.

BAS 500 06 F is a fungicide with protectant and curative properties for disease control in winter and spring wheat, durum, spelt, winter and spring barley, oats, rye and triticale and with protectant properties for disease control in maize (forage and grain).

Details are summarized in the table below:

	Winter wheat	Spring wheat	Winter barley	Spring barley	Oats	Rye	Triticale	Maize (forage and grain)
Septoria spp.	X	X					X	
Yellow rust	X	X	X	X		X	X	
Brown rust	X	X	X	X		X	X	
Crown rust					X			
Net blotch			X	X				
Rhynchosporium			X	X		X		
Kabatiella zea								X
Puccinia sorghi								X
Exserohilum turcicum								X
Cochliobolus carbonum								X

In addition to its fungicidal effects, plant physiology is also effected by the application of BAS 500 06 F.

In cereals a reduction of nonparasitic leaf spots have been observed and a yield response may be obtained in the absence of visual disease.

In maize BAS 500 06 F can delay senescence and can improve the total greenness of the crop towards the end of the growing season. In addition, a grain yield response may be obtained in the absence of visual disease.

Table 1: Supported representative uses for BAS 500 06 F currently registered in the EU

	Representative Uses (for application details see Table 2)				Existing Authorisations								
Crop	Target	Situation of use (e.g. indoor…)	as content & Formulation Type	Application method	Country	Zone	Since	Reg. No.	Product (tradename)	Product application rate per treatment Min and Max	Active substance application rate per treatment Min and Max	Number of treatments per season Min and Max	Active substance Max total dose/ ha Min and Max
Cereals	*Blumeria* spp., *Fusarium* spp., *Puccinia* spp., *Pyrenophora* spp., *Rhynchosporium* spp., *Septoria* spp., nonparasitic leaf spots, other physiological effects	Outdoor	200 g/L EC	Foliar spray	DK	N	01.03.2011	19-184	BAS 500 06 F (Comet Pro)	0.8 – 1.25 L product/ha	160 – 250 g as/ha	1 – 2	500 g as/ha
					EE	N	24.08.2010	383	BAS 500 06 F (Comet Pro)				
					FI	N	03.03.2010	3053	BAS 500 06 F (Comet 200)				
					LT	N	10.11.2010	0396F/10	BAS 500 06 F (Comet Pro)				
					LV	N	22.07.2010	354	BAS 500 06 F (Comet Pro)				
					SE	N	02.01.2014	5163	BAS 500 06 F (Comet Pro)				
					IE	C	29.09.2008	03696	BAS 500 06 F (Comet 200)				
							29.09.2008	03695	BAS 500 06 F (Modem 200)				
					UK	C	14.02.2005	12325	BAS 500 06 F (Platoon)				
							03.03.2005	12338	BAS 500 06				
							22.11.2005	12639	BAS 500 06 F (Comet 200)				
					ES	S	05.10.2012	25.636	BAS 500 06 F (Comet 200)				

	Representative Uses (for application details see Table 2)				Existing Authorisations								
Crop	Target	Situation of use (e.g. indoor…)	as content & Formulation Type	Application method	Country	Zone	Since	Reg. No.	Product (tradename)	Product application rate per treatment Min and Max	Active substance application rate per treatment Min and Max	Number of treatments per season Min and Max	Active substance Max total dose/ ha Min and Max
					FR	S	16.10.2010	2100151	BAS 500 06 F (Comet 200)				
							22.11.2010	2100151	BAS 500 06 F (Solaram 200)				
					GR	S	19.11.2013	60391	BAS 500 06 F (Envelis 20 EC)				
							16.04.2014	60415	BAS 500 06 F (Comet 20 EC)				
					IT	S	19.12.2013	15961	BAS 500 06 F (Retengo New)				
					PT	S	07.02.2012	AV0255	BAS 500 06 F (Comet 200)				
Maize	*Exserohilum turcicum*, *Kabatiella zeae*, *Puccinia sorghi*, physiological effects	Outdoor	200 g/L EC	Foliar spray	SE	N	02.01.2014	5163	BAS 500 06 F (Comet Pro)	0.7 – 1 L/ha	140 – 200 g as/ha	1	140 – 200 g as/ha
					BG	S	28.03.2014	01230	BAS 500 06 F (Retengo)				
					GR	S	16.04.2014	60415	BAS 500 06 F (Comet 10 EC)				
					IT	S	19.12.2013	15961	BAS 500 06 F (Retengo New)				

Table 2: Critical Uses – justification and GAP tables

GAP rev. 1.0, date: 2014-May-05

PPP (code)	**BAS 500 06 F**	**Formulation type:**	**EC**
active substance 1	**Pyraclostrobin**	**Conc. of as:**	**200 g/L**
safener	**-**	**Conc. of safener:**	**-**
synergist	**-**	**Conc. of synergist:**	**-**
Applicant:	**BASF SE**	**professional use**	☒
Zone(s):	**EU**	**non professional use**	☐
Verified by MS:	**y/n**		

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
Cereals												
1	DK, EE, FI, LT, LV, SE, IE, UK, ES, FR, GR, IT, PT	Barley (spring, winter) Oats (spring, winter) Rye Triticale Wheat (spring, winter, durum, spelt)	F	*Blumeria graminis*, *Fusarium spp.*, *Puccinia spp.*, *Pyrenophora teres*, *Rhynchosporium secalis*, *Septoria spp.*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	Including physiological effects (GR, IE, IT, UK)

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
Maize (forage and grain)												
2	SE, BG, GR, IT	Maize (forage and grain)	F	*Exserohilum turcicum* *Kabatiella zeae,* *Puccinia sorghi*	Spraying	30 - 65	a) 1 b) 1	a) 1.00 b) 1.00	a) 0.2 b) 0.2	100 - 400	n.a.	PHI defined by growth stage at application. Including physiological effects (IT)

Note: A table showing a detailed overview of the GAPs for all EU uses of BAS 500 06 F in cereals and maize (already registered and including the ones submitted and still under evaluation by the authorities) can be found in Document D1.

BASF
The Chemical Company

BAS 500 06 F

DOCUMENT M-CP, Section 4

FURTHER INFORMATION ON THE PLANT PROTECTION PRODUCT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162273 (version 1)
27-Feb-2017	Chapters 4.2 and 4.3 were amended by the MSDS of BAS 500 06 F and the text was updated where necessary (new or changed text is marked in yellow).	BASF DocID 2017/1032192 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 4 FURTHER INFORMATION ON THE PLANT PROTECTION PRODUCT

BAS 500 06 F is a new representative formulation, which has not been evaluated during the previous Annex I inclusion process.

CP 4.1 Safety intervals and other precautions to protect humans, animals and the environment

Pre-harvest interval (in days) for each relevant crop:
For cereals maximum two applications are intended at growth stages BBCH 25-69 corresponding to an approximate pre-harvest interval of 35 to 42 days.
For maize including sweet corn one application is intended at growth stages BBCH 30-65. The pre-harvest interval is defined by the growth stage at latest application.

Re-entry period (in days) for livestock, to areas to be grazed:
Because pyraclostrobin is not intended to be used in areas to be grazed, no re-entry period for livestock has to be defined.

Re-entry period (in hours or days) for man to crops, buildings or spaces treated:
The re-entry assessment is discussed in M-CP 7.2. Re-entry is possible after the spray deposits on the crops have dried given the worker is wearing adequate work clothing.

Withholding period (in days) for animal feeding stuffs:
In order to avoid residues above the MRL values proposed for products of animal origin, a withholding period of 35 days (after application) for cereal grains and 21 days for other cereal plant parts to be used as feeding stuff is recommended. In general, maize is fed at different growth stages to livestock. For ripe maize grain, the waiting period is given by the use directions. Green material can be fed to livestock about 1 to 2 weeks after application without exceedance of any MRL in milk or animal tissues. The same period is also applying for cobs with husks.

Waiting period (in days) between application and handling of treated products:
This is not relevant here since a post-harvest treatment is not intended for cereals and maize.

Waiting period (in days) between last application and sowing or planting succeeding crops:
No minimum waiting periods needs to be considered for phytotoxicity or for residues in follow-crops.

Information on specific conditions under which the preparation may or may not be used:
Not relevant

CP 4.2 Recommended methods and precautions

Report:	CP 4.2/1 Anonymous, 2015 a Safety data sheet - Retengo 2015/1280947
Guidelines:	EEC 1907/2006
GLP:	no

Precautions for safe handling

No special measures necessary if stored and handled correctly. Ensure thorough ventilation of stores and work areas. When using do not eat, drink or smoke. Hands and/or face should be washed before breaks and at the end of the shift.

Protection against fire and explosion:
Vapours may form ignitable mixture with air. Prevent electrostatic charge. Sources of ignition should be kept well clear. Fire extinguishers should be kept handy.

Conditions for safe storage including any incompatibilities

Segregate from foods and animal feeds.
Further information on storage conditions:
Keep away from heat. Protect from direct sunlight. Store protected against freezing.

Storage stability:
Storage duration: 60 months
Protect from temperatures below 0 °C and above 40 °C.
Changes in the properties of the product may occur if the product is stored below or above the indicated temperatures for extended periods of time.

Exposure controls

Personal protective equipment

Respiratory protection:
Suitable respiratory protection for higher concentrations or long-term effect: Combination filter for gases/vapours of organic, inorganic, acid inorganic and alkaline compounds (e.g. EN 14387 Type ABEK).

Hand protection:
Suitable chemical resistant safety gloves (EN 374) with prolonged, direct contact. Recommended is protective index 6 corresponding to > 480 minutes of permeation time according to EN 374, e.g. nitrile rubber (0.4 mm), chloroprene rubber (0.5 mm), butyl rubber (0.7 mm) etc.

Eye protection:
Safety glasses with side-shields (frame goggles), e.g. EN 166

Body protection:
Body protection must be chosen depending on activity and possible exposure, e.g. apron, protecting boots, chemical-protection suit (according to EN 14605 in case of splashes or EN ISO 13982 in case of dust).

General safety and hygiene measures
The statements on personal protective equipment in the instructions for use apply when handling crop-protection agents in final consumer packing. Wearing of closed work clothing is recommended. Store work clothing separately. Keep away from food, drink and animal feeding stuffs.

Transport information

Land transport

ADR

UN number:	UN3082
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains SOLVENT NAPHTHA, PYRACLOSTROBIN, FATTY ALCOHOL ETHOXYLATE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	Tunnel code: E

RID

UN number:	UN3082
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains SOLVENT NAPHTHA, PYRACLOSTROBIN, FATTY ALCOHOL ETHOXYLATE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	none known

Inland waterway transport

ADN

UN number:	UN3082
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains SOLVENT NAPHTHA, PYRACLOSTROBIN, FATTY ALCOHOL ETHOXY-LATE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	none known
Transport in inland waterway vessel:	not evaluated

Sea transport

IMDG

UN number:	UN 3082
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains SOLVENT NAPHTHA, PYRACLOSTROBIN, FATTY ALCOHOL ETHOXYLATE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Marine pollutant:	yes
Special precautions for user:	none known

Air transport

IATA/ICAO

UN number:	UN 3082
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (contains SOLVENT NAPHTHA, PYRACLOSTROBIN, FATTY ALCOHOL ETHOXYLATE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	none known

Waste treatment methods

Must be sent to a suitable incineration plant observing local regulations.

Contaminated packaging:
Contaminated packaging should be emptied as far as possible and disposed of in the same manner as the product.

Report: CP 4.2/2
Nolte M., 2010a
BAS 500 06 F: Effectiveness of Procedures for cleaning application equipment and protective clothing
2010/1048392
Guidelines: none
GLP: no

Note: This study was erroneously not contained in the application. It needs to be submitted, because BAS 500 06 F is a new representative formulation, which has not been evaluated during the previous Annex I inclusion process.

Any surplus spray solution containing BAS 500 06 F should be diluted with water at a ratio of 1:10 and sprayed onto the previously treated area according to the use instructions.

The spray equipment should be cleaned thoroughly immediately after use by draining the system completely and by rinsing spray tank, boom and nozzles two to three times with clean water, so that foam and remainders of BAS 500 06 F will be sufficiently removed. No plant damage can be caused when the equipment is used subsequently for other applications.

Protective clothing will be cleaned effectively when washed with usual laundry detergents.

CP 4.3 Emergency measures in the case of an accident

Report:	CP 4.3/1 Anonymous, 2015 a Safety data sheet - Retengo 2015/1280947
Guidelines:	EEC 1907/2006
GLP:	no

First aid measures

First aid personnel should pay attention to their own safety. If the patient is likely to become unconscious, place and transport in stable sideways position (recovery position). Immediately remove contaminated clothing.

If inhaled:
Keep patient calm, remove to fresh air, seek medical attention.

On skin contact:
Immediately wash thoroughly with soap and water, seek medical attention.

On contact with eyes:
Wash affected eyes for at least 15 minutes under running water with eyelids held open, consult an eye specialist.

On ingestion:
Immediately rinse mouth and then drink 200-300 ml of water, seek medical attention. Do not induce vomiting due to aspiration hazard.

Most important symptoms and effects, both acute and delayed
The most important potential symptoms are skin irritation or allergic skin reactions caused by skin contact, or eye irritation caused by eye contact. Furthermore, local irritation of the olfactory epithelium might be caused after repeated inhalation. BAS 500 06 F is classified as harmful if inhaled or swallowed.

Indication of any immediate medical attention and special treatment needed
Treat according to symptoms (decontamination, vital functions), no specific antidote known.

Fire-fighting measures

Suitable extinguishing media
Water spray, carbon dioxide, foam, dry powder

Special hazards arising from the product
The following substances or groups of substances can be released in case of fire:
Carbon monoxide, hydrogen chloride, carbon dioxide, nitrogen oxides, sulphur oxides, organochloric compounds

Advice for fire-fighters
Special protective equipment:
Wear self-contained breathing apparatus and chemical-protective clothing.
Further information:
In case of fire and/or explosion do not breathe fumes. Keep containers cool by spraying with water if exposed to fire. Collect contaminated extinguishing water separately, do not allow to reach sewage or effluent systems. Dispose of fire debris and contaminated extinguishing water in accordance with official regulations.

Accidental release measures

Personal precautions, protective equipment and emergency procedures
Do not breathe vapour or spray. Use personal protective clothing. Avoid contact with the skin, eyes and clothing.

Environmental precautions
Do not discharge into subsoil or soil. Do not discharge into drains, surface waters or ground-water.

Methods and material for containment and cleaning up
For small amounts:
Pick up with suitable absorbent material (e.g. sand, sawdust, general-purpose binder, kieselguhr).
For large amounts:
Dike spillage. Pump off product. Dispose of absorbed material in accordance with regulations. Collect waste in suitable containers, which can be labeled and sealed. Clean contaminated floors and objects thoroughly with water and detergents, observing environmental regulations. Cleaning operations should be carried out only while wearing breathing apparatus.

CP 4.4 Packaging, compatibility of the plant protection product with proposed packaging materials

BAS 500 06 F is marketed in high-density polyethylene containers with an inner barrier, e.g. polyamide (PA/PE). They are sealed by foil seals or by polyamide laminated PE-foam gaskets, protected by screw caps of polyethylene.

0.15 litre bottle	material:	PA/PE (Coex)
	shape/size:	cylindrical / approx. 63 mm diameter x 92 mm
	opening:	42 mm inner diameter
	closure:	polyethylene screw cap
	seal:	HF-seal
0.25 litre bottle	material:	PA/PE (Coex)
	shape/size:	cylindrical / approx. 63 mm diameter x 126 mm
	opening:	42 mm inner diameter
	closure:	polyethylene screw cap
	seal:	HF-seal
0.5 litre bottle	material:	PA/PE (Coex)
	shape/size:	cylindrical / approx. 69 mm diameter x 185.5 mm
	opening:	42 mm inner diameter
	closure:	polyethylene screw cap
	seal:	HF-seal
1 litre bottle	material:	PA/PE (Coex)
	shape/size:	cylindrical / approx. 88.5 mm diameter x 234 mm
	opening:	42 mm inner diameter
	closure:	Polypropylene/Polyethylene screw cap
	seal:	Induction sealed
1 litre eco-bottle	material:	PA/PE (Coex)
	shape/size:	cylindrical / approx. 88.5 mm diameter x 234 mm
	opening:	54 mm mm inner diameter
	closure:	Polypropylene/Polyethylene screw cap
	seal:	gasket
3 litre container	material:	PA/PE (Coex)
	shape/size:	rectangular / approx. 190 mm x 140 mm x 241 mm
	opening:	54 mm inner diameter
	closure:	polypropylene screw cap
	seal:	Induction sealed
5 litre container	material:	PA/PE (Coex)
	shape/size:	rectangular / approx. 190 mm x 140 mm x 313 mm
	opening:	54 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal
5 litre eco-container	material:	PA/PE (Coex)
	shape/size:	rectangular / approx. 185 mm x 136 mm x 313 mm
	opening:	54mm inner diameter
	closure:	polypropylene screw cap
	seal:	gasket

10 litre container	material:	PA/PE (Coex)
	shape/size:	rectangular / approx. 230 mm x 165 mm x 375 mm
	opening:	54 mm inner diameter
	closure:	polypropylene screw cap
	seal:	Induction sealed
10 litre eco-container	material:	PA/PE (Coex)
	shape/size:	rectangular / approx. 230 mm x 187 mm x 358 mm
	opening:	54mm inner diameter
	closure:	polypropylene screw cap
	seal:	gasket

Report:	CP 4.4/1 Schreiner B., 2005a EU performance tests of BAS 500 06 F (Standard-Coex-Bottle, 1 L, Spec.-No. 775 5108) 2005/1025057
Guidelines:	none
GLP:	no

ADR-test 6.1.5.3 was performed for drop resistance. In this test, the tightness of the bottles was successfully tested. The leak test and the hydrostatic test (ADR 6.1.5.3 and 6.1.5.4) were also done. The stacking test (ADR 6.1.5.6) was not applicable (bottles are part of combination packaging). The permeation test according to ADR 6.1.5.8 showed an acceptable value of <0.008 g/Lh. Packagings are supplied in UN-approved combination packs (ADR 6.1.4.21).

These packagings meet the requirements for packaging group III as specified by the ADR/RID regulations for the transport of hazardous goods.

Report:	CP 4.4/2 Kroehl T., 2006a Shelf life at 23°C in original container of the formulation BAS 500 06 F, 24 month storage, analytical results and physical properties 2006/1019717
Guidelines:	OECD Principles of Good Laboratory Practice,GLP Principles of the German Chemikaliengesetz (Chemicals Act),GIFAP Technical Monograph No. 17 (May 1993),EEC 91/414 Annex III 2.7.3
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Note: In the application this study was only mentioned in section 2.

The study results prove that the original container material and the closure/seal resists to the product BAS 500 06 F. No corrosion of the container could be observed during the storage time.

CP 4.5 Procedures for the destruction or decontamination of the plant protection product and its packaging

CP 4.5.1 Neutralisation procedure

The pH of BAS 500 06 F is in a range between 5.6 and 6.8 in aqueous solution. Therefore, the proposal of a neutralisation procedure is not considered to be necessary. Any spilled product and contaminated soil or water has to be absorbed and disposed according to the use instructions.

CP 4.5.2 Controlled incineration

For purposes of disposal, combustion of BAS 500 06 F at a licensed incinerator is recommended. This method of disposal applies also to contaminated packages, which cannot be cleaned or reused.

Although it is possible to incinerate the product at lower temperatures, combustion at approximately 1100°C with a residence time of about 2 seconds is advised. By doing so, i.e. operating the incinerator according to the conditions laid down in council directive 94/67/EEC resp. directive 2000/76/EC of the European Parliament, one will achieve complete combustion and minimize the formation of undesired by-products in the off-gases.

Users are requested to triple rinse empty primary packages as described in the ECPA "Guidelines for the rinsing of agrochemical containers", 1993.

Pressure rinsing or integrated pressure rinsing of the packaging material achieves a similar or even better result. The rinsing water must be added to the spray liquid.

To minimize waste of packages it is recommended that empty and rinsed containers are delivered to local container collection stations. If these do not exist, empty and rinsed containers must be rendered unusable and disposed according to local regulations.

BASF
The Chemical Company

BAS 500 06 F

DOCUMENT M-CP, Section 5

ANALYTICAL METHODS

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162274 (version 1)
27-Feb-2017	Chapter 5.1.1 was amended by a new document. The text and the numbering of the following documents were updated accordingly (new or changed text is marked in yellow).	BASF DocID 2017/1032193 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 5 ANALYTICAL METHODS

BAS 500 06 F is a new representative formulation, which has not been evaluated during the previous Annex I inclusion process.

CP 5.1 Methods for the generation of pre-authorisation data

CP 5.1.1 Analysis of the plant protection product

(a) Methods for the determination of the active substance and/or variant in the plant protection product

Report: CP 5.1.1/1
Ziegler H., Machauer B., 2003a
Analytical method CF-A 669: Quantitative determination of the active ingredient Pyraclostrobin in BAS 500 06 F by HPLC
2003/1022228
Guidelines: none
GLP: no

The method CF-A 669 is applicable for the determination of pyraclostrobin in the EC formulation BAS 500 06 F. A reversed phase HPLC method is used, utilizing a 4µm J'sphere ODS-H80 column. Detection is done by a UV detector at 230 nm, quantification is made by external standard.

Report:	CP 5.1.1/2 Ziegler H., 2004a Validation of the analytical method CF-A 669: Determination of the active ingredient Pyraclostrobin in BAS 500 06 F by HPLC 2004/1004041
Guidelines:	OECD Principles of Good Laboratory Practice, GLP Principles of the German Chemikaliengesetz (Chemicals Act)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Report:	CP 5.1.1/3 Siebecker M., 2016 a BAS 500 06 F - additional document to the validation of the analytical method AFL0669/01 (formerly CF-A 669) 2016/1299807
Guidelines:	none
GLP:	no

Note:
Document BASF DocID 2016/1299807 provides readable chromatograms for the existing HPLC-method. The chromatograms contained in the validation report (BASF DocID 2004/1004041) on page 16 to 18 are unfortunately not well readable and had therefore to be provided in a better quality.

Method principle The active ingredient is dissolved from the samples of BAS 500 06 F by water and acetonitrile. The solution is chromatographed on an HPLC reversed phase system and pyraclostrobin is analyzed by means of UV detection using external calibration.

Findings The validation data of method CF-A 669 with respect to precision, accuracy, linearity and specificity prove that the method is suitable to determine the active ingredient in formulations of the type EC, e.g. in BAS 500 06 F.

Specificity The specificity was demonstrated by:
a) The retention times of the peaks of pyraclostrobin in BAS 500 06 F and the reference items are observed of the same value.
b) Identical UV-spectra of the compound peaks in the preparation and the reference items were measured.
c) The chromatogram of the blank formulation showed no interference with the active ingredient peak.

Linearity The linearity of response to the analyte was determined in a concentration series. The weights amounted to about 0.5, 0.75, 1.0, 1.25 and 1.5 times of the measured concentration. The test demonstrated linearity of response to the analyte concentration over the practical range of the method.

Slope (m): 67609
y axis intercept (b): -2845
Correl. coeff. (r^2): 1.0000

Accuracy Based on six replicates of a sample of blank formulation of BAS 500 06 F fortified with reference grade pyraclostrobin (BAS 500 F), the mean recovery has been determined to be 100.27%. The deviation of the values is given by the analysis errors and errors from preparing the test mixtures. Outliers or stragglers had not been identified.

Repeatability A production sample BAS 500 06 F was weighed six times and the active ingredient pyraclostrobin has been determined from this in accordance with the present analytical method.

Results for the formulation BAS 500 06 F:

Weight % (min, max)	Mean %	RSD %
19.138 – 19.230	19.19	0.194

It is situated in a range which can be expected by the used equipment and the analytical method in general. The RSD values meet the requirements given by the modified Horwitz equation.
Outliers or stragglers had not been identified.

Conclusion **The fortification experiments demonstrate that analytical method CF-A 669 is applicable to determine the content of pyraclostrobin in formulations of the type EC, e.g. in BAS 500 06 F.**

(b) Methods for determination of relevant impurities identified in the technical material or which may be formed during manufacture of the plant protection product or from degradation of the plant protection product during storage

Pyraclostrobin contains < 1mg/kg dimethyl sulfate. Information on the analytical method for determination of this impurity in the technical material can be found in M-CA 4.1.1. In the following, the method for determination of dimethyl sulfate in BAS 500 06 F is outlined.

Report: CP 5.1.1/4
Stegmaier W., 2011a
Gas chromatographic determination of dimethyl sulfate in BAS 500 06 F
2011/1009063
Guidelines: none
GLP: no

The method of analysis was developed in order to determine dimethyl sulfate in BAS 500 06 F. The method applied is a Headspace GC method with quantification by means of standard addition. The method was developed and validated in accordance with current GLP regulations.

Report: CP 5.1.1/5
Stegmaier W., 2011b
Validation of an analytical method for the determination of dimethyl sulfate in BAS 500 06 F
2011/1009064
Guidelines: OECD Principles of Good Laboratory Practice, GLP Principles of the German Chemikaliengesetz (Chemicals Act)
GLP: yes

Apparatus

- Capillary gas chromatograph (HP 6890, Agilent) equipped with headspace autosampler (HS 40 XL, Perkin Elmer) and mass spectrometer detector (MSD, HP 5975A, Agilent), connected to a chromatography data system (ChemStation, Agilent)
- Analytical balance (AT 250 and AT 261, Mettler)

Reagents and reference materials

- Dimethyl sulfate, purity ≥99peak area-% (GC), Merck
- Toluene, purity ≥99.9%, Merck
- "BAS 500 06 F batch 991017", blank formulation, identical with the test item but without active substance, supplied by the sponsor.

All samples, stock solutions and calibration standards were dissolved in toluene.

Headspace conditions

Temperature of equilibration:	90°C
Duration of equilibration:	60 min
Transfer line temperature:	150°C
Duration of pressurization:	0.3 min
Final headspace pressure:	2 bar
Duration of injection:	0.75 min

GC conditions

- Column

Fused silica capillary:	Rtx-1701 (Restek)
Length:	30 m
Internal diameter:	0.25 mm
Film thickness:	0.25 µm

- Carrier gas

Helium	
Column head pressure:	0.9 bar
Splitless injection	
Septum purge:	3 mL/min

- Temperatures

Oven:	50°C isothermal for 1 min 50°C → 160°C, 15 K/min 160°C → 270°C, 35 K/min 270°C isothermal for 22 min
Injector:	210°C

- Detector

MSD	
Acquisition mode:	SIM
EMV:	2435 V
Detection of selected ions:	**m/z = 95.0**, m/z=96.0, m/z=125.0
Temperature of source:	230°C
Temperature of quadrupole:	150°C

Validation

Specificity

Peaks in the gas chromatogram are assigned to the analyte dimethyl sulfate by comparison of retention times and by the selection of characteristic ions in MS-detection. Constituents of the sample which coelute with the analyte which yield the same ions as selected for dimethyl sulfate give rise to excessively high mass fractions.

Linearity

Linearity was tested in the case of external calibration without the influence of the sample matrix and for standard additions to the test item (with influence of active substances) and to the blank formulation “BAS 500 06 F batch 991017” (without influence of active substances).

Linearity (calibration solutions): Verified on 6 concentration levels in the range of 0.0872 mg/kg to 4.42 mg/kg:

Slope (m):	442815
y axis intercept (b):	68561
Correlation coefficient (r^2):	0.9993

Linearity (standard addition to test item): Verified on 7 concentration levels in the range of 0.0428 mg/kg to 4.35 mg/kg:

Slope (m):	454303
y axis intercept (b):	-2106
Correlation coefficient:	0.9991

In principle determinations are possible starting from 0.0428 mg/kg to higher concentrations. However, in order to be compliant to SANCO/3030/99 rev.4 the linear range was limited to a concentration range of 0.189 mg/kg to 4.35 mg/kg with a resulting coefficient of determination of 0.9989.

Linearity (standard addition to blank formulation): Verified on 6 concentration levels in the range of 0.0437 mg/kg to 4.50 mg/kg:

Slope (m):	553206
y axis intercept (b):	-19248
Correlation coefficient:	0.9997

Accuracy

Fortifications of approximately 0.2 and 0.8 mg/kg dimethyl sulfate were added to each - the test item BAS 500 06 F (batch 0003223026) and the blank formulation "BAS 500 06 F batch 991017".

Results:

The mean recovery value for dimethyl sulfate in the test item was 110%, RSD = 15.5% (0.2 ppm fortification) and 107%, RSD = 3.9% (0.8 ppm fortification).

The mean recovery value for dimethyl sulfate in the blank formulation was 99%, RSD = 7.6% (0.2 ppm fortification) and 102%, RSD = 4.3% (0.8 ppm fortification).

Precision	Precision was deduced from the standard deviation of the recoveries of 6 samples spiked with approximately 0.2 and 0.8 mg/kg each. The standard deviation for the 0.2 ppm fortification yielded 17.1% and for the 0.8 ppm fortification 4.2%.
Recovery	Recoveries were almost quantitative (see accuracy data). There is no significant difference in recoveries between the test item and the blank formulation indicating that there are no adverse effects of the active substances on the quantification of the analyte.
Conclusion	**The described method is a valid method for quantification of dimethyl sulfate in EC formulations, e.g. in BAS 500 06 F. Dimethyl sulfate could not be detected in the product.**

(c) Methods for the determination of relevant co-formulants or components of co-formulants, where required by the national competent authorities

Currently not required by EU legislation

CP 5.1.2 Methods for the determination of residues

Methods for the determination of residues are submitted and summarized in M-CA 4.1.2.

CP 5.2 Methods for post-authorisation control and monitoring purposes

Methods for the determination of residues in or on plants, plant products, processed food commodities, food and feed of plant and animal origin

Monitoring methods for plant and animal matrices are discussed in M-CA 4.2.

Methods for the determination of residues in body fluids and tissues

Monitoring methods for body fluids are discussed in M-CA 4.2.

Methods for the determination of residues in soil

Monitoring methods for soil are discussed in M-CA 4.2.

Methods for the determination of residues in water

Monitoring methods for water are discussed in M-CA 4.2.

Methods for the determination of residues in air, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

As pyraclostrobin has a very low vapour pressure of 2.6×10^{-8} Pa (for details see M-CA 2.2), it is considered to be non-volatile. Consequently, inhalation exposure of operators, workers, residents or bystanders to vapours of pyraclostrobin is negligible. Further information can be found in M-CP 7.2.

BASF
The Chemical Company

BAS 500 06 F

DOCUMENT M-CP, Section 7

TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 7 TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

BAS 500 06 F is a new representative formulation, which has not been evaluated during the previous Annex I inclusion process.

This document reviews the toxicological studies for BAS 500 06 F, an EC solo formulation containing 200 g/L pyraclostrobin. In addition, a full risk assessment is provided, which shows that BAS 500 06 F is safe for operators, workers, bystanders and residents (for details please see M-CP 7.2). Information on the detailed composition of BAS 500 06 F can be found in the confidential part of the dossier (Document J).

CP 7.1 Acute toxicity

The acute toxicity of BAS 500 06 F is assessed using the studies listed below in Table 7.1-1. BAS 500 06 F is moderately toxic by the oral and inhalation route of exposure and non-toxic by the dermal route. It is irritant to the skin and the eye and may cause skin sensitization.

Table 7.1-1: Summary of acute toxicity studies conducted with BAS 500 06 F

Type of study	Result Classification	Reference (BASF DocID)
Oral route - rat	LD_{50} ~ 500 mg/kg bw EU classification: Xn, R22 CLP classification: Cat. 4, H302	CP 7.1.1/1 (2007/1053390)
Dermal route - rat	LD_{50} > 5000 mg/kg bw EU classification: not required CLP classification: not required	CP 7.1.2/1 (2009/1084157)
Inhalation route - rat	LC_{50} = 4.48 mg/L (both sexes) LC_{50} = 4.78 mg/L (males) LC_{50} = 4.55 mg/L (females) EU classification: Xn, R20 CLP classification: Cat. 4, H332	CP 7.1.3/1 (2009/1122167)
Skin irritation - rabbit	Irritating to rabbit skin EU classification: Xi, R38 CLP classification: Cat. 2, H315	CP 7.1.4/1 (2009/1100358)
Eye irritation - rabbit	Irritating to rabbit eye EU classification: not required CLP classification: Cat. 2, H 319	CP 7.1.5/1 (2009/1100359)
Skin sensitization - LLNA-Assay	Sensitizing EU classification: Xi, R43 CLP classification: Cat. 1, H317	CP 7.1.6/1 (2007/1053391)
Skin sensitization – Maximization Test		CP 7.1.6/2 (2009/1018498)

CP 7.1.1 Oral toxicity

Report: CP 7.1.1/1
[redacted] 2008a
BAS 500 06 F: Acute oral toxicity study in rats
2007/1053390
Guidelines: OECD 423, EEC 2004/73 B.1 tris, EPA 870.1100
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In an acute oral toxicity study groups of 3 fasted female Wistar rats were administered single doses of 2000, 500 and 300 mg/kg bw of BAS 500 06 F. The high dose was administered undiluted at a volume of 1.92 mL/kg bw. The lower dose levels were administered as diluted emulsion in bidistilled water at a volume of 2 mg/kg bw. The observation period was 14 days.
Administration of 2000 mg/kg bw of BAS 500 06 F resulted in the death of all animals at day 1 (i.e. the day after administration). Whereas the first administration of a dose of 500 mg/kg bw did not result in mortality, the second administration of this dose resulted in the death of 2 animals within 2 hours after administration, i.e. 2 of 6 animals died at 500 mg/kg bw. No mortality was observed after administration of 300 mg/kg bw in two independent experiments. Accordingly, the oral LD_{50} was about 500 mg/kg bw.

oral LD_{50} ~ 500 mg/kg bw

Treatment-related gross necropsy findings were restricted to animals which died. These consisted of lung edema at 500 mg/kg bw and of red and black discoloration of the contents of the small intestines at 2000 mg/kg bw.

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 500 06 F is to be classified as "harmful if swallowed" (Xn, R22 and Cat. 4, H302).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F

Description:	liquid
Density:	1.044 g/mL
Lot/Batch #:	8265
Purity/content:	Pyraclostrobin (= BAS 500 F): 202.7 g/L
Stability of test compound:	Stable (Expiry date: 31-Jan-2008; see Analytical Certificate)

2. Vehicle and/or positive control: bidistilled water

3. Test animals:

Species:	Rat
Strain:	Wistar, HanRcc:WIST(SPF)
Sex:	female
Age:	8 - 12 weeks
Weight at dosing (mean):	184.3 ± 6.7 g
Source:	RCC Ltd Laboratory Animal Services, CH-4414 Füllinsdorf, Switzerland
Acclimation period:	At least 5 days
Diet:	Kliba Laboratory Diet (Maus / Ratte Haltung "GLP"), Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in Makrolon cages, type III; Bedding: Lignocel FS14; SSNIFF; Environmental enrichment: Wooden gnawing blocks (Type NGM E-022); Abedd® Lab. and Vet. Service GmbH Vienna, Austria

Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 19-Jun-2007 to 22-Oct-2007
20-Jun-2007 to 14-Aug-2007 (in-life phase)

2. Animal assignment and treatment:
Groups of 3 female rats received single doses of 2000 mg/kg bw, 500 mg/kg bw (2 groups) and 300 mg/kg bw (2 groups) of the formulation by oral gavage. Prior to administration the animals were deprived of food for 16 hours, however had free access to water.
The top dose was administered undiluted (1.92 mL/kg bw) whereas the lower doses were administered as aqueous dilutions (25 and 15 g/100 mL) at a volume of 2 mg/kg bw. The aqueous dilutions were prepared shortly before administration. Homogeneity was ensured by constant stirring with a magnetic stirrer.
Animals were observed for signs of toxicity and behavioral changes several times on the day of administration, and at least once each workday for the remainder of the 14-day observation period. A check for mortality/morbidity was performed twice each workday and once on Saturdays, Sundays and on public holidays.
Body weights were recorded at day 0 (prior to dosing), weekly thereafter and at the end of the observation period. Additionally, body weights were determined when animals died or were sacrificed moribund.
Necropsy with gross-pathology examination was performed on the last day of the observation period. The animals were sacrificed by CO_2-inhalation. For animals that died prior to the scheduled study termination, necropsy was performed as early as possible.

II. RESULTS AND DISCUSSION

A. MORTALITY

Administration of 2000 mg/kg bw of BAS 500 06 F resulted in the death of all animals at day 1 (day after administration). Whereas the first administration of a dose of 500 mg/kg did not result in mortality, the second administration of this dose resulted in the death of 2 animals within 2 hours after administration. No mortality was observed after administration of 300 mg/kg bw in two independent experiments (see Table 7.1.1-1).

Table 7.1.1-1: Mortality of rats administered BAS 500 06 F by the oral route

Sex	Dose [mg/kg bw]	No. of administration	Cumulative mortality	Time of deaths
Female	2000	1st	3/3	d1 (3x)
	500	1st	0/3	-
	500	2nd	2/3	h2 (2x)
	300	1st	0/3	-
	300	2nd	0/3	-

h = hour after administration, d1 = day after administration

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity were observed at all dose levels. The type, incidence and duration of clinical signs are given in Table 7.1.1-2.

Table 7.1.1-2: Clinical signs in rats administered BAS 500 06 F by the oral route (incidence / duration)

Dose [mg/kg bw]	300	300	500	500	2000
Total number of animals	**3**	**3**	**3**	**3**	**3**
Administration	**1st**	**2nd**	**1st**	**2nd**	**1st**
- Impaired general state	3 / h2 - h4	-	2 / h3 - h4	3 / h1 - h5	3 / h1 - h5
- Poor general state	-	-	-	1 / h2	-
- Dyspnea	3 / h2 - h4	-	2 / h3 - h4	3 / h1 - h5	3 / h1 - h5
- Apathy	-	-	-	1 / h2	-
- Abdominal position	-	-	-	1 / h2	-
- Staggering	-	-	-	-	3 / h1 - h5
- Atonia	-	-	-	1 / h2	-
- Piloerection	2 / h2 - h4	-	2 / h3 - h4	1 / h2 - h5	3 / h1 - h5

h = hour; - not observed

C. BODY WEIGHT

The mean body weights of all surviving animals increased throughout the study period.

D. NECROPSY

Treatment-related gross necropsy findings were restricted to animals which died. These consisted of lung edema at 500 mg/kg bw and of red and black discoloration of the contents of the small intestines at 2000 mg/kg bw (see Table 7.1.1-3).

Table 7.1.1-3: Necropsy findings in rat administered BAS 500 06 F by the oral route

Dose [mg/kg bw]	**300**	**300**	**500**	**500**	**2000**
Administration	**1st**	**2nd**	**1st**	**2nd**	**1st**
Number of affected animals	-	1	-	2	3
Lung:					
- edema in all lobes	-	-	-	2	-
Small intestine:					
- discoloration of contents, black	-	-	-	-	3
- discoloration of contents, red	-	-	-	-	3
Mammary gland:					
- mass, 30 mm diameter, red/beige	-	1	-	-	-

- not observed

One low dose animal (# 701, 2nd administration) displayed a red/beige mass at a mammary gland. Histopathological examination revealed an adenocarcinoma in the mammary gland. This finding is considered to be spontaneous and not related to treatment.

III. CONCLUSION

Under the conditions of this study the oral LD_{50} in rats for BAS 500 06 F was determined to be about 500 mg/kg bw.

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 500 06 F is to be classified as "harmful if swallowed" (Xn, R22 and Cat. 4, H302).

CP 7.1.2 Dermal toxicity

Report: CP 7.1.2/1
[redacted] 2009a
BAS 500 06 F: Acute dermal toxicity study in rats
2009/1084157

Guidelines: OECD 402, EPA 870.1200, JMAFF No 12 Nosan No 8147, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142

GLP: yes
(certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Executive Summary

In an acute dermal toxicity study groups of 5 male and 5 female Wistar rats were administered a single dose of 5000 mg/kg of BAS 500 06 F to the clipped skin under semi-occlusive conditions for 24 hours. The liquid formulation was applied undiluted (4.8 mL/kg bw). The animals were observed for 14 days after administration. Based on the absence of mortality in this study the acute dermal LD_{50} was determined to be

dermal LD_{50} > 5000 mg/kg bw

No clinical observations of systemic toxicity were made. However, local signs of dermal irritation consisting of erythema up to grade 3, edema up to grade 2, scaling, incrustations and severe scaling were observed on study day 1 until day 12. At necropsy no deviations from normal morphology were observed.

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 500 06 F is not to be classified for acute dermal toxicity.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F

Description:	liquid, orange, clear
Lot/Batch #:	8265
Purity/content:	Pyraclostrobin 202.7 g/L
Density:	1.042 g/mL
Stability of test compound:	The stability was guaranteed for the duration of the study

2. Vehicle and/or positive control: none

3. Test animals:

Species:	Rat
Strain:	Wistar, Crl:WI (Han) SPF
Sex:	male and female
Age:	young adult (male animals approx. 8 weeks, female animals approx. 12 weeks)
Weight at dosing (mean):	males: 236.6 ± 7.2 g; females: 200.4 ± 5.13 g
Source:	Charles River Wiga GmbH, Sandhofer Weg 7, 97633 Sulzfeld, Germany
Acclimation period:	At least 5 days
Diet:	VRF1(P); SDS Special Diets Services, 67122 Altrip, Germany), ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in Makrolon cages, type III with enrichment
Temperature:	20 - 26°C
Humidity:	20 - 80%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 04-Aug-2009 to 14-Sep-2009

2. Animal assignment and treatment:
Five male and five female rats were given a single application of the undiluted test substance to the clipped epidermis (dorsal and dorsolateral parts of the trunk; about 40 cm^2, corresponding to at least 10% of the body surface area) for 24 hours. The clipping was performed about 24 hours before administration. The application volume was 4.80 mL/kg bw. The application site was covered with a semi-occlusive dressing (bandage consisting of four layers absorbent gauze, Ph. Eur. Lohmann GmbH & Co. KG and Fixomull stretch (adhesive fleece), Beiersdorf AG). After the exposure period, the dressing was removed and the application site was rinsed with warm water.
Rats were observed for signs and symptoms several times on the day of administration and thereafter at least once each workday. A check for any dead or moribund animals was also made at least once each workday. Individual body weights were recorded shortly before application (day 0), weekly thereafter and on the last day of observation. Skin findings were scored individually 30 – 60 minutes after removal of the semi-occlusive dressing (day 1) and several times until the last day of observation. The skin findings were assessed according to the Draize scheme. Necropsy with gross-pathology examination was performed on the last day of the observation period after sacrifice with CO_2.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality was observed in this study.

B. CLINICAL OBSERVATIONS
No clinical signs of systemic toxicity were observed throughout the study. Skin effects at the application site comprising erythema (grade 1 to 3), edema (grade 1 or 2), scaling and incrustations were observed from study day 1 until day 12 after application.

C. BODY WEIGHT
The mean body weight of the animals increased throughout the study period.

D. NECROPSY
Gross necropsy revealed no abnormal findings in any of the animals.

III. CONCLUSION

Under the conditions of this study the dermal LD_{50} for BAS 500 06 F in rats was determined to be greater than 5000 mg/kg bw.

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 500 06 F is not to be classified for acute dermal toxicity.

CP 7.1.3 Inhalation toxicity

Report:	CP 7.1.3/1 [redacted], 2010a BAS 500 06 F - Acute inhalation toxicity study in Wistar rats - 4-Hour liquid aerosol (head-nose only) 2009/1122167
Guidelines:	OECD 403 (2009), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.2, EPA 870.1300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In an acute inhalation toxicity study, groups of 5 male and 5 female Wistar rats were exposed to liquid aerosols of BAS 500 06 F at actual concentrations of 1.04 ± 0.18 mg/L, 2.02 ± 0.07 and 4.68 ± 0.33 mg/L for 4 hours. The animals were observed for 14 days after exposure.
One of five males and no females died at 1.04 mg/L. No mortality occurred at 2.02 mg/L. At 4.68 mg/L three of five female and three of five male animals died or were sacrificed in a moribund state. Accordingly, the acute inhalation LC_{50} was determined to be

LC_{50} (both sexes combined)	**about 4.48 mg/L**
LC_{50} (male rats)	**about 4.78 mg/L**
LC_{50} (female rats)	**about 4.55 mg/L**

Clinical signs of toxicity in animals exposed to the test substance comprised abdominal, accelerated, depressed, gasping or labored respiration, respiratory sounds, reduced attention, encrusted eyes and nose, semiclosed eylids, contaminated fur, hunched posture, piloerection and/or reduced nutritional conditions. Gross necropsy of animals dying revealed the following findings in the lung: focal dark-red discoloration of all lobes and surface partly sunken (1 female group 3), diffuse dark-red discoloration of lobus dexter cranialis, lobus medialis and pulmo sinister (1 male group 1), focal dark-red discoloration lobus dexter cranialis and pulmo sinister (1 male, group 3) or several red foci of all lobes (1 female, group 3). One female of group 3 showed few black erosion/ulcer in the glandular stomach und one male and one female of group 3 showed furthermore moderated dilation with gaseous content in the jejunum. Necropsy of the animals at termination of the post exposure periods revealed findings only in the lung: in one male animal of test group 1 few red foci in all lobes and in one female animal of test group 3 few red foci, dexter caudalis lobe and pulmo sinister were seen.

The mass mean aerodynamic diameters (MMAD) were in the range of 2.8 to 3.3 µm with geometric standard deviations (GSD) between 2.2 and 2.5. Depending on the concentration 46 to 53% of the particles had a diameter of ≤ 3.0 µm and thus may have reached the alveolar space of the lung.

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 500 06 F is to be classified as "harmful by inhalation / harmful if inhaled" (Xn, R20 and Cat. 4, H332).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F

Description:	liquid, orange, clear
Lot/Batch #:	8265
Purity/content:	Pyraclostrobin 202.7 g/L
Stability of test compound:	The stability was guaranteed for the duration of the study

2. Vehicle and/or positive control: none

3. Test animals:

Species:	Rat
Strain:	Wistar, RccHan:WIST (SPF)
Sex:	male and female
Age:	young adults (male animals approx. 8 to 9 weeks, female animals approx. 10 to 12 weeks)
Weight at dosing (mean):	males: 254.2 ± 10.8 g; females: 211.82 ± 23.6 g
Source:	Harlan Nederland, Kreuzelweg 53, NL-5961 NM Horst
Acclimation period:	At least 5 days
Diet:	Kliba rat/mouse diet (GLP), Provimi Kliba SA, Kaiseraugst, Basel, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing or up to 5 animals in H-Temp (PSU) cages (TECNIPLAST, Germany)

Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 26-Oct-2009 to 22-Jan-2009

2. Animal assignment and treatment:
For determination of the acute inhalation toxicity (single 4-hour-exposure) groups of five male and five female rats were exposed to liquid aerosols of BAS 500 06 F at three concentrations. The animals were randomly selected from a pool of animals.

3. Clinical examinations:
For each test group, the body weight of the animals was determined just prior to exposure (day 0), on study days 1, 3, and 7 and weekly thereafter and at the end of the observation period. A check for overt clinical signs of toxicity or mortality as well as a check for the presence of feed and drinking water was made twice a day on workdays and once daily on weekends and public holidays. Detailed clinical observations were recorded for each animal separately several times during exposure and at least once on each workday of the observation period.

4. Pathology:
At the end of the 14-day observation period the surviving animals were sacrificed with CO_2 and were subjected to gross-pathological examination like those performed for animals which had died prematurely.

5. Statistics/Calculations:
The statistical evaluation of the concentration-response relationship was carried out using a computer program. Depending on the data of the concentration-response relationship obtained by the experiment, this program is used to estimate the LC_{50} or to perform a Probit analysis[1].
The calculation of the particle size distribution was carried out in the inhalation laboratory on basis of mathematical methods for evaluating particle measurements[2].

[1] FINNEY, D.J. (1971): "Probit Analysis" Cambridge University Press, pp. 1 - 50

[2] DIN 661 41: Darstellung von Korngrößenverteilungen, DIN 661 61: Partikelgrößenanalyse (Beuth-Vertrieb GmbH, Berlin 30, FRG und Köln 1, FRG)

6. Generation of the test atmosphere / chamber description:
A head-nose inhalation system INA 20 (glass-steel construction, BASF SE, volume V ≈ 55 L) was used. The animals were restrained in glass tubes and their snouts projected into the inhalation system.

The exposure system was located inside an exhaust cabin in an air-conditioned laboratory. A supply airflow (compressed air) of 0.5 m³/h was used for the exposure. The exhaust airflow was set at 1.35 m³/h for groups 1 and 2 and at 1.00 m³/h for group 3. An air change of about 27 times per hour can be calculated by dividing the supply air flow by the volume of the inhalation system. The lower amount of exhaust air, which was adjusted by means of a separate exhaust air system, achieved a positive pressure inside the exposure system. This ensured that the mixture of test substance and air was not diluted with laboratory air in the breathing zones of the animals.

The animals were exposed to the inhalation atmosphere for 4 hours plus an equilibration time for the inhalation system of about 10 minutes.

7. Analytical investigation:

The flows of supply and exhaust air were adjusted and continuously measured with a flow meter. Air flows, the temperature and the humidity in the exposure system measured were at about 1-hour intervals.
The oxygen content in the inhalation system was not measured. The air change was judged to be sufficient to prevent oxygen depletion by the breathing of the animals, and the concentrations of the test substance used could not have a substantial influence on oxygen partial pressure.

The nominal concentration was calculated from the amount of substance dosed and the supply airflow.

The sampling for determination of the actual aerosol concentration was performed with 3 impingers connected in series and filled with a sorption solvent (acetonitrile) using a sampling probe (diameter 7 mm, with quartz wool). By means of the vacuum pump metered volumes of the inhalation atmospheres were drawn through the impingers.

- Sampling position: immediately adjacent to the animals' noses
- Sampling flow: 3 L/min
- Sampling velocity: 1.25 m/s
- Sampling frequency: 4 samples at about hourly intervals
- Sample volume: Test group 1: 30 L; Test group 2: 15 L; Test group 3: 6 L

The sampling volumes were adjusted to achieve suitable amounts of test substance for analysis.

The quartz wool and the first 2 impingers were analyzed for each sample. The third impinger was used to control the effectiveness of the sorption for all samples of a test group and was analyzed separately at the end of the sampling campaign.

The quantitative determination of the aerosol concentrations were performed by HPLC analysis using the following parameters:

- Column: Gemini 5µ C28, 50 mm x 3 mm
- Injection volume: 10 µL
- Column temperature: ambient
- Mobile phase: 50% A: acetonitrile + HCOOH (1000 mL + 1 mL)
  50% B: highly deionized water + HCOOH (1000 mL + 1 mL)
- Flow rate: 0.6 mL/min
- Detection: UV 274 nm

The particle size analysis was done using a Stack Sampler Mark III (Andersen) and a vacuum pump (Millipore). The sampling probe had an internal diameter of 6.9 mm.
Before sampling, the impactor was assembled with metal collecting discs and a backup filter. The impactor was connected to the vacuum pump and for each test group two samples were taken from the breathing zone of the animals starting not earlier than 30 minutes after the beginning of the exposure. The sample volumes were 36, 15 and 6 L for test groups 1, 2 and 3, respectively.
After sampling the impactor was taken apart. The impactor stages and the backup particle filter were eluted with acetonitrile. The obtained samples were analyzed by HPLC. Additionally, material absorbed on the walls of the impactor and the sampling probe (wall losses) were also determined quantitatively.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality was observed for one of five animals in test group 1 and three of five males and three of five females in test group 3. No lethality was observed in test group 2. Details are given in Table 7.1.3-1.

Table 7.1.3-1: Mortality of rats exposed for 4 hours to a liquid aerosol of BAS 500 06 F

Test group	Concentration [mg/L]	Cumulative mortality		Time of deaths
		Male	Female	
1	1.04	1/5	0/5	d0
2	2.02	0/5	0/5	-
3	4.68	3/5	3/5	d0 – d1

d0: post exposure on the day of exposure

Based on the observed mortality the following LC_{50} values were determined:

- LC50 (both sexes combined) about 4.48 mg/L
- LC50 (male rats) about 4.78 mg/L
- LC50 (female rats) about 4.55 mg/L

B. CLINICAL OBSERVATIONS

Clinical signs observed consisted of abdominal, accelerated, depressed, gasping or labored respiration, respiratory sounds, reduced attention, encrusted eyes and nose, semiclosed eylids, contaminated fur, hunched posture, piloerection and/or reduced nutritional conditions. The maximum incidence and duration of the observations are indicated in Table 7.1.3-2.

Table 7.1.3-2: Clinical signs in rats exposed for 4 hours to a liquid aerosol of BAS 500 06 F (incidence/duration)

Dose [mg/L]	1.04	2.02	4.68	1.04	2.02	4.68
Sex	**Males**			**Females**		
- Total number of animals	5	5	5	5	5	5
- Attention, reduced	d0 – d1	-	d0 – d3	-	-	d0 – d11
- Eye, encrusted, red	d1 – d3	-	d1	-	-	d1
- Eye, semiclosed eyelid	d0	-	d0	-	-	d0
- Fur, contaminated	d0 – d2	d0 – d2	d0 – d4	d0	d0 – d1	d0 – d1
- Hunched posture	d0	-	d0 – d1	-	-	d0 – d3
- Nose, encrusted, red	d2	d1	-	-	-	d1
- Piloerection	d0 – d6	d0 – d5	d0 – d4	d0 – d1	d0 – d1	d0 – d14
- Reduced nutritional condition	-	d1 – d4	-		-	-
- Respiration, abdominal	d0 – d6, d11 – d12	d0 – d5	d0 – d8	d0 – d1	d0 – d3	d0 – d1, d6 – d14
- Respiration, accelerated	h2, d2 – d5, d7 – d8, d10, d13 – d14	d4 – d11	d7 – d9	h2, d0 – d8	d4 – d5	-
- Respiration, depressed	h3 – h4	h1	h1	h3 – h4	h1	h1
- Respiration, gasping	-	d1 – d4	d1	-	-	d2 – d5
- Respiration, labored	-	h2 – h4	h2 – h4	-	h2 – h4	h2 – h4
- Respiration, sounds	d0 – d7, d11 – d12	d0 – d5, d7 – d11	d0 – d6	d0 – d2, d5	d0 – d4	d0 – d14

hn = hour n of exposure; d0 = day of exposure; dn = day n after exposure

C. BODY WEIGHT

In test group 1 and 3 the mean body weight of the surviving animals decreased during the first post exposure observation week, but increased during the second week. In the second test group the mean body weights of the animals did not increase adequately during the first post exposure observation week, but increased during the second week.

D. NECROPSY

Gross necropsy of one male animal exposed to 1.04 mg/L (group 1) that died on study day 0 after exposure revealed diffuse dark-red discoloration of the dexter cranialis lobe, medialis lobe and the pulmo sinister. The following gross pathological abnormality was noted in one male animal necropsy at termination of the post exposure observation period, few red foci in all lung lobes (Ø 2 mm). The remaining animals showed no gross pathological abnormalities.
No gross pathological abnormalities were noted during the necropsy at the termination of the post exposure observation period in animals exposed to 2.02 mg/L (group 2).
Necropsy of one male and one female animal exposed to 4.68 mg/L (group 3) that died on study day 0 during exposure showed no gross pathological abnormality. The female animal that died on study day 1 after exposure showed partly sunken surface and focal dark-red discoloration of the lung and few black erosion/ulcer (Ø 2 mm) in the glandular stomach. From the animals, which were sacrificed in a moribund state on study day 1, two of three animals showed the following findings: in one male animal focal dark-red discoloration in the dexter cranial lobe and the pulmo sinister, in one female animal several red foci in all lobes (Ø 2 mm). Both animals showed additionally a moderate dilation of the jejunum with gaseous content. The third animal (male) showed no gross pathological abnormality. The following gross pathological abnormality was noted in one female animal necropsy at termination of the post exposure observation period, few red foci in the dexter cranial lobe and the pulmo sinister (Ø 2 mm). The remaining animals showed no gross pathological abnormalities.

E. ATMOSPHERE ANALYSIS

The exposure conditions are summarized in Table 7.1.3-3.

Table 7.1.3-3: Exposure conditions

Test group	Supply air (compressed) [m³/h]	Exhaust air [m³/h]	Temp. [°C]	Relative humidity [%]
1	0.5	1.35	20.5 ± 0.0	51.4 ± 0.6
2	0.5	1.35	21.0 ± 0.1	52.2 ± 1.0
3	0.5	1.00	21.0 ± 0.1	52.4 ± 1.6

The results of the concentration measurements (mean of 4 measurements) are presented in Table 7.1.3-4.

Table 7.1.3-4: Measurement of concentrations (4 hourly measurements)

Test group	Mean concentration [mg/L]	Standard deviation	Nominal concentration [mg/L]
1	1.04	0.18	8.8
2	2.02	0.07	20.9
3	4.68	0.33	100.9

The measurements of particle-size distribution revealed mass mean aerodynamic diameters (MMAD) in the range of 2.8 to 3.3 µm with geometric standard deviations in the range of 2.2 to 2.5 (see Table 7.1.3-5).

Depending on the concentration 46 to 53% of the particles had a diameter of ≤ 3.0 µm and thus may have reached the alveolar space of the lung.

Table 7.1.3-5: Particle size measurements

Test group / Sample	% ≤ 3 µm	MMAD [µm]	Geometric standard deviation
1 / 1	52.4	2.9	2.3
1 / 2	53.4	2.8	2.4
2 / 1	51.8	2.9	2.3
2 / 2	46.4	3.3	2.5
3 / 1	49.1	3.1	2.5
3 / 2	51.4	2.9	2.2

III. CONCLUSION

Under the conditions of this study the 4 hour inhalation LC_{50} for BAS 500 06 F was estimated to be

- LC_{50} (both sexes combined) 4.48 mg/L
- LC_{50} (male rats) 4.78 mg/L
- LC_{50} (female rats) 4.55 mg/L

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 500 06 F is to be classified as "harmful by inhalation / harmful if inhaled" (Xn, R20 and Cat. 4, H332).

CP 7.1.4 Skin irritation

Report: CP 7.1.4/1
[redacted] 2010a
BAS 500 06 F - Acute dermal irritation / corrosion in rabbits
2009/1100358

Guidelines: OECD 404 (2002), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, EPA 870.2500, JMAFF No 12 Nosan No 8147

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a primary dermal irritation study the skin irritation/corrosion potential of BAS 500 06 F was tested. An area of 6.25 cm² of clipped skin of 3 New Zealand White rabbits was exposed to 0.5 mL of the test-substance for 4 hours under semi-occlusive conditions. The rabbits were observed for 14 days after removal of the dressings. Signs of skin irritation were scored using the Draize scheme according to guideline requirements.
Administration of BAS 500 06 F to the skin of rabbits caused up to grade 3 (slight to marked) erythema and grade 2 (slight to moderate) edema. The cutaneous reaction was not reversible in all animals within 14 days after removal of the patch. Slight to moderate erythema and scaling, both extending beyond the area of exposure, were still observed in all animals at study termination on day 14. The overall 24 to 72 hour skin irritation scores were 2.8 for erythema and 1.0 for edema.

Based on the findings of this study the formulation BAS 500 06 F has to be classified as "Irritating to skin" (Xi, R38) according to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC). According to Regulation (EC) 1272/2008 (CLP), BAS 500 06 F has to be classified as "Causes skin irritation" (Cat. 2, H315).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F

Description:	liquid, orange, clear
Lot/Batch #:	8265
Purity/content:	BAS 500 F / pyraclostrobin: 202.7 g/L
Stability of test compound:	The stability was guaranteed for the duration of the study

2. Vehicle and/or positive control: none

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, A 1077 INRA (SPF)
Sex:	male and female
Age:	6 – 7 months
Weight at dosing (mean):	3.65 – 3.95 kg
Source:	Centre Logo S.A., 01540 Vonnas, France
Acclimation period:	At least 5 days
Diet:	Kliba Laboratory Diet, Provimi Kliba SA, Kaiseraugst, Basel, Switzerland (approx. 130 g/animal/day)
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages with grating, floor area 3000 cm²

Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 03-Aug-2009 to 09-Sep-2009

2. In-vitro pre-test:
No in-vitro pre-test was performed.

3. Animal assignment and treatment:
The potential of BAS 500 06 F to cause acute dermal irritation or corrosion was assessed by a single topical application of 0.5 mL of the test substance for 4 hours to the intact skin of two male and one female New Zealand White rabbits.

At least 24 hours before treatment, the dorsolateral part of the trunk of the animals was clipped. Only animals without obvious signs of skin irritation were used in the study. A dose of 0.5 mL of the unchanged liquid formulation was applied to a 2.5 x 2.5 cm test patch and applied to the flank for 4 hours. The gauze patch was held in place by means of an adhesive semi-occlusive dressing (Idealbinde, Pfaelzische Verbandstoff-Fabrik, Kaiserslautern and Fixomull® adhesive fleece, Beiersdorf AG, Germany). After a 4 hour exposure period the patch was removed and the application area was washed with Lutrol® (PEG 400) and Lutrol®/water (1:1).
The cutaneous reactions were assessed immediately after removal of the patch, approximately 1, 24, 48 and 72 hours as well as 7 and 14 days after removal of the patch.
Body weights were measured shortly prior to application and after the last reading. The animals were checked for mortality, morbidity and clinical signs twice on working days and once on weekends and public holidays.

II. RESULTS AND DISCUSSION

Administration of BAS 500 06 F to the skin of rabbits caused moderate or marked erythema (grade 2 or 3), observed in all animals immediately after removal of the patch up to day 7, and decreased to slight or moderate (grade 1 or 2) in all animals until 14 days after removal of the patch. Moderate edema (grade 2) was noted in all animals immediately after removal of the patch up to 1 hour and persisted in one animal until 24 hours. Slight edema (grade 1) was observed in two animals after 24 hours and in all animals at the 48-hour reading. Slight edema persisted in two animals from 72 hours up to day 7. Additionally, erythema and edema were extended beyond the area of exposure in all animals during the observation period. Scaling extending beyond the area of exposure was noted in all animals on study days 7 and 14 (see Table 7.1.4-1). The cutaneous reactions were not reversible in all animals within 14 days after removal of the patch. Slight or moderate erythema and scaling, both extending beyond the area of exposure, were still observed in all animals at study termination on day 14.
Mean scores over 24, 48 and 72 hours for each animal were 2.3, 3.0 and 3.0 for erythema and 1.3, 0.7 and 1.0 for edema.

Table 7.1.4-1: Individual and mean skin irritation scores after 4 hour dermal application of BAS 500 06 F

Readings	Animal	Erythema	Edema	Additional findings
0 hour	01 (♂)	2	2	15, 16
	02 (♀)	2	2	15, 16
	03 (♂)	2	2	15, 16
1 hour	01	2	2	15, 16
	02	2	2	15, 16
	03	2	2	15, 16
24 hours	01	3	2	15, 16
	02	3	1	15, 16
	03	3	1	15, 16
48 hours	01	2	1	15, 16
	02	3	1	15, 16
	03	3	1	15, 16
72 hours	01	2	1	15, 16
	02	3	0	15
	03	3	1	15, 16
7 days	01	2	1	S, 15, 16, 17
	02	2	0	S, 15, 17
	03	3	1	S, 15, 16,17
14 days	01	1	0	S, 15, 17
	02	2	0	S, 15, 17
	03	1	0	S, 15, 17
Individual means 24 - 72 hours	01	2.3	1.3	
	02	3.0	0.7	
	03	3.0	1.0	
Group mean 24 - 72 hours		2.8	1.0	

15: Erythema extending beyond the area of exposure
16: Edema extending beyond the area of exposure
17: Scaling extending beyond the area of exposure
S : Scaling

III. CONCLUSION

Based on the findings of this study the formulation BAS 500 06 F has to be classified as "Irritating to skin" (Xi, R38) according to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC). According to Regulation (EC) 1272/2008 (CLP), BAS 500 06 F has to be classified as "Causes skin irritation" (Cat. 2, H315).

CP 7.1.5 Eye irritation

Report: CP 7.1.5/1
[redacted] 2010b
BAS 500 06 F - Acute eye irritation in rabbits
2009/1100359

Guidelines: OECD 405 (2002), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, EPA 870.2400, JMAFF No 12 Nosan No 8147

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a primary eye irritation study, the eye irritation/corrosion potential of BAS 500 06 F was determined by instillation of 0.1 mL of the test substance into the conjunctival sac of the right eye of three rabbits (stepwise procedure starting with one animal and supplementing two additional animals). The left eye, which remained untreated, served as a control. The eyes were rinsed 24 hours after administration of the test substance.
The ocular reactions were assessed approximately 1, 24, 48 and 72 hours as well as 7 and 14 days after the administration of the test substance.
Signs of ocular irritation consisted of up to grade 2 corneal opacity, up to grade 1 iritis, up to grade 3 conjunctival redness and up to grade 3 chemosis. All signs of ocular irritation were reversible within 14 days. The mean scores calculated for all animals over 24, 48 and 72 hours were 1.2 for corneal opacity, 0.7 for iris lesions, 2.3 for redness of the conjunctiva and 1.8 for chemosis.

Based on the results of the study no classification as to eye irritation is warranted according to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC). In contrast, according to Regulation (EC) No 1272/2008 (CLP), the formulation has to be classified as an eye irritant ("Causes serious eye irritation", Cat. 2, H319).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F
Description: liquid, orange, clear
Lot/Batch #: 8265
Purity/content: BAS 500 F / pyraclostrobin: 202.7 g/L
Stability of test compound: The stability was guaranteed for the duration of the study.

2. Vehicle and/or positive control: none

3. Test animals:
Species: Rabbit
Strain: New Zealand White, A 1077 INRA (SPF)
Sex: male and female
Age: 6 – 7 month (animal 01)
ca. 4 months (animals 02 and 03)
Weight at dosing
(mean): 2.86 to 3.82 kg
Source: Centre Lago S. A., 01540 Vonnas, France
Acclimation period: At least 5 days
Diet: Kliba Laboratory Diet, Provimi Kliba SA, Kaiseraugst, Basel, Switzerland (approx.. 130 g/day)
Water: Tap water, ad libitum
Housing: Single housing in stainless steel wire mesh cages with grating, floor area 3000 cm²

Environmental conditions:
Temperature: 20 - 24°C
Humidity: 30 - 70%
Air changes: Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period: 12 h light / 12 h dark
(06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 03-Aug-2009 and 09-Sep-2009

2. In-vitro pre-test:
No in-vitro pre-test was performed.

3. Animal assignment and treatment:
The potential of BAS 500 06 F to cause acute eye irritation was assessed by instillation of 0.1 mL of the unchanged test substance into the conjunctival sac of the right eye. The left eye, which remained untreated, served as a control. The eyes were rinsed with tap water 24 hours after administration of the test substance.
The ocular reactions were assessed approximately 1, 24, 48 and 72 hours as well as 7 days and 14 days after the administration of the test substance.
Body weights were measured shortly prior to application and after the last reading. The animals were checked for mortality, morbidity and clinical signs twice on working days and once on weekends and public holidays.

II. RESULTS AND DISCUSSION

Slight corneal opacity (grade 1) was observed in all animals 24 hours after application and persisted in two animals up to 72 hours. Slight corneal opacity increased to moderate (grade 2) in one animal at the 48-hour reading up to 72 hours. Moderate iritis (grade 1) was observed in two animals 24 and 48 hours after application and persisted in one of these animals up to 72 hours. Another animal showed moderate iritis at the 72-hour reading, only. Moderate or severe conjunctival redness (grade 2 or 3) were noted in all animals from 1 hour up to 72 hours after application. Slight conjunctival redness (grade 1) was observed in one animal on day 7. Moderate or marked conjunctival chemosis (grade 2 or 3), noted in all animals 1 hour after application up to 24 hours, persisted in one animal up to 48 or 72 hours, respectively. Slight conjunctival chemosis (grade 1) was observed in one animal after 48 hours and in two animals at the 72-hour reading. Severe discharge (grade 3) was noted in all animals 1 hour and 24 hours after application and persisted in two animals up to 48 hours. Slight discharge (grade 1) was seen in one animal after 48 hours and in two animals after 72 hours after application. Additional findings like suppuration, contracted pupil and injected scleral vessels in a circumscribed area or circular were noted in the animals during the observation period. The ocular reactions were reversible in two animals within 7 days and in one animal within 14 days after application. The mean 24 to 72 hour scores for corneal opacity, iritis, conjunctival redness and chemosis were 1.2, 0.7, 2.3 and 1.8, respectively (see Table 7.1.5-1).

Table 7.1.5-1: Individual and mean eye irritation scores after ocular application of BAS 500 06 F

Readings	Animal	Cornea		Iris	Conjunctiva			Additional findings
		Opacity	Area involved		Redness	Chemosis	Discharge	
1 h	01 (♂)	0	0	0	2	2	3	49
	02 (♂)	0	0	0	2	2	3	49
	03 (♀)	0	0	0	2	2	3	49
24 h	01	1	4	1	2	3	3	PC, 48
	02	1	4	1	3	2	3	PC, 49
	03	1	4	0	2	2	3	PC, 49
48 h	01	2	2	1	3	2	3	PC, 49
	02	1	4	1	2	2	3	S, PC, 49
	03	1	2	0	2	1	1	48
72 h	01	2	2	1	3	2	1	PC, 49
	02	1	3	0	2	1	1	S, PC, 49
	06	1	2	1	2	1	0	PC, 48
7 d	01	0	0	0	1	0	0	48
	02	0	0	0	0	0	0	SD
	03	0	0	0	0	0	0	SD
14 d	01	0	0	0	0	0	0	
Individual means 24 - 72 h	01	1.7	1.0	1.0	2.7	2.3		
	02	1.0	0.7	0.7	2.3	1.7		
	03	0.3	0.3	0.3	2.0	1.3		
Overall mean 24 - 72 h	all	1.2	0.7	0.7	2.3	1.8		

48 = scleral vessels injected, circumscribed area
49 = scleral vessels injected, circular
PC = pupil contracted
S = suppuration
SD = study discontinued because the animals was free of findings

III. CONCLUSION

Based on the findings of this study a classification of the formulation BAS 500 06 F as an eye irritant is not required according to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC). In contrast, according to Regulation (EC) No 1272/2008 (CLP), the formulation has to be classified as an eye irritant ("Causes serious eye irritation", Cat. 2, H319).

CP 7.1.6 Skin sensitization

Report: CP 7.1.6/1
[redacted] 2008b
BAS 500 06 F: Murine local lymph node assay (LLNA)
2007/1053391
Guidelines: OECD 429, EEC 2004/73 B.42, EPA 870.2600
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

For the determination of potential sensitizing properties of the formulation BAS 500 06 F a mouse local lymph node assay (LLNA) was conducted.
Topical application of the formulation to the ears of groups of 5 female mice at 3 different concentrations (3, 10 and 30% (w/w) in acetone) for three consecutive days resulted in a biologically significant increase of the stimulation indices (SI) for lymph node cell count, ^{3}H-thymidine incorporation into the lymph node cells and lymph node weights, when assessed approximately 2 days after the last application.
A slight increase in ear weights was observed at all concentrations (max SI: 1.47). This may have been caused by a slight irritation of the ears.

The threshold concentration for sensitization induction was < 3%. The estimated concentration that leads to the SI of 1.5 for cell count (EC 1.5) and the estimated concentration that leads to the SI of 3.0 for ^{3}H-thymidine incorporation (EC 3) were calculated by linear regression from the results of the control group and the 3% concentration to be 0.9 and 1%, respectively.

Positive control studies performed twice a year with the sensitizer Hexylcinnamaldehyde proved the sensitivity of the method used.

Based on the results of this study it can be concluded that BAS 500 06 F has sensitizing properties under the test conditions chosen. The formulation has to be classified as a sensitizer ("May cause sensitization by skin contact", Xi, R43) according to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC). According to Regulation (EC) 1272/2008 (CLP), the formulation has to be classified as "May cause an allergic skin reaction" (Cat. 1, H317).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F

Description:	liquid
Lot/Batch #:	8265
Purity/content:	BAS 500 F / pyraclostrobin: 202.7 g/L
Stability of test compound:	According to the CoA the formulation was stable until January 31, 2008 when stored at +5 to +30°C.

2. Vehicle and/or positive control: Acetone

3. Test animals:

Species:	Mouse
Strain:	CBA/J
Sex:	20 females
Age:	6 to 12 weeks
Weight at dosing:	20.6 ± 0.8 g
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Sandhofer Weg 7, 97633 Sulzfeld
Acclimation period:	at least 5 days
Diet:	Kliba-Labordiät (Maus / Ratte Haltung "GLP"), Provimi Kliba SA, Kaiseraugst, Basel, Switzerland, ad libitum
Water:	tap water ad libitum
Housing:	individual housing in Makrolon Type II cages with Lignocel FS 14 (SSNIFF) bedding. Environmental enrichment: Nest-building material (wood wool) (Type NBF E-011); Abedd® Lab. and Vet. Service GmbH Vienna, Austria

Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00/18:00 – 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 28-Aug-2007 to 23-Oct-2007
29-Aug-2007 to 03-Sep-2007 (in life work)

2. Animal assignment and treatment:
The skin sensitizing potential of BAS 500 06 F was assessed using the radioactive Murine Local Lymph Node Assay. For this, female mice were allocated to groups of 5 animals according to the randomization instructions of „Nijenhuis, A. and Wilf, H.S.: Combinatorial Algorithms, Academic Press, New York, San Francisco, London, 1978, pp. 62 – 64".
The groups were treated either with

- the vehicle (acetone),
- a 3% (w/w) dilution in acetone,
- a 10% (w/w) dilution in acetone, or
- a 30% (w/w) dilution in acetone.

The application solutions were prepared shortly before treatment.

3. Analysis of treatment solutions:
The stability of the test substance in the vehicle was determined indirectly by the concentration control or homogeneity analysis. For this purpose, the samples taken were stored at room temperature over the maximum duration of the application period and were subsequently deep-frozen. Afterwards, these samples were analyzed. The homogeneity of the test-substance preparation in the vehicle was not determined since the test-substance was dissolved in the preparation. The actual test substance concentration was determined once for the 3, 10 and 30% dilution.
The correctness of the concentrations of the preparations was confirmed analytically (for details see raw data).

4. Statistics:
Not performed in this study

5. Clinical observation:
Mortality was checked twice daily on working days and once on weekends and public holidays. No detailed clinical examination of the individual animals was performed, but any obvious signs of systemic toxicity and/or local inflammation at the application sites were recorded (see raw data).

6. Body weights:
Individual body weights were determined on day 0 prior to the first application and on day 5 prior to the sacrifice of the animals.

7. Treatment of animals:
The dosing solutions were applied daily to the dorsal part of the ears at a volume of 25 µL per ear for 3 consecutive days. On study day 5, i.e. 66 to 72 hours after the last application, 20 µCi of ^{3}H-thymidine in 250 µL sterile saline was injected into the tail vein of each mouse.

8. Terminal procedures:

Approximately 5 hours after ^{3}H-thymidine injection the animals were killed by cervical dislocation.

Immediately after the death a circular piece of tissue (diameter 0.8 cm) was punched out of the apical part of each ear of all animals. The weight of the pooled punches was determined for each test group. These measurements served for the detection of a potential inflammatory ear swelling.

Immediately after removal of the ear punches the left and right auricular lymph nodes were dissected. The weight of the pooled lymph nodes from both sides was determined for each animal.

After weight determination, a single cell suspension was prepared per test group from the pooled lymph nodes by carefully passing all lymph nodes through an iron mesh (mesh size 200 µm) into 40 mL of phosphate-buffered physiological saline. Subsequently the cell counts were determined with an aliquot of each suspension using a Casy®- Counter.

The remaining cell suspensions were washed twice with phosphate buffered saline (PBS) and precipitated with 5% trichloro-acetic acid. Each precipitate was transferred to scintillation fluid and incorporation of ^{3}H-thymidine into the cells was measured in a β-scintillation counter.

9. Data evaluation and interpretation:

The stimulation indices (SI) of cell count, ^{3}H-thymidine incorporation, lymph node weight and ear weight were calculated as the ratio of the test group values for these parameters divided by those of the vehicle control group.

The lymph node cell count and the ^{3}H-thymidine incorporation into the lymph node cells as well as to a certain extent lymph node weight are used to determine the potential sensitizing properties of a test article. Because not only sensitization but also irritation of the ear skin by the test substance may induce lymph node responses, the weight of ear punches taken from the area of test-substance application is determined as a parameter for inflammatory ear swelling as an indicator for the irritant action of the test substance.

Stimulation indices of >1.5 for cell count and/or of ≥ 3 for ^{3}H-thymidine incorporation are generally considered as indicative for a sensitizing potential of a test substance. If applicable, the EC (estimated concentration) leading to the respective SI values were calculated by linear or semi-logarithmical regression.

If the increase in cell count, ^{3}H-thymidine incorporation and/or lymph node weight is accompanied by a biologically relevant increase in ear weights it cannot be ruled out that the lymph node response was caused by irritation and not by skin sensitization. Depending on the magnitude of lymph node response, based on expert judgment, the evaluation of the sensitizing potential may be modified or additional studies might be necessary.

If a test article – despite of concentration related increase - does not elicit a biological relevant increase in cell count and/or ^{3}H-thymidine incorporation, further investigation of the sensitization potential at higher concentrations may be considered.

10. Positive controls:
A concurrent positive control (reliability check) with a known sensitizer was not included into this study. Studies using the positive control substance Alpha-Hexylcinnamaldehyde are performed twice a year in the laboratory in order to show that the test system is able to detect sensitizing compounds under the test conditions chosen.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS
No clinical observations or mortality were observed.

B. BODY WEIGHTS
Mean body weight gain was observed in control and low dose animals (3% in acetone). However, body weight gain at the low dose level was lower (0.2 ± 0.8 g) than in the controls (1.4 g ± 0.5 g). At the mid dose (10% in acetone; -0.3 ± 0.4 g) and high dose level (30% in acetone; -3.7 ± 1.0 g) body weight loss was observed. This is interpreted as indication for systemic toxicity.

C. STIMULATION INDICES
When applied as 30, 10 and 3% preparations in acetone, the test substance induced a biologically relevant response in the auricular lymph node cell counts and ^{3}H-thymidine incorporation. In addition there was an increase in lymph node weights.

The test-substance preparations caused concentration dependent slight to moderate increases in ear weights as indication of ear skin irritation. Scaling was observed on the day of lymph node removal in all animals applied with the 10% test-substance preparation. Two animals of the 30% test group exhibited scaling and incrustation and slight erythema was noted in all animals.

The irritation probably contributed to the lymph node reaction. However the considerable increase in cell counts and ^{3}H-thymidine incorporation in the 3% test group cannot be fully attributed to the slight ear skin irritation.

The stimulation indices (SI) for lymph node cell counts, ^{3}H-thymidine incorporation and lymph node and ear weights are given in Table 7.1.6-1.

Table 7.1.6-1: Stimulation indices for cell counts, ^{3}H-thymidine incorporation, lymph node and ear weight in mice after treatment with BAS 500 06 F

Test Group	Treatment	Parameter evaluated	Stimulation index (SI)[1]
		Cell count [counts/lymph node pair]	
1	vehicle (acetone)	6085333	1.00
2	3% (w/w) in acetone	16381333	2.69
3	10% (w/w) in acetone	19994667	3.29
4	30% (w/w) in acetone	19416000	3.19
		^{3}H-Thymidine incorporation [DPM/lymph node pair]	
1	vehicle (acetone)	412.5	1.00
2	3% (w/w) in acetone	2839.2	6.88
3	10% (w/w) in acetone	6014.6	14.58
4	30% (w/w) in acetone	6643.4	16.10
		Lymph node weight [mg/lymph node pair]	
1	vehicle (acetone)	4.6	1.00
2	3% (w/w) in acetone	9.2	2.00
3	10% (w/w) in acetone	12.0	2.61
4	30% (w/w) in acetone	11.1	2.39
		Ear weight [mg/animal]	
1	vehicle (acetone)	30.8	1.00
2	3% (w/w) in acetone	36.7	1.19
3	10% (w/w) in acetone	42.2	1.37
4	30% (w/w) in acetone	45.1	1.47

[1] test group x / test group 1 (vehicle control)

The threshold concentration for sensitization induction was < 3%. The estimated concentration that leads to the SI of 1.5 for cell count (EC 1.5) and the estimated concentration that leads to the SI of 3.0 for ^{3}H-thymidine incorporation (EC 3) were calculated by linear regression from the results of the control group and the 3% concentration to be 0.9 and 1%, respectively.

D. POSITIVE CONTROL

The sensitivity of mice (CBA/CaOlaHsd, Harlan Winkelmann GmbH, Borchen, Germany or CBA/J, Charles River Laboratories, Research Models and Services, Germany GmbH, Sandhofer Weg 7, 97633 Sulzfeld) and the reliability of experimental techniques is assessed regularly using a known sensitizer. Positive results were consistently obtained over the years using several variations of the methods and different vehicles. The results of 6 control studies are presented in Table 7.1.6-2.

Table 7.1.6-2: Positive control LLNA studies performed

Project No.	45H0288/ 982036#	45H0288/ 982059#	58H0288/ 982068#	58H0288/ 982075#	58H0508/ 062114#	58H0288/ 982082[6]
Strain used	CBA/CaOlaHsd	CBA/CaOlaHsd	CBA/CaOlaHsd	CBA/CaOlaHsd	CBA/CaOlaHsd	CBA/J
Date of performance	Apr 04	Apr / May 2005	Aug 05	Feb 06	Aug 06	Jun 07
Name of test substance	Alpha-Hexylcinnam-aldehyde, techn. 85%	Alpha-Hexylcinnam-aldehyde, techn. 85%	Alpha-Hexylcinnam-aldehyde, techn. 85%	Alpha-Hexylcinnam-aldehyde, techn. 85%	Alpha-Hexylcinnam aldehyde, 95+%	Alpha-Hexylcinnam-aldehyde, techn. 85%
Concentrations tested	3%, 10%, 30%	1%, 3%, 10%	2.5%, 5%, 10%	3%, 10%, 30%	3%, 10%, 30%	1%, 3%, 10%
Vehicle	1% Pluronic® L92 Surfactant in bi-distilled water	acetone	AOO 4:1 (acetone : olive oil 4:1 v/v)	acetone	1% Pluronic® L92 Surfactant in bi-distilled water	acetone
Stimulation index Cell counts [a]	1.43, 2.28, 2.92	1.28, 1.63, 2.94	1.13, 1.30, 1.83	1.75, 2.36, 2.98	1.13, 2.20, 3.38	1.42, 1.97, 2.75
Stimulation index 3H-thymidine incorporation[b]	-	-	1.12, 1.19, 2.84[1]	4.56, 6.63, 9.86	1.16, 4.64, 17.98	1.81, 3.24, 3.74
Evaluation of study results	Positive	Positive	Positive	Positive	Positive	Positive

[a] = Ratio of test group values to control group values (Stimulation index) greater than 1.5 indicates a positive result
[b] = Ratio of test group values to control group values (Stimulation index) greater than 3.0 indicates a positive result
# = Individual lymph nodes
6 = Pooled lymph nodes
1 = Borderline SI at 10% in accordance with published results using AOO as the vehicle

III. CONCLUSION

Based on the results of this study it can be concluded that BAS 500 06 F has sensitizing properties under the test conditions chosen.

The formulation has to be classified as a sensitizer (“May cause sensitization by skin contact”, Xi, R43) according to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC). According to Regulation (EC) 1272/2008 (CLP), the formulation has to be classified as "May cause an allergic skin reaction" (Cat. 1, H317).

Report:	CP 7.1.6/2 [redacted] 2009a BAS 500 06 F - Maximization test in guinea pigs 2009/1018498
Guidelines:	OECD 406, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH, EPA 870.2600, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

For the determination of potential sensitizing properties of the formulation BAS 500 06 F a Maximization test based on the method of Magnusson and Kligman was conducted using a control and a treated group of 10 and 20 female Dunkin Hartley (Crl:HA) Guinea pigs, respectively.

The test-substance concentrations for the main test were selected based on the results of the pretests and the result of the first challenge. The intradermal induction was performed with a 5% test-substance preparation in 0.9% aqueous NaCl-solution or 5% test-substance preparation in Freund's complete adjuvant / 0.9% aqueous NaCl-solution (1:1). The epicutaneous induction was conducted with a 50% test-substance preparation in highly deionized water.
The intradermal induction caused intense erythema and swelling at the injection sites of the test-substance preparations in all test group animals. After the epicutaneous induction, incrustation, partially open (caused by the intradermal induction) could be observed in addition to intense erythema and swelling in all test group animals.
For the first challenge, a 25% test-substance preparation in highly deionized water was chosen. Skin reactions were observed in 30% of the control and in 70% treated group animals. Therefore, a second challenge with a concentration of 10% was carried out. The second challenge did not cause any skin findings in control but in 7/20 (35%) of the test group animals.

Based on the results of this study it can be concluded that BAS 500 06 F has sensitizing properties under the test conditions chosen. The formulation has to be classified as a sensitizer ("May cause sensitization by skin contact", Xi, R43) according to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC). According to Regulation (EC) 1272/2008 (CLP), the formulation has to be classified as "May cause an allergic skin reaction" (Cat. 1, H317).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F

Description:	liquid
Lot/Batch #:	8265
Purity/content:	BAS 500 F / pyraclostrobin: 202.7 g/L
Stability of test compound:	The stability of the formulation under storage conditions was guaranteed.

2. Vehicle and/or positive control: 0.9% aqueous NaCl-solution for intradermal application and highly deionized water for epidermal application

3. Test animals:

Species:	Guinea pigs
Strain:	Dunkin Hartley, Crl:HA
Sex:	female
Age:	5 to 8 weeks (on day 0)
Weight on day 0:	417 to 510 g; 467.1 ± 24.4 g
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, 88353 Kisslegg
Acclimation period:	at least 5 days
Diet:	Kliba-Labordiät (Kaninchen & Meerschweinchenhaltung "GLP"), Provimi Kliba SA, Kaiseraugst, Basel, Switzerland, ad libitum
Water:	tap water ad libitum
Housing:	groups of 5 animals housed in plastic cages from Tecniplast Deutschland GmbH (Type 3020W009) with perforated base; floor area: 4000 cm^2. Environmental enrichment: Wooden gnawing blocks (Type NGM E-022); Abedd® Lab. and Vet. Service GmbH Vienna, Austria

Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	not indicated in the report
Photo period:	12 h light / 12 h dark (6:00 a.m. - 6:00 p.m. / 6:00 p.m. – 6:00 a.m.)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 22-Dec-2008 to 09-Feb-2009
22-Dec-2008 to 06-Feb-2009 (in life work)

2. Animal assignment and treatment:
The skin sensitizing potential of BAS 500 06 F was assessed using the Guinea Pig Maximization Test. This test consists of a pretest for the determination of suitable induction and challenge concentrations and the Maximization Test itself.
For this, female Guinea pigs were allocated to groups according to the randomization instructions of „Salfi, R.: A Long-Period Random Number Generator with Application to Permutation, Compstat 1974, pp. 28 – 35".

For the intradermal and epicutaneous application, animal fur was clipped at least 2 hours before each test-substance application at the appropriate treatment sites. If necessary, additional hair clipping was performed 2 hours prior to the evaluation of skin reactions.

Evaluations of the skin reactions were performed according to the grading scale of Magnusson and Kligman (The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test. J. Invest. Dermatol. 52, 268 - 276 (1969)).

Individual body weights were determined on study day 0 and on the last day of observation. Mortality was checked twice daily on working days and once on weekends and public holidays.

A: Preliminary tests
Two animals per test substance concentration received six intradermal injections (see below) in groups of two at the neck region in order to determine a test substance concentration that is well-tolerated locally and systemically for the intradermal induction treatment in the main test. The evaluation of the skin reactions was performed 24 h after application.

Intradermal injections:

A) front row: 2 injections each of 0.1 mL Freund's complete adjuvant without test substance emulsified with 0.9% aqueous NaCl-solution in a ratio of 1:1 (w/w)
B) middle row: 2 injections each of 0.1 mL of a test- substance preparation in an appropriate vehicle at the selected concentration
C) back row: 2 injections each of 0.1 mL Freund's complete adjuvant / 0.9% aqueous NaCl-solution (1:1 w/w) with test substance at the selected concentration.

Additionally, for the dermal induction treatment the highest concentration of the test substance that causes slight to moderate irritation and for the challenge the maximum non-irritant concentration was determined. The test-design took into account the possible influence of a previous intradermal treatment with Freund's complete adjuvant on irritating effects. For this, animals were used that received dermal injections of 0.1 mL Freund's complete adjuvant / 0.9% aqueous NaCl-solution at a ratio of 1:1 (w/w) three weeks prior to the epidermal pretest.

Skin reactions were determined by dermal application of 0.5 mL of the test-substance preparations under occlusive conditions to groups of 3 animals for 24 hours. The patch consisted of 6 layers of surgical gauze (2 x 2 cm) covered by patches of rubberized linen (4 x 4 cm) and Idealbinde (5 x 5 cm) which were held in place by Fixomull® adhesive fleece. One hour, 24 and 48 hours after removal of the patch evaluations of the skin reactions were performed.

B: Main study - Induction

The intradermal induction consisted of 6 intradermal injections in groups of two into the neck of the animals according to the scheme given above. Control animals received for the mid row injections of 0.1 mL of 0.9% aqueous NaCl and for back row injections of 0.1 mL of a 50% formulation of 0.9% aqueous NaCl with Freund's complete adjuvant / 0.9% aqueous NaCl-solution (1:1 w/w). Skin reactions were assessed 24 hours after injection. The treated and the control groups consisted of 20 and 10 females, respectively.

Epidermal induction was performed one week after intradermal induction. For this 1 mL of the test substance preparation was applied under occlusive conditions to the neck region previously used for intradermal induction. The patch consisted of 6 layers of surgical gauze (2 x 4 cm) covered by patches of rubberized linen (4 x 6 cm) which was held in place by Fixomull® adhesive fleece for 48 hours. One hour, 24 and 48 hours after removal of the patch evaluations of the skin reactions were performed.

C: Challenge

The 1st challenge was carried out 14 days after epidermal induction. A 2nd challenge was performed one week after the first. For this 0.5 mL of the test substance preparation was applied under occlusive conditions to the intact flank for 24 hours. The patches were prepared as described above for the epidermal pretest. Skin reactions were determined 24 and 48 hours after removal of the patches.

D: Positive controls

A positive control (reliability check) with a known sensitizer was not performed in this study. However, positive control studies are performed twice a year in the laboratory. The positive control with alpha-Hexylcinnamaldehyde techn. 85% showed that the test system was able to detect sensitizing compounds under the laboratory conditions chosen.

E: Evaluation of results

The number of animals with skin findings at 24 and/or 48 hours after the removal of the patch was taken into account for the determination of the sensitization rate. If at least 30% of the test animals exhibit skin reactions in this adjuvant test, the result of the evaluation is "sensitizing".
The evaluation is based on the criteria of the Commission Directive 67/548/EEC and the OECD Harmonized Integrated Classification System that were in place on the date of report signature.

3. Analysis of treatment solutions:

The stability of the test-substance preparations for intradermal induction was not determined, because the preparations using Freund's complete adjuvant + physiological saline (paraffin oil/water emulsion) are required by the test method and impaired stability of the test substance in the preparations, including test substance in the vehicle, is without influence on the procedure of intradermal induction.

The stability of the test substance in highly deionized water was confirmed indirectly by the concentration control or homogeneity analysis. For this purpose, the samples taken were stored at room temperature over the maximum duration of the application period and were subsequently deep-frozen. Afterwards, these samples were analyzed. The results of this analysis are available in the raw data.

The homogeneity of the test-substance preparation used for the first challenge application was confirmed indirectly by the concentration control analysis. The results of this analysis are available in the raw data.

4. Statistics:

Not performed in this study.

II. RESULTS AND DISCUSSION

A. Pre-Test

A first intradermal injection of 1% BAS 500 06 F in 0.9% aqueous NaCl caused only moderate and confluent erythema with swelling (see Table 7.1.6-3).

Table 7.1.6-3: Skin irritation scores 24 hours after intradermal injection - 1st and 2nd preliminary intradermal test

1st preliminary intradermal test				
Animal #	Application site	A) Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1)	B) 1% BAS 500 06 F preparation in 0.9% aqueous NaCl-solution	C) 1% BAS 500 06 F in A)
208	left	3	2 E	3
	right	3	2 E	3
209	left	3	2 E	3
	right	3	2 E	3
2nd preliminary intradermal test				
Animal #	Application site	A) Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1)	B) 5% BAS 500 06 F preparation in 0.9% aqueous NaCl-solution	C) 5% BAS 500 06 F in A)
203	left	3	3	3
	right	3	3	3
207	left	3	3	3
	right	3	3	3

Grading: 2: moderate and confluent erythema
E: swelling
3: intense erythema and swelling

Therefore, a second intradermal pretest was performed using a 5% BAS 500 06 F preparation in 0.9% aqueous NaCl. This time intradermal injection caused intense erythema and swelling at the injection site. Injection of Freund's adjuvant caused intense erythema and swelling irrespective of mixing with 0.9% aqueous NaCl or BAS 500 06 F at concentrations of 1 and 5%.

The dermal irritation scores observed 1, 24 and 48 hours after a 48-hour epidermal exposure of 10, 25 and 50% dilutions in highly deionized water and in undiluted BAS 500 06 F are given in Table 7.1.6-4.

Table 7.1.6-4: Skin irritation scores after epidermal application - Preliminary test

Animal #	Readings 1 hour after removal of the patch		Readings 24 hours after removal of the patch		Readings 48 hours after removal of the patch	
Application site	Right flank middle	Left flank middle	Right flank middle	Left flank middle	Right flank middle	Left flank middle
1st Pretest (conducted 15-Dec-2008)						
BAS 500 06 F in highly deionized water	100%	50%	100%	50%	100%	50%
4	1	1	0	0	0	0
5	1	1	1	1	1	0
6	1	1	1	1	1	1
2nd Pretest (conducted 12-Jan-2009)						
BAS 500 06 F in highly deionized water	25%	10%	25%	10%	25%	10%
83	1	1	0	0	0	0
84	0	0	0	0	0	0
85	0	0	0	0	0	0

Grading: 0: no visible change 1: discrete or patchy erythema

Based on the data from the pre-test a 5% dilution of BAS 500 06 F in 0.9% aqueous NaCl-solution or in Freund's complete adjuvant / 0.9% aqueous NaCl-solution (1:1) was used for intradermal induction, whereas a 50% dilution in highly deionized water was used for epidermal induction. As no skin findings were observed 24 and 48 hours after dermal administration of a 25 and 10% aqueous dilution, a 25% dilution was used for the 1st challenge. Since this concentration caused skin findings in 30% of the control and 70% treated animals (see below), the second challenge was performed with a 10% dilution.

B. Induction reactions
Intradermal injection of

- Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1) in control and treated animals,
- 5% BAS 500 06 F preparation in 0.9% aqueous NaCl-solution in treated animals,
- 5% BAS 500 06 F in Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1) in treated animals, and
- 50% formulation of 0.9% aqueous NaCl-solution with Freund's complete adjuvant / 0.9% aqueous NaCl-solution (1 : 1) in control animals

caused intense erythema and swelling (grade 3) in all animals. No skin reactions were observed in control animals at the mid row injections of 0.9% NaCl-solution (see Table 7.1.6-5).

Table 7.1.6-5: Skin irritation scores 24 hours after intradermal injection - Main test

Control animals				
Animal #	Application site	A) Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1)	B) 0.9% aqueous NaCl-solution	C) 50% formulation of 0.9% aqueous NaCl-solution with Freund's complete adjuvant / 0.9% aqueous NaCl-solution (1 : 1)
all animals	left	3	0	3
	right	3	0	3
Treated animals				
Animal #	Application site	A) Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1)	B) 5% BAS 500 06 F preparation in 0.9% aqueous NaCl-solution	C) 5% BAS 500 06 F in A)
all animals	left	3	3	3
	right	3	3	3

Grading: 3: intense erythema and swelling

The epicutaneous induction with a 50% test-substance preparation in highly deionized water led to incrustation, partially open (caused by the intradermal induction) in addition to intense erythema and swelling (grade 3) in all test group animals.

C. Challenge

Epidermal challenge with a 25% aqueous dilution of BAS 500 06 F in highly deionized water caused grade 1 (discrete or patchy erythema) or Grade 2 skin reactions (moderate and confluent erythema) in certain control and treated animals (see Table 7.1.6-6). Therefore, a second challenge using a 10% aqueous dilution of BAS 500 06 F was performed. This time no skin reactions were observed in control animals whereas grade 1 or grade 2 skin reactions were observed in a total of 7 animals. One of the animals displaying moderate and confluent erythema also displayed skin swelling.

Table 7.1.6-6: Challenge skin reaction scores 24 hours after intradermal injection - Main test

	1st challenge		2nd challenge	
	Test substance 25% in highly deionized water		Test substance 10% in highly deionized water	
	24 h	48 h	24 h	48 h
Control group				
- Grade 1	2 / 10	1 / 10	-	-
- Grade 2	1 / 10	-	-	-
Test group				
- Grade 1	11 / 20	5 / 20	-	1 / 20
- Grade 2	3 / 30	3 / 20	5 / 20	6 / 20
- Swelling	-	-	-	1 / 20

x/y: number of positive reactions/number of animals tested (reading at 24 h and/or 48 h after the removal of the patch)

D. Observations

No clinical signs of systemic toxicity or mortality were observed.

E. Body weights

The expected body weight gain was generally observed in the course of the study.

F. Positive control

The sensitivity of Harlan Guinea pigs (Hsd Poc: DH (SPF), Harlan Winkelmann GmbH, Borchen, Germany) or Charles River Guinea pigs (Dunkin Hartley, Crl:HA, Charles River Laboratories, Research Models and Services, Germany GmbH, 88353 Kisslegg) and the reliability of experimental techniques is assessed regularly using a known sensitizer as recommended by the test guidelines.

The study procedures were based on the methods of Magnusson and Kligmann (1969) (Maximization Test) and Buehler, E.V. (1965) (Buehler Test) and are summarized below.

Name of test substance:	alpha-Hexylcinnamaldehyde, techn. 85%
CAS number:	101-86-0
Substance number:	98/0288-3
Concentrations:	
Maximization Test:	
Intradermal induction:	Test substance 5% in paraffin oil Test substance 5% in Freund's complete adjuvant / 0.9% aqueous NaCl solution (1:1)
Epicutaneous Induction:	Test substance 10% in Lutrol® E 400
Challenge:	Test substance 5% in Lutrol® E 400
Buehler Test:	
Induction:	Test substance 25% in Lutrol® E 400
Challenge:	Test substance 15% in Lutrol® E 400

As evident from Table 7.1.6-7 the test system and the used procedures were capable to detect the sensitizing properties of alpha-Hexylcinnamaldehyde.

Table 7.1.6-7: Positive control studies - Number of animals with skin findings after the challenge in control studies with the test-substance alpha-Hexylcinnamaldehyde (techn. 85%)

				Challenge					
				Test substance in the vehicle			Vehicle		
Project No.	Study type	Date of performance	Group	24 h	48 h	Total	24 h	48 h	Total
			Control group	0/10	0/10	0/10	0/10	0/10	0/10
32H0288/982076	Buehler Test	Mar / Apr 2006	Test group	8/20	4/20	8/20	0/20	0/20	0/20
			Control group	0/5	0/5	0/5	0/5	0/5	0/5
30H0288/982077	Maximization Test	Aug / Sep 2006	Test group	8/10	6/10	8/10	0/10	0/10	0/10
			Control group	1/10	1/10	1/10	1/10	1/10	1/10
32H0288/982078	Buehler Test	Apr / May 2007	Test group	13/20	9/20	13/20	1/20	0/20	1/20
			Control group	0/5	0/5	0/5	0/5	0/5	0/5
30H0288/982083	Maximization Test	Nov / Dec 2007	Test group	10/10	10/10	10/10	0/10	0/10	0/10
			Control group	0/10	0/10	0/10	0/10	0/10	0/10
32H0288/982091	Buehler Test	Feb / Mar 2008	Test group	12/20	8/20	12/20	0/20	0/20	0/20
			Control group	0/5	0/5	0/5	0/5	0/5	0/5
30H0288/982102	Maximization Test	Oct / Nov 2008	Test group	10/10	10/10	10/10	0/10	0/10	0/10

x/y: number of positive reactions/number of animals tested (reading at 24 h and/or 48 h after the removal of the patch)

III. CONCLUSION

Based on the results of this study it can be concluded that BAS 500 06 F has sensitizing properties under the test conditions chosen.

The formulation has to be classified as a sensitizer ("May cause sensitization by skin contact", Xi, R43) according to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC). According to Regulation (EC) 1272/2008 (CLP), the formulation has to be classified as "May cause an allergic skin reaction" (Cat. 1, H317).

CP 7.1.7 Supplementary studies on the plant protection product

No supplementary studies on the plant protection product were deemed necessary and thus none were conducted.

CP 7.1.8 Supplementary studies for combinations of plant protection product

No studies for combinations of BAS 500 06 F with other plant protection products were deemed necessary and thus none were conducted.

CP 7.2 Data on exposure

Exposure assessments and risk evaluations for operators, workers, bystanders and residents are presented below for the new representative formulation BAS 500 06 F, which has not been evaluated during a previous Annex I inclusion process.

CP 7.2.1 Operator exposure

The plant protection product BAS 500 06 F is already registered for the use as fungicide in the representative crops cereals and maize. Among these crops, cereals are the critical use based on the higher application rate (i.e. 250 g/ha per application in cereals versus 200 g/ha in maize) and number of applications (i.e. 2 per season in cereals versus 1 per season in maize). Information on the critical use pattern relevant for operator exposure is summarized in Table 7.2.1-1.
The formulation is an emulsifiable concentrate (EC) containing 200 g/L pyraclostrobin and is mainly commercialized in 5 and 10 L containers.

Table 7.2.1-1: Summary of critical use pattern

Crop (indoor / field)	**Application rate** (g as/ha)		**Spray dilution** (L/ha)	**Application equipment**	**Number of applications**
Cereals (field)	Pyraclostrobin	250	100	Tractor mounted boom sprayers	2

The critical use pattern has been defined based on the already registered uses in cereals and maize (for details see M-CP 3 Table 2).

Estimations of potential operator exposure have been undertaken for BAS 500 06 F using the intended use shown above and the following predictive models:

- Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection), Mitteilungen aus der Biologischen Bundesanstalt, Heft 277, Berlin 1992 ("German model").

- Revised UK POE Model, as available on http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/UK_POEM1.xls [UK Predictive Operator Exposure Model (POEM): Estimation of Exposure and Absorption of Pesticides by Spray Operators, Scientific subcommittee on Pesticides and British Agrochemical association Joint Medical Panel Report (UK MAFF), 1986 and the Predictive Operator Exposure Model (POEM) V 7 of 2008, (UK MAFF), 1992 ("UK Model").

Risk assessment for operator

The toxicological reference value (Acceptable Operator Exposure Level) for pyraclostrobin and dermal absorption values appropriate for BAS 500 06 F, which were used in the operator risk assessment are shown in Table 7.2.1-2 below.

Table 7.2.1-2: Endpoints for pyraclostrobin used in operator risk assessment

Endpoint	Value	Reference
Dermal penetration - Concentrate - Spray dilutions	3% 7%	BASF DocIDs 2010/1059865 and 2014/1001501 (for details see M-CP 7.3 of this dossier)
$AOEL_{systemic}$	0.015 mg/kg bw/day	EU Review Report SANCO/1420/2001-Final, 8. September 2004

The estimated operator exposure to pyraclostrobin for the use of BAS 500 06 F in cereals is shown in Table 7.2.1-3 below.

Table 7.2.1-3: Estimated operator exposure to pyraclostrobin in BAS 500 06 F

Model data	Level of PPE	Total absorbed dose (mg/kg bw/day)[1]	% of AOEL[2]	Reference in Appendix
outdoor tractor operated boom sprayer application with hydraulic nozzles to field crops 1.25 L BAS 500 06 F/ha corresponding to 250 g pyraclostrobin per ha				
German Model - 20 ha/day - 70 kg operator	Gloves during mixing/loading and gloves, coverall and sturdy footwear during application	0.0009	6	**7.2-3**
UK POEM - 50 ha/day - 60 kg operator	Gloves during mixing/loading and gloves during application	0.025	169	**7.2-4**

[1] systemic exposure based on dermal absorption of 3% for mixing/loading and 7% for application for pyraclostrobin
[2] based on a systemic AOEL of 0.015 mg/kg bw/day

For operators exposed to pyraclostrobin when applying BAS 500 06 F to cereals no safe use could be demonstrated based on UK POEM. Therefore a refined risk assessment is presented in M-CP 7.2.1.2. Results are summarised below.

Table 7.2.1-4: Refined assessment: Estimated operator exposure to pyraclostrobin in BAS 500 06 F based on field exposure data

Model data	Level of PPE	Total absorbed dose (mg/kg bw/day)[1]	% of AOEL[2]	Reference in Appendix
outdoor tractor operated boom sprayer application with hydraulic nozzles to field crops 1.25 L BAS 500 06 F/ha corresponding to 250 g pyraclostrobin per ha				
Refined assessment based on field exposure data - 50 ha/day	Coverall and gloves during mixing/loading and application	0.000047	0.31	**7.2-9**

[1] systemic exposure based on dermal absorption of 7% for both mixing/loading and application for pyraclostrobin as a worst case
[2] based on a systemic AOEL of 0.015 mg/kg bw/day

Results and discussion

Following the operator exposure and risk evaluation based on models a safe use could be shown for the BBA model estimates. For UK POEM adequate operator safety could not be demonstrated if PPE is considered. However, refined exposure assessments based on surrogate data, which reflect today's application equipment, have shown that the predicted systemic exposure is within the acceptable limit.

In conclusion operators when exposed to pyraclostrobin under the use conditions of BAS 500 06 F in cereals and maize are considered to be at acceptable risk.

CP 7.2.1.1. Estimation of operator exposure

BAS 500 06 F is applied in field crops (cereals and maize), which is professional use only. The relevant application scenario is outdoor tractor operated boom sprayer with hydraulic nozzles. Risk assessments are presented based on the BBA model and the UK POEM with consideration of the following input parameters.

Table 7.2.1.1-1: German model input parameters for tractor mounted boom sprayer application in cereals

Application method:	tractor-mounted boom sprayers with hydraulic nozzles, field crop
Treated area:	20 ha/day
Max. dose rate:	1.25 L BAS 500 06 F/ha
	corresponding to: 0.25 kg pyraclostrobin/ha
Operator body weight:	70 kg

Table 7.2.1.1-2: UK POEM input parameters for tractor mounted boom sprayer application in cereals

Application method:	Tractor-mounted/trailed boom sprayer: hydraulic nozzles
Treated area:	50 ha/day
Max. dose rate:	1.25 L BAS 500 06 F/ha
	corresponding to: 0.25 kg pyraclostrobin/ha
Spray volume:	100 L/ha
Duration:	6 h
Container:	10 litres 63 mm closure
Operator body weight:	60 kg

Estimation of operator exposure <u>without personal protective equipment</u>

Exposure predictions for operators using no protective equipment (PPE) are summarized in Table 7.2.1.1-3.

Table 7.2.1.1-3: Estimated operator exposure to pyraclostrobin in BAS 500 06 F without using PPE

Model data	Level of PPE	Total absorbed dose (mg/kg bw/day)[1]	% of AOEL[2]	Reference in Appendix
outdoor tractor operated boom sprayer application with hydraulic nozzles to field crops 1.25 L BAS 500 06 F/ha corresponding to 250 g pyraclostrobin per ha				
German Model - 20 ha/day - 70 kg operator	None	0.016	103	**7.2-1**
UK POEM - 50 ha/day - 60 kg operator	None	0.159	1058	**7.2-2**

[1] systemic exposure based on dermal absorption of 3% for mixing/loading and 7% for application for pyraclostrobin
[2] based on a systemic AOEL of 0.015 mg/kg bw/day

A safe use could not be shown for operators without personal protective equipment with any of the models chosen. Detailed information is presented in Appendix 7.2-1 and 7.2-2.

Estimation of operator exposure <u>with personal protective equipment</u>

Exposure predictions for operators using protective equipment (PPE) are summarized in Table 7.2.1.1-4.

Table 7.2.1.1-4: Estimated operator exposure to pyraclostrobin in BAS 500 06 F with using PPE

Model data	Level of PPE	Total absorbed dose (mg/kg bw/day)[1]	% of AOEL[2]	Reference in Appendix
outdoor tractor operated boom sprayer application with hydraulic nozzles to field crops 1.25 L BAS 500 06 F/ha corresponding to 250 g pyraclostrobin per ha				
German Model - 20 ha/day - 70 kg operator	Gloves during mixing/loading and gloves, coverall and sturdy footwear during application	0.0009	6	**7.2-3**
UK POEM - 50 ha/day - 60 kg operator	Gloves during mixing/loading and gloves during application	0.025	165	**7.2-4**

[1] systemic exposure based on dermal absorption of 3% for mixing/loading and 7% for application for pyraclostrobin
[2] based on a systemic AOEL of 0.015 mg/kg bw/day

For operators exposed to pyraclostrobin when applying BAS 500 06 F in cereals no safe use could be demonstrated based on UK POEM. Detailed information is presented in Appendix 7.2-3 and 7.2-4.
Therefore a refined risk assessment is presented which considers higher tier data on the basis of two field exposure studies performed by the applicant.

CP 7.2.1.2. Measurement of operator exposure

Since the risk assessments performed indicate that the health-based limit value (AOEL) for pyraclostrobin will be exceeded under practical conditions of use, two surrogate studies to provide field data on operator exposure to BAS 500 06 F are used, for which study summaries are shown below.

Report:	CP 7.2.1.2/1 Stadler R., 2007a Determination of dermal and inhalation operator exposure for mixing/loading and application of BAS 601 KD F in cereals 2007/1033389
Guidelines:	OECD Series on Testing and Assessment No. 9 - Guidance document on the conduct of studies of occupational exposure to pesticides during agricultural application (Paris 1997)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This study was erroneously not contained in the application. It needs to be submitted, because it is the basis for the refined risk assessment.

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	Epoxiconazole formulated in the preparation BAS 601 KD F
	Description:	Experimental suspension concentrate (SC) formulation containing nominal 100 g/L epoxiconazole
	Batch/purity #:	batch no. 2032: 91.0 g epoxiconazole/L batch no. 2036: 92.2 g epoxiconazole/L batch no. 2037: 94.5 g epoxiconazole/L mean epoxiconazole content: 92.6 g/L
	Stability of test compound:	The stability of the formulation was guaranteed by the sponsor Expiry date (Certificates of Analysis): 30 September 2008

B. STUDY DESIGN AND METHODS

Dates of work: 06-Sep-2006 to 27-Feb-2007

The measurement of operator exposure was conducted with a surrogate formulation (BAS 601 KD F) under field crop conditions. Unlike assessments to the current available models (BBA-model, UK POEM or EUROPOEM), where the data base was generated by means of studies performed before 1990, the existing operator exposure study was performed with application machinery that is more appropriately reflecting the present standard of application technology. Tractors with closed cabins, large sprayers for water volumes in the range of 2000 to 5200 L thus allowing less mixing/loading operations and large spray booms with a spray boom width in the range of 18 to 36 m allowing reduce the time needed for spray operations. The combination of these technical features has been considered to show an operator exposure pattern that is more accurately addressing today's conditions of use.
This operator exposure study was conducted at 10 locations in the Southwest and East of Germany during September and October 2006. The applied product BAS 601 KD F was an experimental suspension concentrate (SC) containing nominal 100 g/L epoxiconazole.
Analytical evaluation of the product revealed, however, that the actual concentration was lower, the mean concentration of the used batches being 92.6 g/L. Therefore the actual mean concentration was used for the calculations. The product was applied at a rate of 1.25 L/ha. The treated daily area per operator was about 50 ha with a range of 48.0 to 53.5 ha. The applications were performed with conventional tractor-mounted or tractor-drawn boom sprayers fitted with hydraulic nozzles. Typical water volumes were used (water rates were in a range of 100 – 200 L/ha with a mean of about 142 L/ha). Water volumes thus appropriately reflect spray practices in Western and Central Europe.
Meteorological conditions were monitored during all workdays. Air temperatures ranged from 12 to 32°C, the range of soil temperatures was 11 to 25°C. A relative humidity of 55–100% was measured, the wind speed ranged from 0 to 4 m/s.
A total of 10 operators were monitored during mixing/loading and application of the spray solution. No differentiation in exposure monitoring between mixing/loading and application was done. The whole study was divided in subset A and B characterised as follows:

A) Mixing/loading was performed as standard procedure to assess operator exposure when handling conventional containers of the product (operator 1B, 2A-10A. Note: In the study conduct the subset designation for operator No 1 was mixed up thus showing the exposure data for the standard mixing/loading process under 1B instead of 1A, and vice-versa. In this subset commercial 5 L containers were used. A summary of the relevant application equipment parameters is provided in Appendix 7.2-5.
B) Mixing/loading was performed with a closed transfer system for the mixing/loading process (operator 1A, 2B-10B). In this subset two closed transfer systems were used: Nine operators used the ECOMATIC system with 50 L product containers. The tenth operator used the CONNECTA system which allowed the transfer of the product from 5 L standard containers into the spray tank under controlled exposure conditions. Subset B was chosen in addition to demonstrate additional operator protection by testing a closed transfer system for the product containers in comparison to standard Mixing/Loading (M/L) [subset B].

   These data were, however, not included into the surrogate database.

Each operator performed two treatments. One treatment was performed according to the conditions of "subset A", the other according to "subset B", about 50 ha having been treated each time. The operator used the same sprayer when performing the two applications.
Operators were considered experienced in pesticide application. Their age was in the range of 32 to 57 years. Their body weight was in the range of 85 kg to 110 kg with a mean of about 93 kg.

Dosimeter

Dermal dosimeter
The exposure was measured by whole body dosimetry for the dermal exposure. Hand exposure was assessed by means of a hand wash procedure following the back rinse method [Durham, W.F. and Wolfe, H.T., 1962: Measurement of the exposure of workers to pesticides, Bull. WHO 26, 75-91]. The operators were equipped with two layers of whole body dosimeters consisting of outer dosimeters and inner dosimeters. A standard work clothing of cotton/polyester fabric was chosen as outer dosimeter. The inner dosimeters consisted of whole cotton underwear with long arms and legs. The hand exposure was assessed by analysis of nitrile protective gloves (outer dosimeter) and hand wash samples (inner dosimeter). Hand wash samples were taken at times when the worker normally washed his hands for reasons such as eating or using the toilet and at the end of the work period when it was reasonable to expect workers to wash hands. The operators were advised to use the protective gloves only for the mixing/loading process. For the application the operator did not need to wear protective gloves as long as he was not doing maintenance works at the sprayer where he could get into contact with spray liquid. In cases where the applicator was likely to get into contact with spray-liquid he wore protective gloves. Right and left gloves used per operator on a working day were pooled and subjected to a common analysis of the active substance contained. Hand wash samples were pooled as well in order to assess the actual hand exposure. Head exposure was measured by face/neck wipes with 100% cotton gauze. Face/neck wipes were taken at the end of the work period. Face and neck wipe samples were performed with 2 pads of cotton gauze wetted with liquid soap solution. If the operator wished to wear a hat or a cap for sun protection or any other reason he was allowed to do so. The hat or a cap was not taken as a dosimeter. At the end of the monitoring period outer and inner dosimeters were separated and sectioned into individual parts as follows for the subsequent analysis.

Outer dosimeter	**Inner dosimeter**
Both lower arms	Both lower arms
Both upper arms	Both upper arms
Chest	Chest
Back	Back
Both lower legs	Both lower legs
Both upper legs	Both upper legs
Protective gloves (right and left pooled)	Hand wash samples (right and left one pooled)
	Face/neck wipes

The potential dermal exposure (PDE) was calculated from residues on the work jacket, the pair of trousers, the underwear (vest & long-johns), face/neck wipes, the hand-wash samples and the protective gloves. The actual dermal exposure (ADE) was calculated from the residues detected on/in the underwear (long sleeved vest & long-johns), the faces/neck wipes and the hand-wash samples.

Inhalation dosimeters

Operators were equipped with personal air sampling pumps (TENAX adsorption tubes) combined with an inhalable fraction sample collector located in the operator's breathing zone. The limit of quantification (LOQ) was 1 ng (0.001 µg). The air pump flow was calibrated to 2 L/min. For a sampling interval of one hour this corresponds to a total air volume of 120 L. For an operator engaged in moderate physical labour a breathing volume of 14 L/min or 840 L/hour was considered. Thus, a conversion factor of 7 [14 : 2 = 7] was used to derive an estimate of inhalation exposure for the operators.

Storage of dosimeters

At the end of the treatment, the body dosimeters were removed and sectioned on site prior to storage in a cool box for the transport to the laboratory. Samples were subsequently stored deep-frozen until analysis. Air samplers were stored in darkness at ambient temperatures until analysis.

Field recoveries and analytical procedures

The stability of the test item under field, storage and transport conditions in or on the sampling materials (coverall, inner dosimeters, facial swabs, protective gloves if necessary and air sampling filters) was assessed for field fortifications, for storage and for transport conditions. For this purpose, the test item was applied under laboratory and under field conditions onto the different sampling materials at two different concentrations (i.e. at the 10-fold and 100-fold of the LOQ) and the recoveries were analytically determined.

Inner and outer dosimeters as well as protective gloves were extracted with methanol. Aliquots of the extract were diluted with equal volumes of water for LC/MS/MS injection. Hand wash solution aliquots were diluted with equal volumes of methanol for LC/MS/MS analysis. Face/Neck wipes were extracted with methanol. Aliquots of the extract were diluted 1: 9 with water : methanol (1:1) for LC/MS/MS injection. The Tenax absorber was extracted with acetone. Extracts were reduced to dryness and subsequently diluted in methanol : water (1:1) for LC/MS/MS analysis.

II. RESULTS AND DISCUSSION

Field and concurrent laboratory fortifications

The results of field, transport and concurrent laboratory fortification analyses demonstrated the storage and transport stability of the test item. Field recovery and lab recovery data for body dosimeters were in the range of 74 to 99% of the test substance initially applied. For air sampler units, recoveries were between 95 to 102%. A short summary of the recovery results is given in Table 7.2.1.2-1 below.

Table 7.2.1.2-1: Field and concurrent laboratory fortifications

Matrix	Field recovery [%]	Lab recovery [%]	Correction factor[1]
Inner dosimeter	74	78	1.35
Face wipe	98	99	n n.
Hand wash solution	92	99	n n.
Outer dosimeter	92	95	n n.
Gloves	88	87	n n.
Air sampler	95	102	n n.

[1] According to guidelines a correction factor needs not be applied if the recovery values are within the 70 - 120% range.

Based on the obtained recovery data a correction factor of 1.35 was applied to account for incomplete field and laboratory recoveries for inner body dosimeters. For all other matrices no correction factor was employed.

Analytical confirmation of the test substance in BAS 601 KD F

Three different batches of BAS 601 KD F were used in the study. The analytical confirmation of test substance control gave the following results (Table 7.2.1.2-2).

Table 7.2.1.2-2: Analytical results of used BAS 601 KD F batches

Batch-No.	Nominal content (epoxiconazole) [g/L]	Analyzed content (epoxiconazole) [g/L]
2032	100	91
2036	100	92.2
2037	100	94.5

The mean analytical recovery was 92.6 g epoxiconazole/L. An additional correction factor was therefore used to account for the difference found in regard to the nominal test substance concentration of 100 g/L. A summary of the obtained exposure levels for outer and inner body dosimeters, hand wash and glove exposure, face and neck wipes as well as inhalation exposure is given in Table 7.2.1.2-3.

Table 7.2.1.2-3: Summary of operator exposure to epoxiconazole when mixing/loading and spraying BAS 601 KD F to field crops

Study	Total Inner Body Dosimeter	Hand Wash	Face/Neck Wipe	Actual Dermal Exposure	Total Outer Body Dosimeter	Glove	Potential Dermal Exposure	Inhalation Exposure
	µg/ operator	µg/ operator	µg/ operator	µg/ operator	µg/ operator	µg/ operator	µg/ operator	µg/ operator
Epoxiconazole in BAS 601 KD F DocID 2007/103389	0.0031098	0.0021332	0.0000567	0.005300	0.01118	0.49863	1.30367	0.0000498
	0.0003537	0.0002413	0.0000596	0.000655	0.02348	0.76442	0.90230	0.0002448
	0.0003495	0.0001752	0.0000122	0.000537	0.01497	0.09824	0.69566	0.0000461
	0.0005299	0.0013121	0.0000779	0.001920	0.08107	0.49892	1.01913	0.0000227
	0.0001535	0.0003396	0.0000071	0.000500	0.00975	0.42697	1.99275	0.0000083
	0.0007086	0.0163456	0.0001269	0.017181	0.12323	1.41512	1.70080	0.0000387
	0.0007047	0.0003608	0.0000534	0.001119	0.00491	0.13925	1.59230	0.0000082
	0.0006835	0.0004510	0.0001935	0.001328	0.11456	1.33114	2.25922	0.0000230
	0.0014542	0.0094168	0.0000730	0.010944	0.21032	0.59093	0.88125	0.0000098
	0.0002347	0.0001387	0.0000373	0.000411	0.00563	0.06302	0.48664	0.0000064
Geometrical mean	0.0005703	0.0008213	0.0000491	0.001703	0.02758	0.38829	1.15865	0.0000234
75th percentile	0.0007077	0.0019279	0.0000767	0.004455	0.10619	0.72105	1.67368	0.0000443
Min	0.0001535	0.0001387	0.0000071	0.000411	0.00491	0.06302	0.48664	0.0000064
Max	0.0031098	0.0163456	0.0001935	0.017181	0.21032	1.41512	2.25922	0.0002448

Results of this study will be further discussed below together with the results of the second field exposure study.

Report:	CP 7.2.1.2/2 Blaschke U., 2010a Determination of operator exposure (passive dosimetry) during typical activities associated with a ground boom application of BAS 480 31 F to cereal crops at farm locations in the United Kingdom and Germany, 2008 2010/1089364
Guidelines:	OECD Series on Testing and Assessment No. 9 - Guidance document on the conduct of studies of occupational exposure to pesticides during agricultural application (Paris 1997)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This study was erroneously not contained in the application. It needs to be submitted, because it is the basis for the refined risk assessment.

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	Epoxiconazole formulated in the preparation BAS 480 31 F
	Description:	Suspension concentrate (SC) formulation containing nominal 125 g/L epoxiconazole
	Batch/purity #:	batch L72-185, epoxiconazole: 126.1 g/L
	Stability of test compound:	The stability of the formulation was guaranteed by the sponsor Expiry data (certificate of analysis): April 01, 2010

B. STUDY DESIGN AND METHODS

Dates of work: 19-May-2008 to 07-August-2008 (experimental data),
30-April-2010 (study completion date)

This operator exposure study was conducted at 13 locations in Germany and the United Kingdom during May and June 2008. At two locations 2 individual operators each were monitored on separate dates. Thus, in total 15 operators were monitored for all tasks related to application of the product BAS 480 31 F in cereals i.e. mixing/loading the appropriate amounts and application to a daily work area of about 50 ha. The applied product BAS 480 31 F was a commercial suspension concentrate (SC) containing nominal 125 g/L epoxiconazole.
In addition to the subset of 15 operators conducting all tasks (mixing/loading and application) a second subset of 14 operators was monitored separately for mixing/loading activities only. Within these two subsets (mixing/loading/application versus mixing/loading 9 operators were members of both subsets, but monitored on separate dates to allow a better comparison.
Although summarized below, the result of the second subset (mixing/loading activity only) will not be taken into consideration for the risk assessment purposes of this dossier.
Meteorological conditions were monitored during all workdays. Air temperatures ranged from 11 to 32°C. A relative humidity of 23–80% was measured, the wind speed ranged from 0 to 2.5 m/s.
A total of 15 operators were monitored during mixing/loading and application of the spray solution. In addition, a total of 14 operators were monitored during mixing/loading only. The subsets were defined as follows.

A) The activities mixing/loading and application were conducted in order to perform an application of BAS 480 31 F according to the recommended GAP of 1 L/ha to an approximate area of 50 ha. Mixing/loading was assessed when handling conventional 5 L containers of the product. Application was performed using conventional boom sprayer equipment with either standard or drift reduced nozzles. A summary of the relevant application equipment parameters is provided in Appendix 7.2-5.
B) For the mixing/loading activity the amount of product handled was intended to cover a daily application area. While 8 operators mixed an amount necessary for application of 50 ha, i.e. 10 containers of product during 1-2 mixing/loading activities corresponding to about 6.25 kg epoxiconazole, the remaining 6 operator mixed 3.75 to 5.63 kg epoxiconazole. Conventional 5 L containers of the product were used.

Operators were considered experienced in pesticide application. Their body weight was in the range of 72 kg to 145 kg with a mean of about 104 kg.

Dosimeter

Dermal dosimeter

The exposure was measured by whole body dosimetry for the dermal exposure. Hand exposure was assessed by means of a hand wash with 2 x 500 ml soap solution (2.5 mL liquid soap in 500 mL water).

The operators were equipped with two layers of whole body dosimeters consisting of outer and inner dosimeters. A standard work coverall of cotton/polyester fabric was chosen as outer dosimeter. The inner dosimeters consisted of whole cotton underwear with long arms and legs.

The hand exposure was assessed by analysis of nitrile protective gloves (outer dosimeter) and hand wash samples (inner dosimeter). Hand wash samples were taken at the end of the monitoring period for each operator prior to removing the body dosimeters. The operators were advised to use the protective gloves only for the mixing/loading process. For the application the operator did not need to wear protective gloves as long as he was not doing maintenance works at the sprayer where he could get into contact with spray liquid. In cases where the applicator was likely to get into contact with spray-liquid he wore protective gloves. Right and left gloves used per operator on a working day were pooled and subjected to a common analysis of the active substance contained. Hand wash samples were pooled as well in order to assess the actual hand exposure. Head exposure was measured by face/neck wipes with 100% cotton gauze. Face/neck wipes were taken at the end of the monitoring period prior to removing of the body dosimeters. Face and neck wipe samples were performed with 2 pads of cotton gauze wetted with 4 mL liquid soap solution. If the operator wished to wear a hat or a cap for sun protection or any other reason he was allowed to do so. The hat or a cap was not taken as a dosimeter. Other protective measures like face shield or mask were allowed at the discretion of the operator and not taken into account as dosimeter.

At the end of the monitoring period outer and inner dosimeters were separated and sectioned into individual parts as follows for the subsequent analysis.

Outer dosimeter	**Inner dosimeter**
Both lower arms	Both lower arms
Both upper arms	Both upper arms
Chest	Chest
Back	Back
Both lower legs	Both lower legs
Both upper legs	Both upper legs
Protective gloves (right and left pooled)	Hand wash samples (right and left one pooled)
	Face/neck wipes

The potential dermal exposure (PDE) was calculated from residues of the coverall, the underwear (vest & long-johns), faces/neck wipes, the hand-wash samples and the protective gloves. The actual dermal exposure (ADE) was calculated from the residues detected on/in the underwear (long sleeved vest & long-johns), the faces/neck wipes and the hand-wash samples.

Inhalation dosimeters

Operators were equipped with personal air sampling tubes (TENAX adsorption tubes) combined with a personal air sampling pump located in the operator's breathing zone. The limit of quantification (LOQ) was 1 ng (0.001 µg). The air pump flow was adjusted to 1.5 L/min and the pumps were calibrated prior and after use. The total volume of air sampled was calculated based on the mean calibration data in conjunction with the total run time. For an operator engaged in moderate physical labour a breathing volume of 14 L/min or 840 L/hour was considered.

Storage of dosimeters

At the end of the treatment, the body dosimeters were removed and sectioned on site prior to storage in a freezer or freezer truck for the transport to the laboratory. Samples were subsequently stored deep-frozen until analysis. Air samplers were stored in darkness at ambient temperatures until analysis.

Field recoveries and analytical procedures

The stability of the test item under field, storage and transport conditions in or on the sampling materials (coverall, inner dosimeters, facial swabs, protective gloves if necessary and air sampling filters) was assessed for field fortifications, for storage and for transport conditions. For this purpose, the test item was applied under laboratory and under field conditions onto the different sampling materials at two different concentrations and the recoveries were analytically determined.

Inner and outer dosimeters as well as protective gloves were extracted with methanol. Aliquots of the extract were diluted with equal amounts of water for LC/MS/MS injection. Hand wash solution aliquots were diluted with equal volumes of methanol for LC/MS/MS analysis. Face/Neck wipes were extracted with methanol. Aliquots of the extract were diluted 1: 9 with water : methanol (1:1) for LC/MS/MS injection. The Tenax absorber was extracted with acetone. Extracts were reduced to dryness and subsequently diluted in methanol : water (1:1) for LC/MS/MS analysis.

II. RESULTS AND DISCUSSION

Field and concurrent laboratory fortifications

The results of field, transport and concurrent laboratory fortification analyses demonstrated the storage and transport stability of the test item. Field recovery and lab recovery data for body dosimeters were in the range of 74 to 99% of the test substance initially applied. For air sampler units, recoveries were between 95 to 102%. A short summary of the recovery results is given in Table 7.2.1.2-4 below.

Table 7.2.1.2-4: Mean field and concurrent laboratory fortifications

Matrix	Field recovery [%]	Lab recovery [%]	Correction factor[2]
Inner dosimeter	100.0 ± 5.5	97.5 ± 8.6	n.n.
Face/Neck wipe	105.7 ± 20.6	93.0 ± 6.0	n.n.
Hand wash solution	109.8 ± 13.4	98.3 ± 8.2	n.n.
Outer dosimeter	93.9 ± 6.1	99.1 ± 7.8	n.n.
Gloves[1]	72.8 ± 19.8	86.6 ± 8.3	Mean of field fortification levels except invalid values
Gloves except field fortification considered invalid	77.5 ± 14.5		1.29
Air sampler	98.3 ± 16.9	106.0 ± 4.3	n.n.

[1] The field fortifications for gloves were in the range of 68 to 89% except for one specific day. The lower values of this day (mean 32%) were considered invalid as the fortified specimens were blown from the table by a sudden wind gust.

[2] According to guidelines a correction factor needs not be applied if the recovery values are within the 70 - 120% range. However, no correction was applied in cases were the upper range was exceeded as the uncorrected values would result in a conservative overestimation of exposure

Based on the obtained recovery data a correction factor of 1.29 was applied to account for field recoveries for gloves. For all other matrices no correction factor was employed.

The occasionally higher field recoveries for face/neck wipes and air samplers were not considered for correction as the uncorrected values would result in an overestimation of exposure.

Analytical confirmation of the test substance in BAS 480 31 F

One batch of BAS 480 31 F was used in the study. The analytical confirmation of test substance control gave the following results (Table 7.2.1.2-5).

Table 7.2.1.2-5: Analytical results of used BAS 480 31 F batch

Batch-No.	Nominal content (epoxiconazole) [g/L]	Analyzed content (epoxiconazole) [g/L]
L72-185	125	126.1

A summary of the obtained exposure levels for outer and inner body dosimeters, hand wash and glove exposure, face and neck wipes as well as inhalation exposure is given in Table 7.2.1.2-6.

Table 7.2.1.2-6: Summary of operator exposure to epoxiconazole when mixing/loading and spraying BAS 480 31 F to field crops

Study	Total Inner Body Dosimeter	Hand Wash	Face/Neck Wipe	Actual Dermal Exposure	Total Outer Body Dosimeter	Glove	Potential Dermal Exposure	Inhalation Exposure
	µg/operator	µg/operator	µg/operator	µg/operator	µg/operator	µg/operator	µg/operator	µg/operator
Epoxiconazole in BAS 480 31 F DocID 2010/1089364	0.0002072	0.0013901	0.0000071	0.001604	0.06911	0.09520	2.71281	0.0000416
	0.0027189	0.0225600	0.0001031	0.025382	0.67547	1.84605	3.02874	0.0000105
	0.0003595	0.0011921	0.0000791	0.001631	0.10331	0.37690	0.82799	0.0000226
	0.0013433	0.0085926	0.0000815	0.010017	0.03885	0.29728	0.58874	0.0000595
	0.0003969	0.0021184	0.0000428	0.002558	0.04519	0.19484	0.38280	0.0000074
	0.0004120	0.0020832	0.0000488	0.002544	0.08548	0.05218	0.91912	0.0000125
	0.0002949	0.0005628	0.0000143	0.000872	0.04545	0.73260	1.38848	0.0000683
	0.0016589	0.0020617	0.0000389	0.003759	0.41816	0.18764	0.79548	0.0000443
	0.0002541	0.0005084	0.0000226	0.000785	0.06117	0.12396	0.65542	0.0015151
	0.0020928	0.0024643	0.0001467	0.004704	0.02382	0.44098	0.61580	0.0000901
	0.0001855	0.0055321	0.0000433	0.005761	0.02294	0.11760	0.84266	0.0001269
	0.0002833	0.0019033	0.0000521	0.002239	0.03536	0.65876	12.58724	0.0000783
	0.0010171	0.0031233	0.0000809	0.004221	0.17347	11.71319	17.49922	0.0000560
	0.0011794	0.1394636	0.0075891	0.148232	0.99997	4.46015	11.37720	0.0000275
	0.0005231	0.0323466	0.0005249	0.033395	0.29605	5.43942	6.37514	0.0000205
Geometrical mean	0.0005917	0.0036787	0.0000750	0.004750	0.09880	0.50273	1.82732	0.0000453
75th percentile	0.0012614	0.0070623	0.0000923	0.007889	0.23476	1.28932	4.70194	0.0000733
Min	0.0001855	0.0005084	0.0000071	0.000785	0.02294	0.05218	0.38280	0.0000074
Max	0.0027189	0.1394636	0.0075891	0.148232	0.99997	11.71319	17.49922	0.0015151

The results of the two field exposure studies, which are summarized above, will be further discussed in the following chapters.

General conclusion on the applicability of the surrogate studies to assess operator exposure under the proposed use conditions of BAS 500 06 F

The surrogate field exposure studies conducted used liquid formulations applied to field crops using ground-boom sprayers with hydraulic nozzles. In the studies the exposure during mixing/loading and application was monitored together. The results are considered representative of equipment intended for large areas, i.e. corresponding to the data used in the UK agronomic parameters (50 ha/day). The application rate was 125 g a.s./ha with application volumes of 100 to 200 L/ha. The exposure data were generated using current standards of application equipment (e.g. tractors with cabins in combination with large-tank ground-boom sprayers and wide spray booms (for details see Appendix 7.2-5). Thus, the use conditions of the field exposure study is representative for the use conditions of BAS 500 06 F as it is applied today.

Methodology

In the studies the operator exposure was investigated using the whole body dosimetry method, which is considered to be more reliable than the patch method. In all cases, the inhalation exposure was measured by means of pumps connected to suitable filters or cartridges. The pumps were adjusted to an air volume that allowed capturing airborne particles in the respirable range. The exposure of the hands was measured by performing hand washing with an appropriate solution after taking off the protective gloves. Gloves were worn in all the cases during the mixing/loading phase. During the application phase protective gloves were worn when operators identified the need (e.g. during operations like fixing blocked nozzles). Therefore, since gloves were not worn throughout the application phase, it is not possible to evaluate a transfer through gloves from the available data.

Agronomic parameters

Treated areas were in the range of 48.0 to 55.4 ha for operators (details are shown in Appendix 7.2-5). The geometric mean and the 75th percentile is about 50 ha which corresponds to the recommended area of the UK-POEM and EUROPOEM. Treated areas are thus well representing agricultural practice in European countries, where treated areas have increased since the time when the BBA model was developed. On the other hand, for these areas the average duration of application was only 3.8 hours, which is about half the duration recommended in the agronomic parameter guide in UK-POEM or BBA.

The agricultural equipment was of a size suitable to spray large areas and all the tractors were equipped with cabins (open or closed). Application rates were defined in the protocol of the studies being 0.125 kg a.s./ha, respectively. Water application volumes were variable and comprised between 90 and 180 L/ha, which corresponds to the current standards. Therefore, this exposure database is considered to be representative for the uses on large field crop areas with modern application equipment.

Standardisation and adjustment of surrogate data

The data obtained from the operator exposures studies were standardized in order to simplify the extrapolation of surrogate data to application rates of other products or of other conditions of use.

Normalisation of the application rate

Data generated in the studies were normalized to an application of 1 g active substance per 1 ha allowing subsequent adoption to product specific application. Furthermore adjustments were made to work rates of 50 ha per day and to exposure per kg operator body weight to meet the respective input data for standard model assessments (e.g. UK POEM). A summary of the normalized study data is presented in Table 7.2.1.2-7; details are given in Appendix 7.2-6.

Table 7.2.1.2-7: Extrapolated dermal and inhalation exposure of recently conducted operator exposure studies with ground boom sprayers standardized for a work rate of 50 ha/day and for 1 g a.s./ha and normalized to the individual body weight

	Dermal exposure		Inhalative exposure
	no PPE	**complete PPE**	**no PPE**
	Potential dermal exposure	**Actual dermal exposure**	
	Sum of inner + outer body dosimeter + hand (inner) + gloves (outer) + head	Sum of inner body dosimeter + hand (inner) + head	
	[µg/kg bw/day when applying 1 g a.s./ha to 50 ha/day]		
Geometric Mean	**0.2903**	**0.0016**	**0.0000177**
75th Percentile	**0.4778**	**0.0032**	**0.0000289**

Personal protective equipment

For the evaluation below two scenarios with regard to different levels of personal protective equipment (PPE) were taken into consideration:

"no PPE":

It is assumed that operators completely neglect the label advice on protective equipment. The potential dermal exposure reflects the sum of total outer and inner body dosimeters, the head and hands including gloves. The “no PPE” scenario assumes permeation of 50% of the findings on dosimeters of outer clothing. Dermal exposure estimates thus have been based on the aggregation of findings on inner dosimeters including hand wash and face/neck wipes (head), findings on gloves plus 50% of findings on outer clothing dosimeters. Individual results and percentile values over the data set are presented as ‘Scenario 1’ in Appendix 7.2-7.

"complete PPE":

Complete protective equipment as used in the operator exposure study (standard protective working clothing during mixing/loading and application and protective gloves during mixing/loading). Dermal exposure estimates thus have been based on the aggregation of findings on inner dosimeters including hand wash and face/neck wipes (head). Individual results and percentile values over the data set are presented as ‘Scenario 2’ in Appendix 7.2-7.

Note: In the studies, operators wore protective gloves during mixing/loading and usually no gloves during spray application, except when performing maintenance work that would likely result in contact with the spray liquid.

Operator body weight

The individual operator body weights determined in the exposure study clearly indicate that the standard value used in the UK model is at the low end of distribution for actual operator weights. The exposure levels are thus normalized to µg a.s. per kg bodyweight on an individual basis (see Appendix 7.2-6).

Conclusion on the surrogate data of the two operator exposure studies in field crops

Exposure data points from two field studies comprising 25 operators on 23 farms were generated. State-of-the-Art technology of application equipment was used and individual work rates appropriately reflect today's agronomic conditions for field crop growers. The data are therefore considered to provide an adequate data base for the assessment of operator exposure during mixing/loading of liquid formulations and spray applications with field crop sprayers. Therefore, this database can be regarded as well adapted for extrapolation to other active substances applied within liquid formulations on field crops.

Exposure estimates and risk assessment for pyraclostrobin when used in BAS 500 06 F based on the surrogate operator exposure data

The following parameters were used for the estimations of operator exposure from the product BAS 500 06 F (see Table 7.2.1.2-8).

Table 7.2.1.2-8: Product specific parameters

	Pyraclostrobin
Application rate (kg a.s./ha)	0.25
Systemic AOEL (mg/kg bw/day)	0.015
Dermal penetration (%)	7

The normalized surrogate data were adjusted to account for the maximum recommended application rate of 250 g pyraclostrobin/ha. Furthermore a daily work rate of 50 ha/day was taken into account. The obtained dermal and inhalation exposure values (75th percentile estimates) are presented in Table 7.2.1.2-9 below. Detailed information is presented in Appendix 7.2-8 for the scenario "no PPE" and in Appendix 7.2-9 for the scenario "complete PPE".

Table 7.2.1.2-9: Operator risk assessment for the product BAS 500 06 F based on surrogate operator exposure data adapted to a work rate of 50 ha/day

Level of personal protection	Active ingredient	Systemic exposure[1]			Total systemic exposure	Account of AOEL[2]	Reference Appendix
		Dermal	Inhalation	Total			
		mg/kg bw/d			mg/kg bw/d		
75th percentile							
no PPE [3]	pyraclostrobin	0.0084	0.0000073	5.362	0.00841	56%	7.2-8
complete PPE [4]	pyraclostrobin	0.00004	0.0000073	0.000047	0.000045	0.31%	7.2-9

[1] systemic exposure based on dermal absorption for both mixing/loading and application of 7% for pyraclostrobin as a worst case
[2] total systemic exposure x 100 / systemic AOEL (AOEL for pyraclostrobin = 0.015 mg/kg bw/d)
[3] scenario 1, 50% permeation of outer body dosimeters
[4] scenario 2, actual dermal exposure
Note: Calculations were made using non rounded values, therefore deviations may occur when calculating with rounded values as shown above

Following the concept of the UK POEM with a work rate of 50 ha/day, the 75th percentile exposure values derived for pyraclostrobin correspond to about 56% of the AOEL and is thus within the acceptable range for an operator not being adequately dressed and not applying PPE.

The exposure values derived for pyraclostrobin for a protected operator wearing gloves during mixing/loading and adequate working clothing correspond to about 0.31% of the respective AOEL.

Conclusion

A generic data base consisting of recently generated field exposure data was set up and adjusted to the conditions of use for the formulation BAS 500 06 F (200 g pyraclostrobin/L). The basis for the generic data has been formed by two operator exposure field studies performed by BASF and described above. The studies were conducted under the current conditions of field crop applications using fungicide formulations.

In conclusion, operators are considered to be at acceptable risk when exposed to pyraclostrobin under the use conditions of BAS 500 06 F in cereals.

CP 7.2.2. Bystander and resident exposure

The plant protection product BAS 500 06 F is already registered for the use as fungicide in the representative crops cereals and maize. Information on the formulation and the critical use pattern relevant for the bystander and resident risk assessment can be found in chapter 7.2.1. The critical GAP is summarized in Table 7.2.1-1.

Exposure assessments and risk evaluations for bystanders and residents for the representative formulation BAS 500 06 F are presented below. According to the EU requirements they have been based on the following model:

- Martin S. et al. (2008): Guidance for Exposure and Risk Evaluation for Bystanders and Residents exposed to Plant Protection Products during and after Application. J. Verbr. Lebensm., drift distances adopted according to the recommendation published by the German BVL: B. Nolting (2012) Bekanntmachung ueber Mindestabstaende, die bei der Anwendung von Pflanzenschutzmitteln zum Schutz von Umstehenden und Anwohnern einzuhalten sind. Bundesanzeiger 2012 No. 4 Pp. 75-76 for the bystander and resident.

Risk assessment for bystander and resident

A summary of the bystander risk assessment is provided in Table 7.2.2-1.

Table 7.2.2-1: Summary of bystander exposure during application of BAS 500 06 F in cereals

	Active ingredient	Estimated bystander exposure[1] (mg/kg bw/day)	% of AOEL[2]	Reference in Appendix
Adults	pyraclostrobin	0.000813	5.42	7.2-10
Children		0.000635	4.23	

[1] according to the German model for bystander and resident exposure assessment; Martin S. et al. (2008)
[2] based on a systemic AOEL of 0.015 mg/kg bw/day for pyraclostrobin

A summary of the risk assessment for residents is provided in Table 7.2.2-2.

Table 7.2.2-2: Summary of residential exposure during application of BAS 500 06 F in cereals

	Active ingredient	Estimated residential exposure[1] (mg/kg bw/day)	% of AOEL[2]	Reference in Appendix
Adults	pyraclostrobin	0.0001	0.7	7.2-11
Children		0.00022	1.5	

[1] based on the German model for bystander and resident exposure assessment; Martin S. et al. (2008)
[2] based on a systemic AOEL of 0.015 mg/kg bw/day for pyraclostrobin

Conclusion
It is concluded that there is no unacceptable risk to bystanders or residents after accidental exposure of BAS 500 06 F under the use conditions in cereals and maize.

CP 7.2.2.1. Estimation of bystander and resident exposure

Bystanders and residents are not involved in application or handling of plant protection products or the professional handling of treated crops. Therefore, exposure differs significantly from operator exposure levels. The exposure assessment presented below is based on the German guidance paper for evaluation of bystander and resident exposure (Martin S. et al., 2008) with adopted drift distances (B. Nolting, 2012).

A. Bystander exposure

The presence of bystanders is incidental within or directly adjacent to an area where plant protection products are applied. A situation in which bystander exposure could occur would be a person walking alongside an area being treated at the same time. Under these conditions the bystander would never walk directly next to the outer spraying nozzle. A distance of some meters from the spraying device can always be expected. It can further be assumed that any bystander, as soon as becoming aware of an exposure will leave the spraying area. Therefore, bystander exposure is of short duration, typically a matter of minutes. Thus, the exposure duration of 5 minutes is assumed.

Bystander exposure results from spray drift that deposits on the body surface or passes the breathing zone. Assuming that bystanders wear only light clothing (i.e. short-sleeved shirt and shorts), the exposed, uncovered body surface of an adult (head, face, neck front and back, forearms, half upper arms, hands, lower half of thighs, lower legs and feet) is amounting to about 1 m². For children the exposed body surface with the same level of clothing amounts to 0.21 m².

For the scenario of professional agricultural use it is assumed that the bystander is located at a distance of 1 m, downwind from the spraying source. The extent of spray drift and the consequent deposition depends on the plant protection product application rate, the particular crop being treated and the method of application. Measurements of spray drift following different crop/equipment combinations are available from Rautmann D. et al., 2001: New basic drift values in the authorisation procedure for plant protection products. In: Forster, B. and Streloke, M. (eds.), Workshop on Risk Assessment and Risk Mitigation Measures (WORMM). 27–29 September 1999, Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, booklet 383, 2001; actual version of 27th March 2006: Rautmann, D., 2006: Aktuelle Abdrifteckwerte (Current Drift Values). http://www.jki.bund.de/cln_045/nn_926124/SharedDocs/10_FA/Publikationen/Pflanzenschutzgeraete/abdrifteckwerte_xls.html.
Thus, for the corresponding application scenario in cereals the proposed drift value at 1 m distance of 2.77% (90th percentile value) is used for estimations of dermal exposure. However, the drift deposition data by Rautmann D. et al., 2001 cannot easily be transposed into airborne concentrations and consequent inhalation exposure values. Therefore, to ensure a conservative evaluation, measured inhalation exposure data for the unprotected operator during spray applications (Lundehn J.R. et al., 1992, German BBA model) are used for the bystander inhalation exposure estimation.

The parameters used for bystander exposure calculations are summarized in Table 7.2.2.1-1.

Table 7.2.2.1-1: Parameters used for bystander exposure calculations

	Parameter	**Pyraclostrobin**	
AR	Maximum application rate (kg a.s./ha)	0.25	
ar	Maximum application rate (mg a.s./m²)	25	
DA	Dermal absorption (%)	7	
IA	Inhalation absorption (%)	100	
		Default values	
D	Drift at 1 meter distance for field crop (% of application rate)	2.77	
TB	Exposure duration bystander (minutes)	5	
TO	Exposure duration operator (hours)	6	
TF	Exposure duration factor (TB / TO) [1]	0.0139	
A	Area treated (ha/day)	50	
		Adults	Children
BSA	Exposed body surface (m²)	1	0.21
IA	Specific inhalation exposure (mg a.s./kg a.s. handled) [2]	0.001	0.00057
BW	Bystander body weight (kg)	60	16.15

[1] since the German model values are based on an application period of 6 hours/day, adjustment to 5 minutes is required for exposure calculations.
[2] based on geometric mean values proposed by the German BBA Model (Lundehn et al., 1992) and inhalation rates of 1.74 and 1.0 m³/h for adults and children, respectively

Bystander exposure for adults and children is estimated according the following equations:

Systemic dermal exposure $$SDE_B = \frac{ar \times D \times BSA \times DA}{BW}$$

Systemic inhalation exposure $$SIE_B = \frac{I_A \times AR \times A \times TF \times IA}{BW}$$

Total systemic exposure $$SE_B = SDE_B + SI_B$$

Assessment

For the exposure of a bystander passing by the field treated with BAS 500 06 F the systemic exposure to pyraclostrobin was assessed based on 5 minutes exposure applying generic spray drift deposits of 2.77% of the application rate at 1 meter distance and air borne spray concentrations of 0.001 and 0.00057 mg/kg a.s./person for adults and children, respectively.

The result of the bystander exposure estimation is presented in Table 7.2.2.1-2. Details of the estimations are presented in Appendix 7.2-10.

Table 7.2.2.1-2: Estimated bystander exposure to pyraclostrobin and % of the AOEL

	Active Substance	
	Pyraclostrobin	
	AOEL = 0.015 mg/kg bw/day	
	Adults	**Children**
Systemic exposure via dermal route (mg/kg bw/day)	0.00081	0.00063
Systemic exposure via inhalation (mg/kg bw/day)	0.000003	0.000006
Total systemic exposure [1] (mg/kg bw/day)	**0.000813**	**0.000635**
% of AOEL [2]	**5.42**	**4.23**

[1] according to the German model for bystander and resident exposure assessment; Martin S. et al. (2008)
[2] based on a systemic AOEL of 0.015 mg/kg bw/day for pyraclostrobin

This estimate results in 5.4 and 4.2% usage of the AOEL for pyraclostrobin for adults and children, respectively. Thus, the exposure of adult and child bystanders passing by a field treated with BAS 500 06 F is considered to be safe.

In conclusion, bystanders are not considered to be at risk when exposed to spray drift of pyraclostrobin under the use conditions of BAS 500 06 F in cereals and maize.

B. Resident exposure

Residents are persons who live, work or attend any institution adjacent to an area that has been treated with a plant protection product. Possible situations are persons who are standing, working, or sitting in a garden in the vicinity of the application. They may be exposed to the plant protection products mainly via the dermal route from spray drift deposits and by inhalation of vapour drift depending on the vapour pressure of the active substances. For infants and toddlers oral exposure via hand-to-mouth transfer or object-to-mouse transfer has to be considered, too.
As for the bystander it can be assumed that residents are unlikely to take actions to avoid or control exposure, and they wear only light clothing and no protective equipment. In addition, as conservative approach it is assumed that residents are located directly downwind of the centre of the treatment area from the point of spray emission caused by professional agricultural uses.
The field crop application is here considered to represent the worst case scenario for which a distance of 1 m from the spraying device is taken into consideration.

It can be assumed that the exposure duration of residents being in a garden is longer than the exposure duration of bystanders. Therefore, the default exposure duration of 2 hours is adopted for risk evaluations.

According to the German model for bystander and resident exposure assessments, inhalation exposure has only to be considered for semi-volatile and volatile active substances (vapour pressures of 1×10^{-5} – 5×10^{-3} Pa and $> 5 \times 10^{-3}$ Pa, respectively). As pyraclostrobin has a vapour pressure of 2.6×10^{-8} it is considered non-volatile. Consequently, inhalation exposure to vapours is not taken into account.

For assessment of the oral exposure of children and toddlers in a first-tier approach the default values proposed by the German model for bystander and resident exposure assessments are used (Martin S. et al. (2008) as derived from a US-EPA policy paper). Table 7.2.2.1-3 summarizes the parameters used for resident exposure of adults and children.

Table 7.2.2.1-3: Parameters used for resident exposure estimations

	Parameter	Pyraclostrobin	
	Vapour pressure (Pa)	2.6×10^{-8}	
	Volatility	non-volatile	
	Maximum application rate (kg a.s./ha)[1]	0.25	
	Maximum number of applications	2	
AR	Application rate relevant for resident (kg a.s./ha)	0.50	
Ar	Maximum application rate (mg a.s./cm²)	0.005	
DA	Dermal absorption (%)	7	
IA	Inhalation absorption (%)	100	
OA	Oral absorption (%)	50	
ACV	Airborne concentration of vapour (mg/m³) [2]	-	
		Default values	
D	Drift (%) at 1 m distance - 90th percentile values[3] for field crop	2.77	
H	Duration (hours)	2	
		Adults	Children
TTR	Turf transferable residues hand (%)	5	5
TC	Transfer coefficient (cm²/hour)	7300	2600
IR	Inhalation rate (m³/day)	16.57	8.31
SE	Salivation extraction factor (%)		50
SA	Surface area of hands (cm²)		20
Freq	Frequency of hand-to-mouth events (events/hour)		20
DFR	Dislodgeable foliar residue object to mouth (%)		20
IgR	Ingestion rate for mouthing of grass (cm²)		25
BW	Resident body weight (kg)	60	16.15

[1] As BAS 500 06 F will be applied twice per season at maximum, the double application rate is considered relevant for residential exposure according to the German model for bystander and resident exposure assessment.

[2] Since pyraclostrobin is non-volatile, i.e. the vapour pressure is 2.6×10^{-8} Pa, the ACV value is 0.0 mg/m³.

[3] 90th percentile of drift recommended for one application, 82nd percentile of drift for double application rate recommended for more than one application according to Martin S. et al. (2008)

Resident exposure for adults and children is estimated according the following equations:

Systemic dermal exposure

$$SDE_R = \frac{AR \times D \times TTR \times TC \times H \times DA}{BW}$$

Systemic inhalation exposure

$$SIE_R = \frac{AC \times IR \times IA}{BW}$$

Systemic exposure due to hand-to-mouth transfer (children only)

$$SOE_H = \frac{AR \times D \times TTR \times SE \times SA \times F \times H \times OA}{BW}$$

Systemic exposure due to mouthing (children only)

$$SOE_O = \frac{AR \times D \times DFR \times IgR \times OA}{BW}$$

Total systemic exposure (adults)

$$SE_R = SDE_R + SIE_R$$

Total systemic exposure (children)

$$SE_R = SDE_R + SIE_R + SOE_H + SOE_O$$

The resulting predicted exposures are summarized below.

Assessment

For the exposure of a resident located next to a field treated with BAS 500 06 F the systemic exposure to pyraclostrobin was assessed based on 2 hour exposure to spray drifts via dermal and oral route. Exposure to airborne concentration of vapour for non-volatile compounds such as pyraclostrobin is not considered.

The results of the resident exposure calculations are summarized in Table 7.2.2.1-4 below. Details of the estimations are presented in Appendix 7.2.11.

Table 7.2.2.1-4: Estimated resident exposure to pyraclostrobin and % of the AOEL for the use of BAS 500 06 F in cereals

	Active Substance	
	Pyraclostrobin	
	AOEL = 0.015 mg/kg bw/day	
	Adults	**Children**
Systemic exposure via dermal route (mg/kg bw/day)	0.0001	0.00013
Systemic exposure via inhalation (mg/kg bw/day)	-	-
Systemic exposure via oral route (mg/kg bw/day)	-	0.000094
Total systemic exposure[1] (mg/kg bw/day)	**0.0001**	**0.00022**
% of AOEL[2]	**0.7**	**1.5**

[1] based on the German model for bystander and resident exposure assessment; Martin S. et al. (2008)
[2] based on a systemic AOEL of 0.015 mg/kg bw/day for pyraclostrobin

The estimate results for adults in 0.7 and for children in 1.5% of the AOEL for pyraclostrobin. Thus, the exposure of adult and child residents living next to a field treated with BAS 500 06 F is considered to be safe.

In conclusion, residents are not considered to be at risk when exposed to spray drift of pyraclostrobin under the use conditions of BAS 500 06 F in cereals.

CP 7.2.2.2. Measurement of bystander and resident exposure

Since the risk assessment performed indicates that the health-based limit values (AOEL) will not be exceeded under practical conditions of use, studies to provide field data on bystander or residential exposure to BAS 500 06 F were not considered to be necessary and were thus not performed.

CP 7.2.3. Worker exposure

The plant protection product BAS 500 06 F is already registered for the use as fungicide in the representative crops cereals and maize. Information on the formulation and the critical use pattern relevant for the re-entry worker risk assessment can be found in chapter 7.2.1. The critical GAP is summarized in Table 7.2.1-1.

Exposure assessments and risk evaluations for re-entry workers for the representative formulation BAS 500 06 F are presented below. Estimations of potential worker exposure have been undertaken applying the following guidance for exposure prediction:

- EUROPOEM - Re-entry exposure model final draft adopted by more specific US EPA agricultural transfer coefficients

Risk assessment for worker

A summary of the worker risk assessment is provided Table 7.2.3-1.

Table 7.2.3-1: Summary of re-entry workers exposure following application of BAS 500 06 F without protective equipment (standard assessment)

	Active ingredient	Estimated worker exposure (mg/kg bw/day)	% of AOEL[1]	Reference in Appendix
2 hour/day scouting and crop inspection in cereals	pyraclostrobin	0.00525	35	**7.2-12**

[1] based on a systemic AOEL of 0.015 mg/kg bw/day for pyraclostrobin

Conclusion
It is concluded that there is no unacceptable risk anticipated for the worker wearing adequate working clothing when re-entering cereal crops treated with BAS 500 06 F after the spray dilute has dried.

CP 7.2.3.1. Estimation of worker exposure

Worker exposure to pyraclostrobin from the product BAS 500 06 F

BAS 500 06 F will be used as a fungicide in cereals during growth stages BBCH 25-69 with a maximum of two applications per season. Thus, the considered reasonable worst case for the maximum applied amount of product per season is 2.5 L/ha corresponding to 0.5 kg pyraclostrobin per ha.

Hand operations in cereals (and maize), which may result in re-entry exposure do not belong to standard growing procedures after the application of the product. Exposure scenarios one may think of as a worst case may be scouting and crop inspection. These operations are considered to be of limited duration and of limited direct contact to the treated plants. For these operations a working period of 2 hours per day is considered a reasonable approach.

Exposure Estimation Models used

The exposure estimation of the re-entry worker presented below is based on the:

- EUROPOEM - Re-entry exposure model final draft adopted by more specific US EPA agricultural transfer coefficients

The default exposure assessment is based on the following assumptions:

- Re-entry exposure is predominantly via the dermal route (contact with the foliage)
- Residues on the foliage depend on:
  - application rate
  - the crop habitat (total size of foliage compared to surface area, Leaf Area Index (LAI))
- Transfer of residues from the foliage to clothes or skin of workers is more or less independent of the product applied, but depend mainly on the intensity of contact with foliage (work activity).
- Activities with a similar pattern can be grouped and generic Transfer Coefficients (TC) can be used for one group.
- Based on the EUROPOEM proposal an Dislodgeable Foliar Residue (DFR) default value of 3 µg a.s./cm² is taken into account.

It is reasonably presumed that workers re-enter the treated crop after the spray has dried.

For pyraclostrobin a cumulated application rate of 0.5 kg a.s./ha is taken into account.

Within the EU, there are no commonly accepted specific transfer coefficients (TC) available to assess scouting and crop inspection activities in cereals. Whereas the RMS widely accepted a TC of 1500 cm²/hour for crop inspection, the EFSA draft guidance on operator, bystander, resident and worker exposure assessment has proposed a transfer coefficient of 1100 cm²/hour for crop inspection activities instead.
Following the US EPA agricultural transfer coefficient, a TC of 1500 cm²/hour based on Central value from ARF021 -- scouting dry peas (range 486 to 2760) has been used in this evaluation as the more conservative approach.

In the following Table 7.2.3.1-1 the parameters used in the re-entry worker risk assessment are presented.

Table 7.2.3.1-1: Parameters used for the worker risk assessment

	Parameter	**Pyraclostrobin**	
MR	Application rate considered for default worker exposure	0.500	kg a.s./ha
DF	Dermal absorption[1]	7	%
DFR	Default dislodgeable foliar residue	3	µg/cm² x kg a.s. applied
		Default values	
BW	Re-entry worker body weight	60	kg
TC	Transfer coefficient[2]	1,500	cm²/h x person
A	Working period	2	h/day
TR	Transmission to skin for unprotected worker	1.00	(factor, equal to 100%)

[1] The given value for dermal absorption of pyraclostrobin represent the estimate for spray dilutes. Exposure during re-entry occurs to dry residues for which it is adequate to expect a very low dermal absorption. Therefore, it is considered to be a conservative approach to use the dermal absorption value determined for the liquid spray dilute.
[2] A TC of 1500 cm²/h x person was used (according to US EPA agricultural transfer coefficients) for activities like scouting and irrigation in cereals and maize based on recommendations for Central value from ARF021 -- scouting dry peas (range 486 to 2760).

Based on the assumptions and consideration made above, worker exposure is calculated as follows:

External dermal exposure ED_W

$$ED_W = MR \times A \times DFR \times TC \times TR$$

Total systemic exposure SE_W

$$SE_W = \frac{ED_W}{BW} \times DF$$

Estimatimation of worker exposure without personal protective equipment

The results of the re-entry worker risk assessment without PPE is presented in Table 7.2.3.1-2 below, details are given in Appendix 7.2-12.

Table 7.2.3.1-2: Estimated worker exposure to pyraclostrobin following application of BAS 500 06 F in cereals (standard assessment)

		Exposure parameter	
Active substance	**AOEL (mg/kg bw/day)**	**Absorbed dose (mg/kg bw/day)**	**% of AOEL**
unprotected worker[1]			
Pyraclostrobin	0.015	0.00525	35

[1] worker wearing working clothing

Based on the proposed exposure estimation, the predicted exposure to pyraclostrobin is 35% of the AOEL. Thus, for a worker performing post-treatment activities (cereals: scouting and crop inspection) without gloves or protective clothing for 2 hours, the estimated worker exposure levels for pyraclostrobin is within the acceptable range.

It is concluded that there is no unacceptable risk anticipated for the worker wearing adequate working clothing when re-entering cereal crops treated with BAS 500 06 F after the spray dilute has dried.

CP 7.2.3.2. Measurement of worker exposure

Since the risk assessment performed indicates that the health-based limit value (AOEL) will not be exceeded under practical conditions of use, studies to provide field data on worker exposure to BAS 500 06 F were not considered to be necessary and were thus not performed.

Appendices for section 7.2

Appendix 7.2-1: Pyraclostrobin: BBA model estimations for tractor mounted boom sprayer application without PPE

Product:	BAS 500 06 F		**Formulation type:**	EC	
Active ingredient:	Pyraclostrobin		**Concentration:**	200.0	g/L
AOELsys:	0.015	mg/kg bw/day	**Assessment factor:**	100	
Maximum Rate:	0.250	kg a.i. per ha	**Area treated per day:**	20	ha
Amount of a.i. handled / day:	5.00	kg a.i. per day	**Dermal absorption (M/L):**	3.0%	
Application technique	tractor-mounted boom sprayers with hydraulic nozzles, field crop		**Dermal absorption (Spray):**	7.0%	
Personal protective equipment:	None				

$D_{M(H)}$ =	2.4	mg / person x kg a.i.	x	5.0	=	12.000	mg / person / day
$D_{A(H)}$ =	0.38	mg / person x kg a.i.	x	5.0	=	1.900	mg / person / day
$D_{A(B)}$ =	1.6	mg / person x kg a.i.	x	5.0	=	8.000	mg / person / day
$D_{A(C)}$ =	0.06	mg / person x kg a.i.	x	5.0	=	0.300	mg / person / day
I_M =	0.0006	mg / person x kg a.i.	x	5.0	=	0.003	mg / person / day
I_A =	0.001	mg / person x kg a.i.	x	5.0	=	0.005	mg / person / day

			External exposure		Abs. factor	Systemic exposure		
Inhalation	mix/load	I_M =	0.0030	x	100%	=	0.003	mg / person / day
	spray	I_A =	0.0050	x	100%	=	0.005	mg / person / day
Dermal	mix/load	D_M =	12.0000	x	3.0%	=	0.360	mg / person / day
	spray	D_A =	10.2000	x	7.0%	=	0.714	mg / person / day

Total exposure (assuming person weighing 70 kg):	=	**1.08**	**mg / person / day**
Total exposure (mg/kg bw/day):	=	**0.016**	**mg/kg bw/day**
Total exposure (% AOEL):	=	**103%**	

Appendix 7.2-2: Pyraclostrobin: UK POEM estimations for tractor mounted boom sprayer application without PPE

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles		
Product	**BAS 500 06 F**	Active ingredient	**Pyraclostrobin**
Formulation type	organic solvent-based	a.i. concentration	**200.0** mg/ml
Dermal absorption from product	**3** %	Dermal absorption from spray	**7.0** %
Container	10 litres 63 mm closure		
PPE during mix/loading	None	PPE during application	None
Dose	**1.25** l/ha	Work rate/day	**50** ha
Application volume	**100** litres spray/ha	Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	10	litres
Hand contamination/operation	0.05	ml
Application dose	1.25	litres product/ha
Work rate	50	ha/day
Number of operations	7	per day
Hand contamination	0.35	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.35	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	100	litres spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	5%	15%	
Dermal exposure	6.5	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	ml/day		

ABSORBED DERMAL DOSE

	Mixing/loading		Spray application	
Dermal exposure	0.35	ml/day	41.55	ml/day
Concen. of a.i. in product or spray	200	mg/ml	2.5	mg/ml
Dermal exposure to a.i.	70	mg/day	103.875	mg/day
Percent absorbed	3	%	7	%
Absorbed dose	**2.1**	**mg/day**	**7.271**	**mg/day**

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.i. in spray	2.5	mg/ml
Inhalation exposure to a.i.	0.15	mg/day
Percent absorbed	100	%
Absorbed dose	**0.15**	**mg/day**

PREDICTED EXPOSURE

Total absorbed dose	9.52	mg/day
Operator body weight	60	kg
Operator exposure	0.159	mg/kg bw/day
Total exposure (% AOEL)	**1058%**	

Appendix 7.2-3: Pyraclostrobin: BBA model estimations for tractor mounted boom sprayer application with PPE during mixing/loading and application

Product:	BAS 500 06 F		**Formulation type:**	EC	
Active ingredient:	Pyraclostrobin		**Concentration:**	200.0	g/L
AOELsys:	0.015	mg/kg bw/day	**Assessment factor:**	100	
Maximum Rate:	0.250	kg a.i. per ha	**Area treated per day:**	20	ha
Amount of a.i. handled / day:	5.00	kg a.i. per day	**Dermal absorption (M/L):**	3.0%	
Application technique	tractor-mounted boom sprayers with hydraulic nozzles, field crop		**Dermal absorption (Spray):**	7.0%	
Personal protective equipment:	gloves during mixing/loading and gloves and coverall and sturdy footwear during application				

$D_{M(H)}$ =	2.4	mg / person x kg a.i.	x	5.0	x	1%	=	0.120	mg / person / day
$D_{A(H)}$ =	0.38	mg / person x kg a.i.	x	5.0	x	1%	=	0.019	mg / person / day
$D_{A(B)}$ =	1.6	mg / person x kg a.i.	x	5.0	x	5%	=	0.400	mg / person / day
$D_{A(C)}$ =	0.06	mg / person x kg a.i.	x	5.0			=	0.300	mg / person / day
I_M =	0.0006	mg / person x kg a.i.	x	5.0			=	0.003	mg / person / day
I_A =	0.001	mg / person x kg a.i.	x	5.0			=	0.005	mg / person / day

			External exposure		Abs. factor		Systemic exposure	
Inhalation	mix/load	I_M =	0.0030	x	100%	=	0.003	mg / person / day
	spray	I_A =	0.0050	x	100%	=	0.005	mg / person / day
Dermal	mix/load	D_M =	0.1200	x	3.0%	=	0.004	mg / person / day
	spray	D_A =	0.7190	x	7.0%	=	0.050	mg / person / day

Total exposure (assuming person weighing 70 kg):	=	**0.06**	**mg / person / day**
Total exposure (mg/kg bw/day):	=	**0.0009**	**mg/kg bw/day**
Total exposure (% AOEL):	=	**6%**	

Appendix 7.2-4: Pyraclostrobin: UK POEM estimations for tractor mounted boom sprayer application with PPE during mixing/loading and application

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles		
Product	**BAS 500 06 F**	Active ingredient	**Pyraclostrobin**
Formulation type	organic solvent-based	a.i. concentration	**200.0** mg/ml
Dermal absorption from product	**3** %	Dermal absorption from spray	**7.0** %
Container	10 litres 63 mm closure		
PPE during mix/loading	gloves	PPE during application	gloves
Dose	**1.25** l/ha	Work rate/day	**50** ha
Application volume	**100** litres spray/ha	Duration of spraying	**6** h

EXPOSURE DURING MIXING AND LOADING

Container size	10	litres
Hand contamination/operation	0.05	ml
Application dose	1.25	litres product/ha
Work rate	50	ha/day
Number of operations	7	per day
Hand contamination	0.35	ml/day
Protective clothing	gloves	
Transmission to skin	10	%
Dermal exposure to formulation	0.035	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	100	litres spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	gloves	Permeable	Permeable	
Penetration	10%	5%	15%	
Dermal exposure	0.65	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	6.45	ml/day		

ABSORBED DERMAL DOSE

	Mixing/loading		Spray application	
Dermal exposure	0.035	ml/day	6.45	ml/day
Concen. of a.i. in product or spray	200	mg/ml	2.5	mg/ml
Dermal exposure to a.i.	7	mg/day	16.125	mg/day
Percent absorbed	3	%	7	%
Absorbed dose	**0.21**	**mg/day**	**1.12875**	**mg/day**

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.i. in spray	2.5	mg/ml
Inhalation exposure to a.i.	0.15	mg/day
Percent absorbed	100	%
Absorbed dose	**0.1500**	**mg/day**

PREDICTED EXPOSURE

Total absorbed dose	1.49	mg/day
Operator body weight	60	kg
Operator exposure	0.025	mg/kg bw/day
Total exposure (% AOEL)	**165%**	

Appendix 7.2-5: Agronomic conditions of the surrogate operator exposure study data

Study	Operator No.	Treated area [ha]	Treatment duration [h]	Treated area [ha/h]	Tank capacity [L]	Nozzle type	Spray boom width [m]	Application volume [L/ha]
Epoxiconazole in BAS 601 KD F DocID 2007/103389	1	48.6	3.80	12.8	4000	IDK 04	21	160
	2	49.4	3.17	15.6	4400	Twinfluid 035	30	100
	3	49.8	4.15	12.0	4400	Twinfluid 035	30	160
	4	48	5.38	8.9	2000	Air Jet 035	18	100
	5	51.9	2.67	19.5	4400	ID 120 02/03	36	125
	6	50	2.92	17.1	3000	IDN 025	24	120
	7	51	2.42	21.1	5000	IDK 04	36	180
	8	50	3.17	15.8	4000	IDN 120 025	30	100
	9	50	2.52	19.9	5200	ID 120 03	36	170
	10	50	2.70	18.5	4000	ID 120 04	27	160
Epoxiconazole in BAS 480 31 F DocID 2010/1089364	1	50.3	4.43	11.4	3000	Lechler flat fan IDK 120-03 (anti-drift)	24	99
	2	50	3.42	14.6	3600	Bubble jet 03 ABJ (anti-drift)	24	100
	3	50.6	3.17	16.0	2000	Flat fan 120-025 (standard)	24	98.8
	4	48.6	3.72	13.1	2500	Flat fan 110 03 VK (low drift)	24	100.2
	5	50	3.17	15.8	2500	Flat fan 110 03 VK (low drift)	24	99.9
	6	50	3.75	13.3	2000	Lurmark 110-03 (standard)	18	100
	7	50.6	5.27	9.6	2000	Flat fan 110/1.0/3 (low drift)	20	98.8
	8	50.6	3.37	15.0	3000	Lechler flat fan IDK 120-03 (low drift)	24	98.8
	9	51.3	3.13	16.4	2000	Flat fan 110-04 (standard)	24	97.5
	10	49.8	7.60	6.6	2200	Air jet 23216 (low drift)	18	100.4
	11	48.3	3.80	12.7	4000	Twin fluid 35 (low drift)	30	101
	12	48	3.78	12.7	3200	Lechler flat fan IDN 120-025	24	104.2
	13	48	3.63	13.2	3200	Lechler flat fan IDN 120-025	24	104.2
	14	52.2	3.05	17.1	4000	Lechler ID 02 (low drift)	30	95.8
	15	55.4	3.33	16.6	4000	Lechler ID 02 (low drift)	30	90.2
	Geometric Mean	50.07	3.54	14.16	3191		25	112
	75th Percentile	50.60	3.80	16.64	4000		30	120
	Minimum	48.00	2.42	6.55	2000		18	90
	Maximum	55.40	7.60	21.10	5200		36	180

Appendix 7.2-6: Study data standardized to 1 g a.s./ha and treatment of 1 ha/day and normalized to individual body weight

Study	Operator No.	Body Weight	Treated Area	Sum (outer top + bottom)	Glove (outer)	Sum (outer) + glove (outer)	Sum (inner top + bottom)	Head	Hand (inner)	Sum (inner) + head + hand	Inhalation
		[kg]	[ha]	[µg/kg bw/day]							
Epoxiconazole in BAS 601 KD F DocID 2007/1033389	1	86	48.6	0.000130	0.005798	0.005928	0.000036	0.000001	0.000025	0.000062	0.0000006
	2	87	49.4	0.000270	0.008786	0.009056	0.000004	0.000001	0.000003	0.000008	0.0000028
	3	90	49.8	0.000166	0.001092	0.001258	0.000004	0.000000	0.000002	0.000006	0.0000005
	4	96	48	0.000844	0.005197	0.006042	0.000006	0.000001	0.000014	0.000020	0.0000002
	5	85	51.9	0.000115	0.005023	0.005138	0.000002	0.000000	0.000004	0.000006	0.0000001
	6	96	50	0.001284	0.014741	0.016024	0.000007	0.000001	0.000170	0.000179	0.0000004
	7	110	51	0.000045	0.001266	0.001310	0.000006	0.000000	0.000003	0.000010	0.0000001
	8	100	50	0.001146	0.013311	0.014457	0.000007	0.000002	0.000005	0.000013	0.0000002
	9	85	50	0.002474	0.006952	0.009426	0.000017	0.000001	0.000111	0.000129	0.0000001
	10	95	50	0.000059	0.000663	0.000723	0.000002	0.000000	0.000001	0.000004	0.0000001
Epoxiconazole in BAS 480 31 F DocID 2010/1089364	1	85.1	50.3	0.000812	0.001119	0.001931	0.000002	0.000000	0.000016	0.000019	0.0000005
	2	119.3	50	0.005662	0.015474	0.021136	0.000023	0.000001	0.000189	0.000213	0.0000001
	3	80.9	50.6	0.001277	0.004659	0.005936	0.000004	0.000001	0.000015	0.000020	0.0000003
	4	123.8	48.6	0.000314	0.002401	0.002715	0.000011	0.000001	0.000069	0.000081	0.0000005
	5	95	50	0.000476	0.002051	0.002527	0.000004	0.000000	0.000022	0.000027	0.0000001
	6	137.9	50	0.000620	0.000378	0.000998	0.000003	0.000000	0.000015	0.000018	0.0000001
	7	102.1	50.6	0.000445	0.007175	0.007620	0.000003	0.000000	0.000006	0.000009	0.0000007
	8	104.3	50.6	0.004009	0.001799	0.005808	0.000016	0.000000	0.000020	0.000036	0.0000004
	9	145.1	51.3	0.000422	0.000854	0.001276	0.000002	0.000000	0.000004	0.000005	0.0000104
	10	100.4	49.8	0.000237	0.004392	0.004629	0.000021	0.000001	0.000025	0.000047	0.0000009
	11	91	48.3	0.000252	0.001292	0.001544	0.000002	0.000000	0.000061	0.000063	0.0000014
	12	102.1	48	0.000346	0.006452	0.006798	0.000003	0.000001	0.000019	0.000022	0.0000008
	13	102.2	48	0.001697	0.114611	0.116308	0.000010	0.000001	0.000031	0.000041	0.0000005
	14	101.9	52.2	0.009813	0.043770	0.053583	0.000012	0.000074	0.001369	0.001455	0.0000003
	15	71.7	55.4	0.004129	0.075864	0.079993	0.000007	0.000007	0.000451	0.000466	0.0000003
	Geometric Mean	98.42	50.07	0.0006026	0.0046066	0.0057507	0.0000059	0.0000006	0.0000205	0.0000320	0.0000004
	75th Percentile	102.2	50.60	0.0012836	0.0087865	0.0094265	0.0000109	0.0000009	0.0000608	0.0000633	0.0000006
	Minimum	71.7	48.00	0.0000446	0.0003784	0.0007226	0.0000018	0.0000001	0.0000015	0.0000043	0.0000001
	Maximum	145.1	55.40	0.0098132	0.1146105	0.1163078	0.0000362	0.0000745	0.0013686	0.0014547	0.0000104

Appendix 7.2-7: Summary of data standardized to 1 g a.s./ha and 1 ha/day according to different PPE level (data normalized to individual body weight)

Study	Operator No.	Treated Area	Dermal exposure		Inhalative exposure
			Scenario 1	Scenario 2	
			no PPE	complete PPE	no PPE
			Sum (inner) + hand wash + face/neck wipes (head) + 50% sum (outer) + gloves	Sum (inner) + hand wash + face/neck wipes (head)	
		[ha]	[µg/kg bw when applying 1 g a.s. on 1 ha/day]		
Epoxiconazole in BAS 601 KD F DocID 2007/103389	1	48.6	0.0060	0.000062	0.00000058
	2	49.4	0.0091	0.000008	0.00000281
	3	49.8	0.0013	0.000006	0.00000051
	4	48	0.0061	0.000020	0.00000024
	5	51.9	0.0051	0.000006	0.00000010
	6	50	0.0162	0.000179	0.00000040
	7	51	0.0013	0.000010	0.00000007
	8	50	0.0145	0.000013	0.00000023
	9	50	0.0096	0.000129	0.00000012
	10	50	0.0007	0.000004	0.00000007
Epoxiconazole in BAS 480 31 F DocID 2010/1089364	1	50.3	0.0019	0.000019	0.00000049
	2	50	0.0213	0.000213	0.00000009
	3	50.6	0.0060	0.000020	0.00000028
	4	48.6	0.0028	0.000081	0.00000048
	5	50	0.0026	0.000027	0.00000008
	6	50	0.0010	0.000018	0.00000009
	7	50.6	0.0076	0.000009	0.00000067
	8	50.6	0.0058	0.000036	0.00000042
	9	51.3	0.0013	0.000005	0.00001044
	10	49.8	0.0047	0.000047	0.00000090
	11	48.3	0.0016	0.000063	0.00000139
	12	48	0.0068	0.000022	0.00000077
	13	48	0.1163	0.000041	0.00000055
	14	52.2	0.0550	0.001455	0.00000027
	15	55.4	0.0805	0.000466	0.00000029
	Geometric Mean	48.00	0.006	0.000022	0.0000004
	75th Percentile	51.90	0.0096	0.000063	0.00000058
	Minimum	48.00	0.0007	0.0000	0.0000001
	Maximum	55.40	0.1163	0.0015	0.0000104

Appendix 7.2-8: Pyraclostrobin: Estimates for tractor mounted ground-boom application to field crops based on operator exposure study data without PPE (Scenario 1)

Product:	**BAS 500 06 F**				Formulation type	**EC**	
Active ingredient:	**pyraclostrobin**				AOEL of a.s.	**0.015**	mg/kg bw/day
a.i. concentration:	**200**	g a.s./l product					
Dose rate:	**1.25**	l product/ha			Dermal absorption of spray dilution	**7%**	
Use rate:	**0.25**	kg a.s./ha			PPE during M&L	**None**	
Work rate:	**50**	ha/day			PPE during application	**None**	

		Calculation					**Exposure estimate**
		specific exposure	**x**	**use rate**	**x**	**work rate**	
Task	**Route of exposure**	[mg/(kg a.s. handled*kg bw)]		[kg a.s./ha]		[ha/day]	[mg/kg bw/day]
Mixing / loading & application*	Inhalation	0.00000058	x	0.25	x	50	0.0000073
	Dermal (hands)	0.0096	x	0.25	x	50	0.12

		Mixing/Loading and Application Exposure	
		Dermal	Inhalation
Route specific exposure [mg/kg bw/day]		0.12	0.0000073
Dermal absorption / inhalation absorption [%]		7%	100%
Route specific systemic dose [mg/kg bw/day]		0.0084	0.0000073
Total systemic exposure [mg/kg bw/day]	**0.00841**		
Percentage of AOEL [%]	**56%**		
***Based on 75th percentile estimates**			

Appendix 7.2-9: Pyraclostrobin: Estimates for tractor mounted ground-boom application to field crops based on operator exposure study data with complete PPE (Scenario 2)

Product:	**BAS 500 06 F**			Formulation type	**EC**	
Active ingredient:	**pyraclostrobin**			AOEL of a.s.	**0.015**	mg/kg bw/day
a.i. concentration:	**200**	g a.s./kg product				
Dose rate:	**1.25**	l product/ha		Dermal absorption of spray dilution	**7%**	
Use rate:	**0.25**	kg a.s./ha		PPE during M&L	**gloves during mixing/loading**	
Work rate:	**50**	ha/day		PPE during application	**Gloves and working clothing**	

		Calculation					**Exposure estimate**
		specific exposure	**x**	**use rate**	**x**	**work rate**	
Task	**Route of exposure**	[mg/(kg a.s. handled*kg bw)]		[kg a.s./ha]		[ha/day]	[mg/kg bw/day]
Mixing / loading & application	Inhalation	0.00000058	x	0.25	x	50	0.0000073
	Dermal (hands)	0.000063	x	0.25	x	50	0.000788

		Mixing/Loading and Application Exposure	
		Dermal	Inhalation
Route specific exposure [mg/kg bw/day]		0.000788	0.0000073
Dermal absorption / inhalation absorption [%]		7%	100%
Route specific systemic dose [mg/kg bw/day]		0.00004	0.0000073
Total systemic exposure [mg/kg bw/day]	**0.000047**		
Percentage of AOEL [%]	**0.31%**		
***Based on 75th percentile estimates**			

Appendix 7.2-10: Estimated exposure and risk assessment for bystanders exposed to pyraclostrobin during the product application of BAS 500 06 F

Product:	BAS 500 06 F	**Active ingredient:**	pyraclostrobin
Crop:	field crop	**AOELsys:**	0.015 mg/kg bw/day
Max. application rate (AR):	0.25 kg/ha	**Dermal absorption spray (DA):**	7.0%
Max. application rate (ar):	25.0 mg a.i./m²	**Inhalation absorption (IA):**	100%
Area treated (A):	50 ha		
Drift deposition at 10-m distance (D):	2.77 % of application rate		
Exposure duration operator during spraying (TO):	6 h		
Exposure duration bystander (TB):	5 min		
Exposure duration factor (TF = TB / TO):	0.014		

		Adults	**Children**
Exposed body surface area (BSA):		1 m²	0.21 m²
Specific inhalation exposure operator (IA*):		0.001 mg/kg a.i.	0.00057 mg/kg a.i
Respiration rate (R):		1.74 m³/h	1 m³/h
Body weight:		60 kg	16.15 kg
		mg / person / day	mg / person / day
External exposure of bystanders via the dermal route			
ar x D x BSA	=	0.6925	0.1452
External exposure of bystanders via the inhalative route			
IA* x AR x A x TF	=	0.0002	0.0001
		mg / kg bw / day	mg / kg bw / day
Systemic exposure via the dermal route (SDE_B)			
(ar x D x BSA x DA) / BW	=	0.00081	0.00063
Systemic exposure via the inhalation route (SIE_B)			
(IA* x AR x A x TF) / BW	=	0.000003	0.000006
Total systemic exposure (SE_R)		**Adults** mg / kg bw / day	**Children** mg / kg bw / day
$= SDE_B + SIE_B$	=	0.000813	0.000635
% AOEL	=	**5.42%**	**4.23%**

According to the bystander exposure model proposed by Martin et al. 2008

Appendix 7.2-11: Estimated exposure and risk assessment for residents exposed to pyraclostrobin during the product application of BAS 500 06 F

Product:	BAS 500 06 F	**Active ingredient:**	pyraclostrobin
Crop:	field crop	**AOELsys:**	0.015 mg/kg bw/day
Applications per season:	2		
Max. application rate (x2):	0.5 kg a.i./ha	**Oral absorption (OA):**	50%
Max. application rate (Ar):	0.0050 mg a.i./cm²	**Dermal absorption spray (DA):**	7.0%
Drift deposition at 1-m distance (D):		**Inhalation absorption (IA):**	100%
2.38% of application rate	(82nd percentile)	**Vapour pressure:**	2.60E-08 Pa
Dislodgeable foliar residue (DFR):	20%	**Volatility of pyraclostrobin:**	non-volatile
Turf Transferable Residues (TTR):	5%	**Airborne vapour conc. (ACv):**	0 mg/m³

		Adults	Children
Duration of exposure			
- dermal (H):		2 h	2 h
- inhalation:		24 h	24 h
- mouthing (H):		----	2 h
Transfer Coefficient (TC):		7300 cm²/h	2600 cm²/h
Body weight (BW):		60 kg bw	16 kg bw
Inhalation rate (IR):		16.6 m³/day	8.3 m³/day
Saliva extraction factor (SE):		----	50%
Hand surface area (SA):		----	20 cm²
Hand-to-mouth frequency (Freq):		----	20 events/h
Ingestion rate for mouthing of grass/day (IgR):		----	25 cm²
		mg / person / day	mg / person / day
External exposure of residents via the dermal route			
Ar x D x TTR x TC x H	=	0.087	0.031
External exposure of residents via the inhalative route			
ACv x IR (a.i. is considered non-volatile)	=	0.0000	0.0000
Exposure via hand to mouth route			
Ar x D x TTR x SE x SA x Freq x H	=	----	0.002
Exposure via object to mouth route			
Ar x D x DFR x IgR	=	----	0.001
		mg / kg bw / day	mg / kg bw / day
Systemic exposure via the dermal route (SDE_R)			
(Ar x D x TTR x TC x H x DA) / BW	=	0.00010	0.00013
Systemic exposure via the inhalation route (SIE_R)			
(ACv x IR x IA) / BW	=	0.000000	0.000000
Systemic exposure via hand-to-mouth (SOE_H)			
(Ar x D x TTR x SE x SA x Freq x H x OA) / BW	=	----	0.000063
Systemic exposure via object-to-mouth (SOE_O)			
(Ar x D x DFR x IgR x OA) / BW	=	----	0.000031
Total systemic exposure (SE_R)		**Adults**	**Children**
		mg / kg bw / day	mg / kg bw / day
$= SDE_R + SIE_R$	=	0.0001	----
$= SDE_R + SIE_R + SOE_H + SOE_O$	=	----	0.00022
% AOEL	=	**0.7%**	**1.5%**

According to the bystander exposure model proposed by Martin et al. 2008

Appendix 7.2-12: Estimated exposure and risk assessment for an unprotected worker exposed to pyraclostrobin from the product BAS 500 06 F (standard assessment)

Product:	BAS 500 06 F	**Active ingredient:**		Pyraclostrobin	
Maximum application rate (MR):	0.5 kg a.i. per ha	**AOELsys:**		0.015	mg/kg bw/day
Crop:	Cerealst	**Assessment factor:**		100	
Growth stage BBCH:	*29-69)*	**Dermal absorption (DF):**		7%	
Activity:	Cereal: scouting and crop inspection	**Working duration (A):**		2	hours/day
		Worker bodyweight:		60	kg
Dislodgeable Foliar Residue (DFR):	0.003 mg/cm²	**Transfer coefficient (TC):**		1,500	cm²/hour
Protective clothing:	**None**	**Transmission to skin (TR):**		100%	
Dermal exposure (mg/person/d):		MR x A x DFR x TC x TR	=	4.5	mg/person/day
Systemic exposure (mg/person/d):		MR x A x DFR x TC x TR x DF	=	0.315	mg/person/day
Total systemic exposure (assuming person of 60 kg):				**0.005251**	**mg/kg bw/day**
Total exposure (% AOEL):				**35%**	

Based on the Europoem - Re-entry exposure model final draft adopted by more specific US EPA agricultural transfer coefficients.

CP 7.3 Dermal absorption

Report: CP 7.3/1
Fabian E.,Landsiedel R., 2010a
^{14}C-BAS 500 F in BAS 500 06 F - Study of penetration through human skin in vitro
2010/1059865

Guidelines: OECD Guideline for testing of chemicals No. 428 (Skin absorption: In vitro method (2004)), OECD Guidance Document No. 28 for the conduct of skin absorption studies (March 2004)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This reference was erroneously not included in the study list of the application. It is submitted, because the proposed dermal absorption value for the formulation concentrate is based on this study.

Executive Summary

In an in-vitro experiment, the dermal penetration of pyraclostrobin (BAS 500 F) formulated as BAS 500 06 F through human skin was determined. For this a high dose (formulation concentrate; nominal dose 2000 µg pyraclostrobin per cm²) and a low dose (1:200 spray dilution; 10 µg pyraclostrobin per cm²) was applied to human dermatomed skin at a volume of 10 µL/cm². The skin was mounted into Franz-type diffusion cells operated in static mode. The exposure of the skin to the test material lasted 6 hours; thereafter the skin was thoroughly washed. Samples of the receptor fluid were taken 0.5, 1, 2, 4, 6, 10 and 24 hours after the start of the exposure in order to determine kinetic parameters (lag phase, absorption rate and permeability constant).

The in-vitro dermal absorption of ^{14}C-pyraclostrobin formulated as BAS 500 06 F through human skin as determined in this study was low. The absorption estimates (sum of the absorbed dose and the remaining dose associated to the skin membrane and the 3rd to 6th tape strip) for pyraclostrobin were 1.64 ± 1.12% for the formulation concentrate and 4.77 ± 1.35% of the 1:200 spray dilution. The dermal penetration estimates to be used for risk assessment are based on the results of this as well as the results of a 2014 study summarized below (M-CP 7.3/2).

Considering both in-vitro dermal penetration studies, the dermal penetration estimates to be used for risk assessment were therefore calculated to be 3 and 7% for the formulation concentrate and the spray dilution (1:400), respectively. The value for the formulation concentrate was derived from this study, whereas the value for the 1:400 spray dilution was derived from the 2014 study (see M-CP 7.3/2).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

	a) ^{14}C-BAS 500 F (chlorophenyl-U-C14)
	b) BAS 500 06 F (formulated BAS 500 F)
Description:	a) not indicated
	b) not indicated
Lot/Batch #:	a) 579-2301
	b) 8265
Purity:	a) radiochemical purity: 99.4%; specific activity: 4.38 MBq/mg
	b) Content: 202.7 g pyraclostrobin per liter
Stability of test compound:	a) The storage stability of the test substance was confirmed by HPLC analysis prior to and after termination of the study
	b) The test substance is stable in the formulation; the expiry date of the formulation: 31-Dec-2010.

2. Vehicle: tap water

3. Human skin preparations:

Source:	BIOPREDIC International, Rennes, France
Preparation:	Dermatomed (split thickness) human skin membranes with a thickness of 390-420 µm were prepared and supplied frozen by BIOPREDIC International.
Storage of skin samples:	Human skin samples were stored vacuum-wrapped at -20°C until use.

4. Reagents:

Receptor fluid:	Ethanol/tap water (1:1 (v/v)) for the high dose (formulation concentrate) Ethanol/tap water (2:8 (v/v)) for the low dose (spray dilution) The solubility of pyraclostrobin was determined analytically in several mixtures of ethanol/tap water. It was for the low dose 8.2 mg/L in ethanol/tap water (2:8 (v/v) and at a maximum of 2710 mg/L in ethanol/tap water (1:1 (v/v)). The maximum solubility of the test substance in water is 0.41 mg/L.
Extraction media:	Soluene®-350 and ethanol
Washing solution:	Texapon® N 70 (sodium-laurylethersulfate), 1:140 w/w in bi-distilled water

5. Preparation of the dosing solutions

High dose (undiluted formulation):
The applied radioactivity was 37 kBq/cm² for the formulation concentrate. This corresponds to a desired specific radioactivity of the test substance preparation of 3.7 MBq/g. To obtain the desired specific activity, a respective aliquot of the radio-labeled stock solution in toluene was taken and the organic solvent was evaporated to dryness. The dried residue was taken up in the required amount of liquid formulation concentrate. The preparations were stirred and sonicated in order to ensure homogeneity.

Low dose (1:200 spray dilution):
Due to the low concentration of active ingredient in the use dilution (1:200 dilution of the formulation concentrate), an applied radioactivity of about 10 kBq/cm² was achieved. This corresponds to a desired specific radioactivity of the test substance preparation of 1 MBq/g. To obtain the desired specific activity, a respective aliquot of the radio-labeled test-substance solution was taken and the organic solvent was evaporated. The required amount of the liquid formulation concentrate was added to the residual radio-labeled test substance. This mixture was filled up to the final volume with the required volume of tap water. The preparations were stirred and sonicated in order to produce homogeneity.

Analyses:
Liquid scintillation counting and/or HPLC verified the homogeneity and accuracy of the test substance preparations. Taking and analyzing samples before and after the application period confirmed the stability of the test-substance in the preparation.

The conditions of the HPLC analysis are described in the table below:

Column:	J'sphere ODS-H80 4µm, 250 mm x 4.6 mm
Eluent:	A: acetonitrile + formic acid (1 mL/L) B: highly de-ionized water + formic acid (1 mL/L)
Gradient:	0 – 10 min: 30% B 10 – 15 min: 30% – 10% B 15 – 25 min: 10% B 25 – 26 min: 30% – 10% B 26 – 35 min: 30% B
Flow:	1.2 mL/min
Wavelength:	230 nm
Detection:	Agilent 1100 with variable wavelength detector, Radioflow Detector Berthold LB 509 (Cell: YG 75 – S6M)

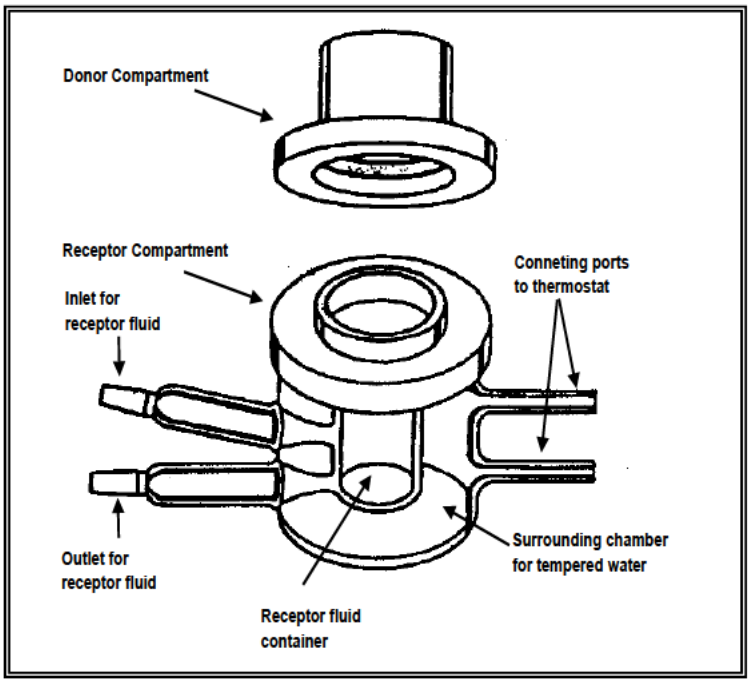
B. TEST PERFORMANCE

1. Dates of experimental work: 23-Mar-2010 - 25-May-2010

2. Study design:

The penetration of pyraclostrobin through human dermatomed skin was determined using a modified Franz cell (see Figure 7.3-1). The study was carried out in consecutive steps using a maximum of 10 skin preparations per step.

Figure 7.3-1: Schematic depiction of a Franz cell



After introducing the skin preparation, the upper donor compartment and the lower receptor compartment were assembled and fixed with a stainless steel clamp and four screws. Specifically, the receptor compartment was filled with the physiological saline (for the assessment of skin integrity). Subsequently, the skin was placed with the external surface of the stratum corneum upmost on the fluid avoiding air bubbles underneath. The donor compartment was carefully placed on top so that the skin sample was stretched between the donor and receptor compartments.

The receptor compartment was jacketed with a second chamber to maintain the receptor fluid at the 32 ± 1°C with the aid of thermostated water circulating in the surrounding second chamber. The exposed skin area was about 1 cm^2 and the receptor volume was about 4 ml. Mixing of receptor fluid was provided by magnetic stirrers.

The diffusion cells were operated in a static mode. Sampling and replacement of removed receptor fluid was carried out by outlet (bottom) and inlet (top) pipe connections. The inlet ports were connected to a receptor fluid stock container, the outlet ports to an automated fraction sampler.

Before the actual penetration experiment, the integrity of the skin preparations was determined by measuring electrical resistance. For intact skin preparations using physiological saline electrical resistances in the range of > 1 kΩ were expected. In addition, the skin preparations integrity was checked by visual inspection. In case of low resistance skin preparations were used unless the visual check revealed any damage.

The following findings were reasons for not using a skin preparation in the study or rejecting results from calculation of the mean penetration parameters:

- A skin preparation showed low resistance and physical damage or leakage of receptor medium to the surface.
- A skin preparation displayed clearly aberrant test substance recovery rates (according to the guidelines of OECD a total recovery per membrane of 100 ± 10% is considered to be acceptable).
- A membrane displayed clearly aberrant penetration kinetics.

The test substance preparation was applied to the skin preparations using a displacement pipette. The exact amount applied per diffusion cell was determined by reweighing the pipette after application, the weight of which had been previously determined after filling. The application time was documented for each cell. The donor compartment was covered with a sheet of semiocclusive adhesive fleece (Fixomull® Stretch, Beiersdorf AG). After 6 hours the skin surface was washed twice using approx. 250 µL Texapon® N70 diluted 1:140 (w/w) in highly de-ionized water and once with 250 µL pure water. The skin was then wiped dry using cotton swabs. The rinsing fluids, the pipette tips and the cotton swabs were stored for analysis. The upper chamber was covered again with the fleece until the end of the run.

Samples of the receptor fluid were taken 0.5, 1, 2, 4, 6, 10 and 24 hours after application. For this a volume of about 0.6 ml was removed from the receptor cell within 60 seconds by means of a peristaltic pump connected to the outlet port of the receptor cell (see above). The removed volume was hydrostatically replaced with fresh receptor fluid from the reservoir via the inlet port.

The sampling was divided into a 30-second flush period to remove receptor medium remaining in the tubes from the previous sampling, and an actual 30-second sampling period for determination of test-substance concentration in the receptor medium. The void volumes were pooled per diffusion cell and the actual sample volumes were collected in separate vessels.

After the last sampling of receptor fluid, the feed of the receptor medium was stopped and the contents of the individual receptor compartments was sampled by emptying the receptor compartments with the fraction collector. The receptor chamber was washed and the skin was removed from the diffusion cell, put onto aluminum foil and pinned onto a Styrofoam support. The skin surface was washed using cotton swabs soaked with afore mentioned washing fluid and dried using an untreated swab. When the skin surface had dried, the stratum corneum was removed by tape stripping. The tapes were pooled into two samples (1st and 2nd tape strip (sample 1) and 3rd to 6th tape strip (sample 2)) for analysis. The remaining skin and the tape strips were analyzed separately.

The diffusion cells were dismantled and all parts (with the exception of the stainless steel clamp and tubes) were extracted in ethanol.

Weighed aliquots of each native receptor fluid sample or of the solvents used for extraction were analyzed by liquid scintillation counting for 10 min.

The cumulative absorbed dose [µg/cm²] was presented graphically over the time. The absorption rate [µg/(cm²·h)] was determined from the slope of the penetration curve in the linear region by means of linear regression, and the lag phase [h] of the test substance penetration was calculated (intersection point of the regression line with the x-axis). The permeability constant Kp [cm/h] was determined by dividing the absorption rate by the concentration of the applied test substance.

In this summary the recovery rate of the test substance applied in the various compartments was determined as non-absorbed fraction (donor compartment wash and the dislodgeable test substance fraction at the first and second skin wash), the dose associated to the skin (1st and 2nd tape strip, 3rd to 6th tape strip and the remaining skin preparation) and the absorbed fraction (contents of the receptor compartment, receptor compartment wash and receptor fluid samples taken).

II. RESULTS AND DISCUSSION

A. STABILITY, HOMOGENEITY AND CONTENT OF PYRACLOSTROBIN IN THE APPLICATION MEDIUM

The stability, homogeneity and content of pyraclostrobin in the application medium were confirmed by analysis. Details are available in the raw-data.

B. APPLIED DOSE AND RECOVERY OF DOSE

The applied doses and the number of usable skin samples per dose group are given in Table 7.3-1.

Table 7.3-1: Applied doses

Dose group	Target dose [µg/cm²]	Actual nominal dose [µg/cm²]
1 (concentrate)	2000	2002 ± 64 (n=4/5) [a]
2 (1:200 spray dilution)	10	10.8 ± 0.2 (n=5/5)

[a] (number of valid cells/total number of cells); cell no 4 was excluded from calculation due to an invalid first skin wash (58.2% recovery compared to 84.6 ±9.9% for the remaining 4 cells)

The mean recoveries were 100.3 and 98.7% for the high and the low dose levels, respectively (see Table 7.3-2). All individual cell recoveries were within the OECD acceptance criteria (100 ± 10%). The individual values ranged between 96.5 and 103.1% for the high dose and 96.7 and 100.9% for the low dose. Furthermore, the group mean total recovery for all groups was > 95%, therefore no adjustment to the dermal penetration values was necessary.

Nearly the entire dose, i.e. 98.7% was recovered from the skin washing, donor chamber and first two tape strips at the high dose. In case of the cells treated with the low dose this value was 93.9%. The amount associated with the skin preparations and the 3rd to 6th skin strip was 1.42% and 3.16% for the high and low dose, respectively.

The absorbed dose, i.e. the sum of receptor samples, the receptor fluid recovered at the end of the experiment as well as the receptor chamber, amounted to 0.22 and 1.61% at the high and low doses, respectively. The absorption estimate, which corresponds to the amount recovered from skin, the tape strips 3-6 and the absorbed dose, thus, sum up to 1.64 and 4.77% for the high and low dose, respectively.

Table 7.3-2: In-vitro dermal penetration of BAS 500 F formulated as BAS 500 06 F through human skin - Recovery data

Dose group	High dose* (Formulation concentrate)		Low dose (Spray dilution 1:200)	
Target concentration [mg/mL]	200		1	
Target dose [µg/cm²]	2000		10.0	
Mean actual applied dose [µg/cm²]	2002 ± 64		10.8 ± 0.2	
Number of cells used/Valid cells	4/5		5/5	
	Recovery [%]		Recovery [%]	
	Mean	S.D.	Mean	S.D.
Unabsorbed dose				
Skin washing after 6 hours	84.6	9.9	56.0	8.4
Skin washing after 24 hours	8.62	8.03	35.5	8.2
Donor chamber	5.35	1.69	2.24	1.60
Dose associated to skin[a]				
Tape strip (1st pool, strips 1-2)	0.13	0.13	0.22	0.11
Tape strips (2nd pool; strips 3 - 6)	0.14	0.09	0.37	0.21
Skin preparation	1.28	1.06	2.79	1.07
Absorbed dose				
Sum receptor samples incl. wash out	0.02	0.00	0.22	0.10
Receptor fluid	0.18	0.07	0.80	0.37
Receptor chamber wash	0.02	0.02	0.59	0.23
Total recovery[#]	**100.3**	**2.8**	**98.7**	**2.1**
Absorption essentially complete at end of study (>75% absorption within half the study duration)	**No**		**No**	
Absorption estimates when absorption not essentially completed (= absorbed dose + dose associated to skin - tape strips 1 and 2)	1.64	1.12	4.77	1.35
Absorption estimates when absorption essentially completed (= absorbed dose + dose associated to skin - all tapes)	**n.a.**	**n.a.**	**n.a.**	**n.a.**
Absorption estimate normalized[b]	**n.a.**	**n.a.**	**n.a.**	**n.a.**
Absorption estimates used for risk assessment[c]	**3**		**6**	

* Results of one cell not used for calculation due to aberrant test substance recovery in the 1st skin wash

\# values may not calculate exactly due to rounding of figures

[a] Grouping is different than in the report: In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2012;10(4):2665) the radioactivity in the second tape-strip pool (3rd to 6th tape strip) is considered potentially absorbable if less than 75% of the absorption occurred in the first half of the study (see Table 7.3-3). Finally, the skin preparation is also considered potentially absorbable.

[b] Cells with insufficient recovery (<95%) were corrected by normalization of absorption estimate to 100% recovery.

[c] In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2012;10(4):2665) one standard deviation was added to the mean % dermal penetration in cases where the standard deviation was ≥ 25% of the mean value. This value was then rounded to the required number of significant figures.

n.a.: not applicable

The total amount of pyraclostrobin recovered in the receptor fluid after 24 hours was 4.38 and 0.173 µg for the formulation concentrate and the spray dilution, respectively. When compared to the maximum solubilisation capacity in the total receptor medium volume of 8.2 mL of 22222 µg and 67.2 µg, the solubility in the receptor media was ~5000 and ~390 fold higher than actually needed for the formulation concentrate and the spray dilution, respectively. Even if the receptor volume of 4 mL is considered only, the solubility was at least 190-fold higher than actually needed. These results show that the maximum concentration of the test substance in the receptor chamber does not exceed 10% of the saturation concentration as recommended by EFSA.

C. ABSORPTION KINETICS

The mean values of the kinetic parameters determined from the linear region of the cumulative absorbed dose curve are presented in Table 7.3-3. An absorption rate of 0.140 µg/cm²·h and a permeability coefficient (Kp) of $0.069 \cdot 10^{-5}$ cm/h was obtained for the formulation concentrate of BAS 500 06 F. At the approx. 200-fold lower concentration of applied test substance an absorption rate of 0.007 µg/cm²·h and a permeability coefficient of $0.662 \cdot 10^{-5}$ cm/h was determined. A lag time of 8.2 and 1.8 hours was determined for the formulation concentrate and the 1:200 spray dilution, respectively.

Table 7.3-3: In-vitro dermal penetration of BAS 500 F formulated as BAS 500 06 F through human skin - Penetration kinetics

Dose group	**High dose* (Formulation concentrate)**		**Low dose (Spray dilution 1:200)**	
Target concentration [mg/mL]	200		1	
Target dose [µg/cm²]	2000		10.0	
Mean actual applied dose [µg/cm²]	2002 ± 64		10.8 ± 0.2	
Number of cells used/Valid cells	4/5		5/5	
	Mean cumulative absorption		Mean cumulative absorption	
	[µg/cm²]	[%]	[µg/cm²]	[%]
Sample time [h]				
0.5	0.000	0.00	0.000	0.00
1	0.000	0.00	0.000	0.00
2	0.000	0.00	0.002	0.02
4	0.000	0.00	0.016	0.15
6	0.000	0.00	0.025	0.23
10	0.174	0.01	0.042	0.39
24	2.138	0.11	0.073	0.68
Kp[*10^{-5} cm/h]	0.069		0.662	
Absorption rate [µg/cm²·h]	0.140		0.007	
Lag time [h]	8.6		1.8	
% absorbed within 10 hours[a]	8.2		57.4	

[a] No receptor medium sample was taken after 12 hours.
* Results of one cell not used for calculation due to aberrant test substance recovery in the 1st skin wash

III. CONCLUSION

The in-vitro dermal absorption of ^{14}C-pyraclostrobin formulated as BAS 500 06 F through human skin is low. The absorption estimates (sum of the absorbed dose, the remaining dose associated to the skin membrane and the remaining test substance in the 3rd to 6th tape strip) for pyraclostrobin were 1.64 ± 1.12% for the formulation concentrate and 4.77 ± 1.35% for the 1:200 spray dilution.

Report:	CP 7.3/2 Fabian E.,Landsiedel R., 2014a ^{14}C-BAS 500 F in BAS 500 06 F - Study of penetration through human skin in vitro 2014/1001501
Guidelines:	OECD 428, OECD Guidance Document No. 28 for the conduct of skin absorption studies (March 2004)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In an in-vitro experiment, the dermal penetration of pyraclostrobin formulated as BAS 500 06 F through human skin was determined. For this a high dose (formulation concentrate; nominal dose 2000 µg pyraclostrobin per cm²) and a low dose (spray dilution; 5 µg pyraclostrobin per cm²) was applied to human dermatomed skin at 10 µL/cm². The skin was mounted into Franz-type diffusion cells operated in static mode. The exposure of the skin to the test material lasted 8 hours; thereafter the skin was thoroughly washed. Samples of the receptor fluid were taken 1, 2, 4, 6, 8, 12 and 24 hours after the start of the exposure in order to determine kinetic parameters (lag phase, absorption rate and permeability constant).

The in-vitro dermal absorption of ^{14}C-pyraclostrobin formulated as BAS 500 06 F through human skin as determined in this study was low. The absorption estimates (sum of the absorbed dose and the remaining dose associated to the skin membrane) for pyraclostrobin were 0.18 ± 0.14% for the formulation concentrate and 4.13 ± 2.67% of the 1:400 spray dilution. The dermal penetration estimates to be used for risk assessment are based on the results of this as well as the results a 2010 study summarized above (CP 7.3/1).

Considering both in-vitro dermal penetration studies, the dermal penetration estimates to be used for risk assessment were therefore calculated to be 3 and 7% for the formulation concentrate and the spray dilution (1:400), respectively. The value for the formulation concentrate was derived from the 2010 study (see M-CP 7.3/1) whereas the value for the 1:400 spray dilution was derived from this study.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

	a) ^{14}C- pyraclostrobin (tolyl-ring-U-C14)
	b) BAS 500 06 F (formulated pyraclostrobin)
	c) Blank formulation (BAS 500 06 F without pyraclostrobin)
Description:	a) not indicated
	b) not indicated
	c) not indicated
Lot/Batch #:	a) 566-5101
	b) FRE-000904
	c) FD-130726-0004
Purity:	a) radiochemical purity: 99.9%; specific activity: 6.6 MBq/mg
	b) Content: 200.2 g pyraclostrobin per liter
	c) not applicable
Stability of test compound:	a) The storage stability of the test substance was confirmed by HPLC analysis prior to and after termination of the study
	b) The test substance is stable in the formulation; the expiry date of the formulation: 31-May-2015.
	c) expiry date: 23-Jan-2018

2. Vehicle: tap water

3. Human skin preparations:

Source:	BIOPREDIC International, Saint-Grégoire, France
Preparation:	Dermatomed (split thickness) human skin membranes with a thickness of 300-400 µm were prepared and supplied frozen by BIOPREDIC International.
Storage of skin samples:	Human skin samples were stored vacuum-wrapped at -20°C until use.

4. Reagents:

Receptor fluid:	Ethanol/tap water (4:6 (v/v); pyraclostrobin solubility 600 mg/L) for the high dose (formulation concentrate)
	Ethanol/tap water (2:8 (v/v); pyraclostrobin solubility 8.2 mg/L) for the low dose (spray dilution)
Extraction media:	Soluene®-350, ethanol and ethanol/ultrapure water (90%)
Washing solution:	Texapon® N 70 (sodium-laurylethersulfate), 1:140 w/w in tap water

5. Preparation of the dosing solutions

High dose (undiluted formulation):
The applied radioactivity was 37 kBq/cm² for the formulation concentrate. This corresponds to a desired specific radioactivity of the test substance preparation of 3.57 MBq/g. To obtain the desired specific activity, about 162.4 mg of the radio-labeled stock solution in toluene (10.4 mg/g) was taken and the organic solvent was evaporate to dryness. The dried residue (~1.69 mg) was taken up in ~3.1242 g BAS 500 06 F formulation concentrate. The preparation was stirred and sonicated in order to ensure homogeneity.

Low dose (1:400 spray dilution):
Due to the low concentration of active ingredient in the use dilution (1:400 dilution of the formulation concentrate), an applied radioactivity of about 33.7 kBq/cm² was achieved. This corresponds to a desired specific radioactivity of the test substance preparation of 3.30 MBq/g. To obtain the desired specific activity, ~144.3 mg of the radio-labeled test-substance solution was taken (~ 1.5 mg ^{14}C-pyraclostrobin) and the organic solvent was evaporated. Approximate 1 g of the blank formulation was diluted with ~ 399 g tap water. From this dilution ~3.00 g was added to the residual radio-labeled test substance. The preparations were stirred and sonicated in order to ensure homogeneity.

Analyses:
Liquid scintillation counting and/or HPLC verified the homogeneity and accuracy of the test substance preparations. Taking and analyzing samples before and after the application period confirmed the stability of the test-substance in the preparation.

The conditions of the HPLC analysis are described in the table below:

Column:	Ascentis Express C18, 150 mm x 4.6 mm, 2.7µm
Eluent:	A: ultrapure water / formic acid (1 L + 1 mL) B: acetonitrile / formic acid (1 L + 1 mL) isocratic: 70% B
Flow:	0.8 mL/min
Wavelength:	225 nm
Detection:	variable wavelength detector HP 1100 serial, radioflow detector Berthold LB 509 (Cell: YG 75 – S6M)

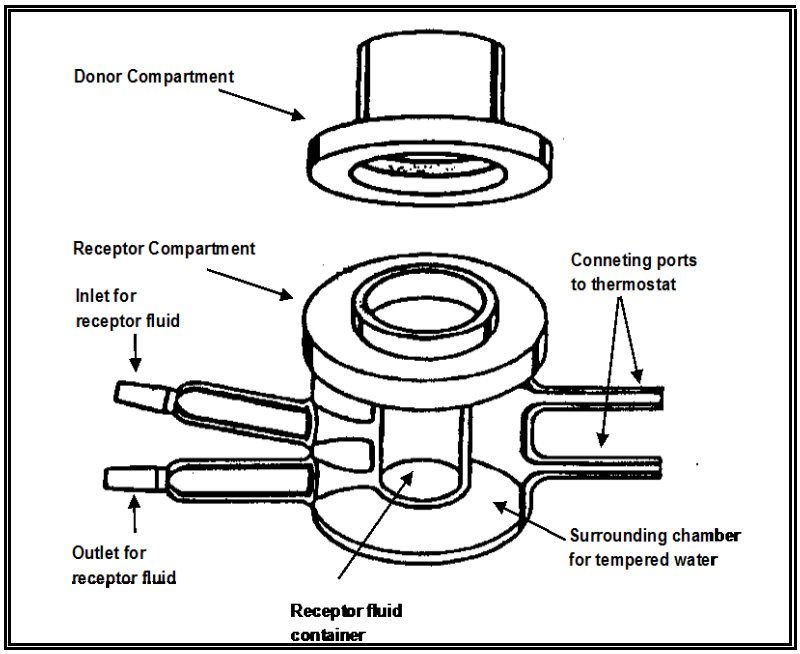
B. TEST PERFORMANCE

1. Dates of experimental work: 14-Jan-2014 - 25-Feb-2014

2. Study design:

The penetration of pyraclostrobin through human dermatomed skin was determined using a modified Franz cell (see Figure 7.3-2). The study was carried out in consecutive steps using 8 skin preparations per step.

Figure 7.3-2: Schematic depiction of a Franz cell



After introducing the skin preparation, the upper donor compartment and the lower receptor compartment were assembled and fixed with a stainless steel clamp and four screws. Specifically, the receptor compartment was filled with the physiological saline (for the assessment of skin integrity). Subsequently, the skin was placed with the external surface of the stratum corneum upmost on the fluid avoiding air bubbles underneath. The donor compartment was carefully placed on top so that the skin sample was stretched between the donor and receptor compartments.

The receptor compartment was jacketed with a second chamber to maintain the receptor fluid at the 32 ± 1°C with the aid of thermostated water circulating in the surrounding second chamber. The exposed skin area was about 1 cm² and the receptor volume was about 4 mL. Mixing of receptor fluid was provided by magnetic stirrers.

The diffusion cells were operated in a static mode. Sampling and replacement of removed receptor fluid was carried out by outlet (bottom) and inlet (top) pipe connections. The inlet ports were connected to a receptor fluid stock container, the outlet ports to an automated fraction sampler.

Before the actual penetration experiment, the integrity of the skin preparations was determined by measuring the transepidermal electrical resistance (TEER) and the transepidermal water loss (TEWL). For intact skin preparations using physiological saline TEER values in the range of > 1 kΩ and TEWL values below 15 g/m² were expected. In addition, the skin preparations integrity was checked by visual inspection. In case of low resistance skin preparations were used unless the visual check revealed any damage.
After the experiment, cells were assessed to be valid, if total recoveries generally fulfilled guideline requirements (according to the guidelines of OECD a total recovery per membrane of 100 ± 10% is considered to be acceptable) and if clearly aberrant penetration kinetics and aberrant skin wash after the 8h exposure period could be excluded.

The test substance preparation was applied to the skin preparations using a displacement pipette. The exact amount applied per diffusion cell was determined by reweighing the pipette after application, the weight of which had been previously determined after filling. In addition, so called exemplary applications were performed in parallel by pipetting 10µl of the preparations directly into scintillation vials. Samples with high specific activity were appropriately diluted. The results of these exemplary applications are not taken into account for calculations unless preliminary results indicate that (depending on e.g. the phys./chem. properties of the test substance preparations) the weights taken as described before may be unacceptable in respect of variability and the there from derived actual doses.
The application time was documented for each cell. The donor compartment was covered with a sheet of semiocclusive adhesive fleece (Fixomull® Stretch, Beiersdorf AG). After 8 hours the skin surface was washed twice using approx. 250 µL Texapon® N70 diluted 1:140 (w/w) in tap water and once with 250 µL tap water. The skin was then wiped dry using cotton swabs. The rinsing fluids, the pipette tips and the cotton swabs were stored for analysis. The upper chamber was covered again with the fleece until the end of the run.

Samples of the receptor fluid were taken 1, 2, 4, 6, 8, 12 and 24 hours after application. For this a volume of about 0.6 mL was removed from the receptor cell within 60 seconds by means of a peristaltic pump connected to the outlet port of the receptor cell (see above). The removed volume was hydrostatically replaced with fresh receptor fluid from the reservoir via the inlet port.
The sampling was divided into a 30-second flush period to remove receptor medium remaining in the tubes from the previous sampling, and an actual 30-second sampling period for determination of test-substance concentration in the receptor medium. The void volumes were pooled per diffusion cell and the actual sample volumes were collected in separate vessels.

After the last sampling of receptor fluid, the feed of the receptor medium was stopped and the contents of the individual receptor compartments was sampled by emptying the receptor compartments with the fraction collector. The receptor chamber was washed and the skin was removed from the diffusion cell, put onto aluminum foil and pinned onto a Styrofoam support. The skin surface was washed using cotton swabs soaked with afore mentioned washing fluid and dried using an untreated swab. When the skin surface had dried, the stratum corneum was removed by tape stripping by using Scotch Crystal Clear Tape 600. The tapes were pooled into two samples (1st and 2nd tape and 3rd to 6th tape) for analysis. The remaining skin and the tape strips were analyzed separately.

The diffusion cells were dismantled and all parts (with the exception of the stainless steel clamp) were extracted in ethanol.

Weighed aliquots of each native receptor fluid sample or of the solvents used for extraction were analyzed by liquid scintillation counting for 10 min.

The cumulative absorbed dose [µg/cm²] was presented graphically over the time. The absorption rate [µg/(cm²·h)] was determined from the slope of the penetration curve in the linear region by means of linear regression, and the lag phase [h] of the test substance penetration was calculated (intersection point of the regression line with the x-axis). The permeability constant Kp [cm/h] was determined by dividing the absorption rate by the concentration of the applied test substance.

In this summary the recovery rate of the test substance applied in the various compartments was determined as non-absorbed fraction (donor compartment wash and the dislodgeable test substance fraction at the first and second skin wash), the dose associated to the skin (1st and 2nd tape strip, 3rd to 6th tape strip and the remaining skin preparation) and the absorbed fraction (contents of the receptor compartment, receptor compartment wash and receptor fluid samples taken).

II. RESULTS AND DISCUSSION

A. STABILITY, HOMOGENEITY AND CONTENT OF PYRACLOSTROBIN IN THE APPLICATION MEDIUM

The stability, homogeneity and content of pyraclostrobin in the application medium were confirmed by analysis. Details are available in the raw-data.

B. APPLIED DOSE AND RECOVERY OF DOSE

The applied doses and the number of usable skin samples per dose group are given in Table 7.3-4.

Table 7.3-4: Applied doses

Dose group	Target dose [µg/cm²]	Actual nominal dose [µg/cm²]
1 (concentrate)	2000	2120 ± 27 (7/8)[a]
2 (spray dilution)	5.0	4.8 ± 0.1 (8/8)

[a] (number of valid cells/total number of cells); cell no 8 was excluded from calculation due to an invalid first skin wash (21.6% recovery compared to 94.3 ± 1.2% for the remaining 7 cells and to an invalid recovery of < 90%, i.e. 88.93%)

The mean recoveries were 94.8 and 103.3% for the high and the low dose levels, respectively (see Table 7.3-5). With regard to the high dose group, the mean total recovery was below 95% (94.80%) with individual values ranging from 93.54 to 98.33%. Six cells which displayed an insufficient recovery of < 95% were corrected by normalization of absorption estimate to 100% recovery. As group mean total recovery for the low dose group (1:400 spray dilution) was 103.32%, no adjustment of the dermal penetration values was necessary. The individual recovery was in the range of 100.23 – 107.17%.

In both dose groups absorption was essentially complete. After 12 hours 80.5 or 89.1% of the total penetrated radioactivity was recovered in the receptor media for the formulation concentrate and the spray dilution, respectively (see Table 7.3-6). Accordingly, neither the first two nor the following four tape strips were added to the absorption estimate.

Nearly the entire dose, i.e. 94.62% was recovered from the skin washing, donor chamber and the first two tape strips at the high dose. In case of the cells treated with the low dose this value was 98.55%. The amount associated with the skin preparations was 0.06 and 2.12% for the high and low dose, respectively.

The absorbed dose, i.e. the sum of receptor samples, the receptor fluid recovered at the end of the experiment as well as the receptor chamber, amounted to 0.13 and 2.65% at the high and low dose, respectively. The total absorption, which corresponds to the amount recovered from skin and the absorbed dose, thus, sum up to 0.18 and 4.13% for the high and low dose, respectively.

The total amount of pyraclostrobin recovered in the receptor fluid after 24 hours was 2.70 µg and 0.13 µg for the formulation concentrate and the spray dilution, respectively. When compared to the maximum solubilization capacity in the total receptor medium volume of 8.2 mL of 4920 µg and 67.2 µg, the solubility in the receptor media was ~1820 and 517 fold higher than actually needed for the formulation concentrate and the spray dilution, respectively. Even if the receptor volume of 4 mL is considered only, the solubility was at least 252-fold higher than actually needed. These results show that the maximum concentration of the test substance in the receptor chamber did not exceed 10% of the saturation concentration as recommended by EFSA.

Table 7.3-5: In-vitro dermal penetration of BAS 500 F formulated as BAS 500 06 F through human skin - Recovery data

Dose group	High dose* (Formulation concentrate)		Low dose (Spray dilution 1:400)	
Target concentration [mg/mL]	200		0.5	
Target dose [µg/cm²]	2000		5.0	
Mean actual applied dose [µg/cm²]	2120 ± 27		4.8 ± 0.1	
Number of cells used/Valid cells	7/8		8/8	
	Recovery [%]		Recovery [%]	
	Mean	S.D.	Mean	S.D.
Unabsorbed dose				
Skin washing after 8 hours	94.26	1.15	93.09	7.18
Skin washing after 24 hours	0.16	0.16	3.87	3.48
Donor chamber	0.18	0.41	1.03	1.89
Dose associated to skin[a]				
Tape strip (1st pool, strips 1-2)	0.02	0.02	0.56	0.46
Tape strips (2nd pool; strips 3 - 6)	0.01	0.01	0.64	0.55
Skin preparation	0.04	0.04	1.48	1.54
Absorbed dose				
Sum receptor samples incl. wash out	0.03	0.03	0.80	0.24
Receptor fluid	0.09	0.06	0.73	0.37
Receptor chamber wash	0.01	0.01	1.11	0.61
Total recovery[#]	**94.80**	**1.62**	**103.32**	**2.13**
Absorption essentially complete at end of study (>75% absorption within half the study duration)	**Yes**		**Yes**	
Absorption estimates when absorption not essentially completed (= absorbed dose + dose associated to skin - tape strips 1 and 2)	**n.a.**	**n.a.**	**n.a.**	**n.a.**
Absorption estimates when absorption essentially completed (= absorbed dose + dose associated to skin - all tapes)	**0.17**	**0.14**	**4.13**	**2.67**
Absorption estimate normalized[b]	**0.18**	**0.14**	**n.a.**	**n.a.**
Absorption estimates used for risk assessment[c]	**0.3**		**7.0**	

* Results of one cell not used for calculation due to aberrant test substance recovery in the 1st skin wash

[#] values may not calculate exactly due to rounding of figures

[a] Grouping is different than in the report: In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2012;10(4):2665) the radioactivity in the second tape-strip pool (3rd to 6th tape strip) is considered potentially absorbable if less than 75% of the absorption occurred in the first half of the study (see Table 7.3-6). Finally, the skin preparation is also considered potentially absorbable.

[b] Cells with insufficient recovery (<95%) were corrected by normalization of absorption estimate to 100% recovery.

[c] In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2012;10(4):2665) one standard deviation was added to the mean % dermal penetration in cases where the standard deviation was ≥ 25% of the mean value. This value was then rounded to the required number of significant figures.

n.a.: not applicable

C. ABSORPTION KINETICS

The mean values of the kinetic parameters determined from the linear region of the cumulative absorbed dose curve are presented in Table 7.3-6. An absorption rate of 0.49 µg/cm²·h and a permeability coefficient (Kp) of $0.25 \cdot 10^{-5}$ cm/h was observed for the formulation concentrate of pyraclostrobin. At the 400-fold lower amount of applied test substance, the absorption rate was 0.02 µg/cm²·h and an approx. 13-fold higher permeability coefficient of $3.30 * 10^{-5}$ cm/h was determined.

Table 7.3-6: In-vitro dermal penetration of BAS 500 F formulated as BAS 500 06 F through human skin - Penetration kinetics

Dose group	High dose* (Formulation concentrate)		Low dose (Spray dilution 1:400)	
Target concentration [mg/mL]	200		0.5	
Target dose [µg/cm²]	2000		5.0	
Mean actual applied dose [µg/cm²]	2120 ± 27		4.8 ± 0.1	
Number of cells used/Valid cells	7/8		8/8	
	Mean cumulative absorption		Mean cumulative absorption	
	[µg/cm²]	[%]	[µg/cm²]	[%]
Sample time [h]				
1	0.00	0.00	0.01	0.18
2	0.00	0.00	0.03	0.53
4	0.00	0.00	0.04	0.93
6	0.30	0.01	0.05	1.11
8	0.68	0.03	0.06	1.28
12	1.30	0.06	0.07	1.38
24	1.61	0.08	0.07	1.55
Kp [$*10^{-5}$ cm/h]	0.25		3.30	
Absorption rate [µg/cm²·h]	0.49		0.02	
Lag time [h]	4.73		0.80	
% absorbed within 12 hours	80.5		89.1	

* Mean values for Kp, absorption rate and lag time are calculated from cell 5-7. For cell 1-4, no meaningful kinetic values could be calculated. Cell 8 is not included in statistics due to insufficient first skin and invalid recovery wash.

III. CONCLUSION

The in-vitro dermal absorption of ^{14}C-pyraclostrobin formulated as BAS 500 06 F through human skin is low. The absorption estimates (sum of the absorbed dose and the remaining dose associated to the skin membrane) for pyraclostrobin were 0.18 ± 0.14% for the formulation concentrate and 4.13 ± 2.67% of the 1:400 spray dilution.

Considering both in-vitro dermal penetration studies, the dermal penetration estimates to be used for risk assessment were therefore calculated to be 3 and 7% for the formulation concentrate and the spray dilution (1:400), respectively. The value for the formulation concentrate was derived from the 2010 study (see M-CP 7.3/1), whereas the value for the 1:400 spray dilution was derived from the 2014 study (see M-CP 7.3/2).

CP 7.4 Available toxicological data relating to co-formulants

Confidential information - data provided in Document J.

BASF
The Chemical Company

BAS 500 06 F

DOCUMENT M-CP, Section 8

RESIDUES IN OR ON TREATED PRODUCTS, FOOD OR FEED

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

CP 8 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

Supplementary data and information on residues in or on treated products, food and feed are discussed in M-CA 6.

BASF
The Chemical Company

BAS 500 06 F

DOCUMENT M-CP, Section 9

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Table of Contents

CP 9 FATE AND BEHAVIOUR IN THE ENVIRONMENT

BAS 500 06 F is a new representative formulation, which has not been evaluated during the previous Annex I inclusion process.

CP 9.1 Fate and behaviour in soil

No studies were performed with BAS 500 06 F. The fate and behavior in soil is sufficiently addressed by information given in M-CA 7.1.

CP 9.1.1 Rate of degradation in soil

No studies were performed with BAS 500 06 F. The rate of degradation in soil is sufficiently addressed by information given in M-CA 7.1.2.

CP 9.1.1.1 Laboratory studies

No laboratory studies were performed with BAS 500 06 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.1.2.1.

CP 9.1.1.2 Field studies

No field studies were performed with BAS 500 06 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.1.2.2.

CP 9.1.1.2.1 Soil dissipation studies

No soil dissipation studies were performed with BAS 500 06 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.1.2.2.1.

CP 9.1.1.2.2 Soil accumulation studies

No soil accumulation studies were performed with BAS 500 06 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.1.2.2.2.

CP 9.1.2 Mobility in the soil

No studies were performed with BAS 500 06 F. The mobility in soil is sufficiently addressed by information given in M-CA 7.1.4.

CP 9.1.2.1 Laboratory studies

No laboratory studies were performed with BAS 500 06 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.1.4.1.

CP 9.1.2.2 Lysimeter studies

Due to the negligible leaching risk of pyraclostrobin and its metabolites, lysimeter studies are not considered necessary.

CP 9.1.2.3 Field leaching studies

Due to the negligible leaching risk of pyraclostrobin and its metabolites, field leaching studies are not considered necessary.

CP 9.1.3 Estimation of concentrations in soil

Predicted environmental concentrations in soil (PEC_S)

Report: CP 9.1.3/1
Kallweit W., 2014a
Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes
2014/1000685

Guidelines: FOCUS Groundwater (2000) Sanco/321/2000, FOCUS groundwater (2009): SANCO/13144/2010, FOCUS Groundwater (2012) Generic guidance for FOCUS groundwater scenarios v 2.1, FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011, FOCUS Surface Water Report SANCO/4802/2001 rev. 2, FOCUS Surface Water (2012) Generic guidance for FOCUS surface water scenarios v 1.1, FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 139 pp., FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 169 pp.

GLP: no

Executive Summary

PEC_{soil} were calculated for worst-case application scenarios of pyraclostrobin and its aerobic soil metabolites BF 500-6 and BF 500-7 applied to cereals and maize. Anaerobic soil metabolites (BF 500-3, BF 500-4 and BF 500-5) are not considered relevant for the assessment in soil, since soil organisms are not viable under these conditions. In addition, the anaerobic soil metabolites are immediately further degraded when aerobic conditions are re-established. Conservative crop interception values were selected in accordance with the guidance of the FOCUS groundwater scenarios workgroup (2012). For cereals, interception values of 50% for the first and 70% for the second application were selected. For the application to maize, a crop interception value of 50% was considered.

A soil bulk density of 1.5 g cm^{-3} and a soil layer depth of 5 cm were assumed for the calculations.

Maximum PEC_{soil} for pyraclostrobin and its soil metabolites were calculated (Table 9.1.3-1). No accumulation is triggered for pyraclostrobin (DT_{90} of trigger endpoints < 365 days). However, $PEC_{soil,plateau}$ and $PEC_{soil,accu}$ of the metabolites were taken into account due to multi-year application of pyraclostrobin to cereals and maize.

Table 9.1.3-1: PEC_{soil} concentrations of pyraclostrobin and its soil metabolites BF 500-6 and BF 500-7 after application to cereals and maize

Compound	Crop	$PEC_{soil,plateau}$ [mg kg^{-1}] in 0 - 20 cm depth	$PEC_{soil,max}$ [mg kg^{-1}] in 0 - 5 cm depth	$PEC_{soil,accu}$ [mg kg^{-1}] (= $PEC_{soil,plateau}$ + $PEC_{soil,max}$) in 0 - 5 cm depth
Pyraclostrobin	Cereals	-	0.228	-
	Maize	-	0.133	-
BF 500-6	Cereals	0.057[a]	0.065[a]	0.121[a]
	Maize	0.028	0.033	0.061
BF 500-7	Cereals	0.034	0.039	0.073
	Maize	0.017[a]	0.019[a]	0.038[a]

[a] Rounded values

I. MATERIAL AND METHODS

Application scenarios

Calculations were carried out for worst-case application scenarios of pyraclostrobin applied to cereals and maize. Conservative crop interception values were chosen as recommended by the FOCUS groundwater scenarios workgroup [*FOCUS (2012): Generic guidance for Tier 1 FOCUS Ground Water Assessments, v2.1, 64 pp.*]. The aerobic soil metabolites BF 500-6 and BF 500-7 were additionally considered in the assessment. Anaerobic soil metabolites (BF 500-3, BF 500-4 and BF 500-5) are not considered relevant for the assessment in soil, since soil organisms are not viable under these conditions. In addition, the anaerobic soil metabolites are immediately further degraded when aerobic conditions are re-established.

Table 9.1.3-2 summarizes the worst-case application scenarios of pyraclostrobin applied to cereals and maize. The minimum application interval for the respective crop was considered as conservative scenario.

Table 9.1.3-2: Worst-case application scenarios of pyraclostrobin applied to various crops considered for the PEC_{soil} calculations

Crop	Cereals	Maize
Growth stage at first application [BBCH]	25	30
Application rate [g a.s. ha^{-1}]	250	200
No. of applications [-]	2	1
Interval [d]	21	-
Interception [%]	50 / 70	50
Amount reaching the soil surface [g a.s. ha^{-1}]	125 / 75	100
Total yearly soil load [g a.s. ha^{-1}]	200	100

Environmental fate parameters

Degradation of pyraclostrobin in soil

The aerobic soil degradation of pyraclostrobin under laboratory conditions was investigated in five studies with altogether 10 soils for 120 to 360 days at a temperature of 20°C and a soil moisture of 40 to 53% of maximum water holding capacity (MWHC) (see M-CA 7.1.2.1). The degradation kinetics of these studies were re-evaluated in a separate study [*CA 7.1.2.1.1/3, Eickler B. – BASF DocID 2014/1093424*]. An overview of the non-normalized DT_{50} values is given in Table 7.1.2.1.1-26.

The dissipation behavior of pyraclostrobin was investigated in two different field dissipation studies conducted in Europe between 1997 and 1999 with altogether six trials located in Germany (n=3), Spain (n=2) and Sweden (n=1) (see M-CA 7.1.2.2). The degradation kinetics were evaluated in a separate study [*CA 7.1.2.2.1/1, Eickler B. – BASF DocID 2014/1093423*]. An overview of the DT_{50} values is given in Table 7.1.2.2.1-24.

In addition four terrestrial field dissipation trials were conducted in Europe between 2011 and 2012 [*CA 7.1.2.2.1/2, Bayer H., Marwitz A. - BASF DocID 2013/1348661*]. These trials were located in Denmark, Germany, Italy and France, and the plots were covered with sand to exclude surface processes according to the requirements of the EFSA guidance for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ (2010) [*EFSA (2010): Guidance for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ values of plant protection products in soil. EFSA Journal 2010;8(12):1936, 67 pp.*]. Due to the specific design of the studies, the obtained degradation half-lives in the soil matrix are not appropriate for derivation of trigger endpoints and were therefore not considered for PEC_{soil} calculation.

For the calculations of PEC_{soil} the worst-case DT_{50} from the field studies of 55.8 days (SFO) was used.

Formation and degradation of BF 500-6 and BF 500-7 in soil

Two metabolites of pyraclostrobin, BF 500-6 and BF 500-7, were found in laboratory aerobic soil metabolism and degradation studies with maximum occurrences of 12 to 31% and of 1 to 14% of the applied radioactivity (AR), respectively.

In the older pyraclostrobin field dissipation trials [*old EU Dossier, A II M 7.1.1.2.2/1, Kellner O., Zangmeister W. – BASF DocID 1999/11301; old EU Dossier, A II M 7.1.1.2.2/2, Kellner O., Zangmeister W. – BASF DocID 1999/11292*] BF 500-6 was found only rarely at a single trial site, and BF 500-7 was not detected (limit of quantification 0.01 mg kg^{-1}). In the new field dissipation study [*CA 7.1.2.2.1/2, Bayer H., Marwitz A. – BASF DocID 2013/1348661*], the metabolites BF 500-6 and BF 500-7 were detected at maximum occurrences of 28 and 19% of applied parent amount, respectively.

For PEC_{soil} calculations, the maximum occurrences of 31% observed in the laboratory and 19% observed in the field study were used for BF 500-6 and BF 500-7, respectively.

The $DegT_{50}$ values of the metabolites calculated from laboratory studies with the parent or with the metabolites ranged from 95.9 to 971.2 days and from 81.5 to >1000 days for BF 500-6 and BF 500-7, respectively. As the $DegT_{50}$ values of BF 500-6 and BF 500-7 were close to the worst-case default value of 1000 days this default value was used for PEC_{soil} calculations.

Calculation methods

Maximum, actual and time-weighted average concentrations in soil ($PEC_{soil,max}$, $PEC_{soil,act}$, $PEC_{soil,twa}$) were calculated for the parent substance and its soil metabolites. The calculations were carried out based on the approach given in the guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 of November 2011, 436 pp.*]. In addition, for the metabolites the potential of accumulation in soil after multi-year use was addressed.

Calculation of maximum concentrations in soil ($PEC_{soil,max}$)

The metabolites BF 500-6 and BF 500-7 are formed by a dimeric reaction, i.e. one metabolite molecule is formed out of two parent molecules. Hence, for the calculation of the respective f_{mol} the molar mass of the parent has to be considered twice (i.e. $f_{mol} = M_{met} / (2 \cdot M_{par})$). The resulting f_{mol} are 0.788 and 0.768 for the metabolites BF 500-6 and BF 500-7, respectively.

The maximum PEC values in soil ($PEC_{soil,max}$) were calculated considering the respective application rate, a soil bulk density of 1.5 g cm^{-3} and a thickness of the soil layer of 5 cm (Equation 9.1.3-1).

Equation 9.1.3-1 Calculation of the maximum PEC_{soil}

$$PEC_{soil,max,n} = \sum_{i=1}^{n} \left[\left(\frac{A_i \cdot (1 - f_{int,i})}{100 \cdot d \cdot bd_{soil}} \cdot f_{max,soil} \cdot f_{mol} \right) \cdot e^{-k \cdot (t_n - t_i)} \right]$$

with:			
	$PEC_{soil,max}$	maximum concentration in soil after n applications	[mg kg^{-1}]
	n	number of applications	[-]
	A_i	application rate at i-th application	[g a.s. ha^{-1}]
	$f_{int,i}$	fraction intercepted by plant cover at i-th application	[-]
	d	depth of soil layer (5 cm)	[cm]
	bd_{soil}	soil bulk density (1.5 g cm^{-3})	[g cm^{-3}]
	k	degradation rate (= ln (2) / DT_{50})	[d^{-1}]
	t_i	time of i-th application	[d]
	t_n	time of n-th (last) application	[d]
	$f_{max,soil}$	maximum observed formation in soil	[-]
	f_{mol}	correction factor for molar mass difference	[-]

Actual and time-weighted average concentrations ($PEC_{soil,act}$ and $PEC_{soil,twa}$)

The actual concentration in soil ($PEC_{soil,act}$) was calculated for different time points t = 1, 2, 4, 7, 14, 21, 28, 50 and 100 days after the occurrence of the maximum concentration according to Equation 9.1.3-2. First order kinetics (SFO) is assumed, since this type of kinetics was observed in laboratory studies and field trials.

Equation 9.1.3-2 Calculation of the actual PEC_{soil}

$$PEC_{soil,act} = PEC_{soil,max} \cdot e^{-k \cdot t}$$

with:			
	$PEC_{soil,act}$	actual concentration in soil	[mg kg^{-1}]
	$PEC_{soil,max}$	maximum concentration in soil	[mg kg^{-1}]
	k	degradation rate (= ln (2) / DT_{50})	[d^{-1}]
	t	time after last application	[d]

The maximum time-weighted average concentrations ($PEC_{soil,twa}$) for exposure periods of 1, 2, 4, 7, 14, 21, 28, 50 and 100 days were derived from the time series of PEC values ranging from day of the first application up to 200 DAT (days after first treatment) using a moving time-frame Excel spreadsheet. For a given exposure period t, the spreadsheet calculates the respective set of time-weighted average concentrations from the time series first (moving time-frame) and scans for the highest value in the set afterwards, see Equation 9.1.3-3.

Equation 9.1.3-3 Calculation of the time-weighted average PEC_{soil}

$$PEC_{soil,twa}(\Delta t) = \max\left[\frac{1}{m}\sum_{t=t_j}^{t_j+\Delta t} PEC_{soil}(t)\right] \qquad \text{for } j = 0,...,200$$

with:			
	$PEC_{soil,twa}$	worst-case time-weighted average concentration in soil for time interval Δt	[mg kg^{-1}]
	PEC_{soil}	concentration in soil at time t	[mg kg^{-1}]
	t	time	[d]
	t_j	start time point for integration	[d]
	Δt	time interval	[d]
	j	running variable for time step	[-]
	m	number of time steps in time interval t	[-]

<u>Maximum PEC_{soil} due to multi-year application ($PEC_{soil,accu}$)</u>

The potential of accumulation in soil was assessed for metabolites BF 500-6 and BF 500-7. For this purpose, the plateau concentration in soil at steady state ($PEC_{soil,plateau}$) and the overall accumulation PEC in soil ($PEC_{soil,accu}$) after application of pyraclostrobin over many years were determined following Equation 9.1.3-4 and Equation 9.1.3-5. The ploughing depth was set to 20 cm to represent the depth of soil cultivation in arable crops.

Equation 9.1.3-4 Calculation of the plateau PEC_{soil}

$$PEC_{soil,plateau} = \frac{PEC_{soil,max,20}}{1-e^{-kt}} \cdot e^{-k\cdot(t-i)}$$

with:			
	$PEC_{soil,plateau}$	plateau concentration at steady state	[mg kg^{-1}]
	$PEC_{soil,max,20}$	maximum soil concentration following last application considering a mixing depth of 20 cm	[mg kg^{-1}]
	t	interval between application seasons (365 days)	[d]
	i	interval between first application and last application in the cropping season	[d]
	k	degradation rate (= ln(2) / DT_{50})	[d^{-1}]

The overall accumulation PEC in soil ($PEC_{soil,accu}$) represents the highest potential soil concentration considering the multi-year accumulation load as background concentration ($PEC_{soil,plateau}$) plus the peak concentration ($PEC_{soil,max}$) after application in the top soil layer. For this purpose, the $PEC_{soil,plateau}$ and the $PEC_{soil,max}$ in the top soil layer of 5 cm were added (see Equation 9.1.3-5).

Equation 9.1.3-5 Calculation of the overall accumulation PEC_{soil}

$$PEC_{soil,accu} = PEC_{soil,plateau} + PEC_{soil,max}$$

with:			
	$PEC_{soil,accu}$	maximum concentration in soil for the accumulation risk assessment	[mg kg^{-1}]
	$PEC_{soil,plateau}$	concentration at steady state (plateau concentration) related to the plough layer depth of 20 cm	[mg kg^{-1}]
	$PEC_{soil,max}$	maximum concentration that gives respect to the soil load after one application period related to a soil layer depth of 5 cm	[mg kg^{-1}]

II. RESULTS AND DISCUSSION

The maximum, actual and time weighted average PEC_{soil} values of pyraclostrobin and its metabolites for a soil layer depth of 5 cm are shown in Table 9.1.3-3.

Table 9.1.3-3: PEC_{soil} of pyraclostrobin, BF 500-6 and BF 500-7 after application to cereals and maize

	Time[a] [d]	PEC_{soil} [mg kg^{-1}]											
		Pyraclostrobin				BF 500-6				BF 500-7			
		Cereals		Maize		Cereals		Maize		Cereals		Maize	
		Act	TWA	Act	TWA	Act	TWA	Act	TWA	Act	TWA	Act	TWA
Global maximum	0	0.228	-	0.133	-	0.065	-	0.033	-	0.039	-	0.019	-
Short-term	1	0.226	0.227	0.132	0.133	0.065	0.065	0.033	0.033	0.039	0.039	0.019	0.019
	2	0.223	0.226	0.130	0.132	0.064	0.065	0.033	0.033	0.039	0.039	0.019	0.019
	4	0.217	0.223	0.127	0.130	0.064	0.064	0.032	0.033	0.038	0.039	0.019	0.019
Long-term	7	0.209	0.219	0.122	0.128	0.064	0.064	0.032	0.032	0.038	0.038	0.019	0.019
	14	0.192	0.210	0.112	0.122	0.064	0.064	0.032	0.032	0.038	0.038	0.019	0.019
	21	0.176	0.201	0.103	0.117	0.064	0.064	0.032	0.032	0.038	0.038	0.019	0.019
	28	0.161	0.193	0.094	0.113	0.063	0.064	0.032	0.032	0.038	0.038	0.019	0.019
	50	0.123	0.174	0.072	0.099	0.062	0.063	0.031	0.032	0.037	0.038	0.019	0.019
	100	0.066	0.146	0.038	0.076	0.060	0.062	0.030	0.031	0.036	0.037	0.018	0.019

[a] Time: days after maximum concentration ($PEC_{soil,act}$) or time interval ($PEC_{soil,twa}$)

$PEC_{soil,accu}$ of the metabolites BF 500-6 and BF 500-7 after application for a period of many years are given in Table 9.1.3-4 and Table 9.1.3-5, respectively.

Table 9.1.3-4: $PEC_{soil,plateau}$ and $PEC_{soil,accu}$ of BF 500-6 following multi-year use in cereals and maize

Crop	$PEC_{soil,plateau}$ [mg kg^{-1}] in 0 - 20 cm depth	$PEC_{soil,max}$ [mg kg^{-1}] in 0 - 5 cm depth	$PEC_{soil,accu}$ [mg kg^{-1}] (= $PEC_{soil,plateau}$ + $PEC_{soil,max}$) in 0 - 5 cm depth
Cereals	0.057[a]	0.065[a]	0.121[a]
Maize	0.028	0.033	0.061

[a] Rounded values

Table 9.1.3-5: $PEC_{soil,plateau}$ and $PEC_{soil,accu}$ of BF 500-7 following multi-year use in cereals and maize

Crop	$PEC_{soil,plateau}$ [mg kg^{-1}] in 0 - 20 cm depth	$PEC_{soil,max}$ [mg kg^{-1}] in 0 - 5 cm depth	$PEC_{soil,accu}$ [mg kg^{-1}] (= $PEC_{soil,plateau}$ + $PEC_{soil,max}$) in 0 - 5 cm depth
Cereals	0.034	0.039	0.073
Maize	0.017[a]	0.019[a]	0.038[a]

[a] Rounded values

III. CONCLUSION

Maximum, short-term and long-term actual and time-weighted average PEC_{soil} were calculated for worst-case application scenarios of pyraclostrobin in the formulated product BAS 500 06 F, i.e. twofold application of 250 g a.s. ha^{-1} to cereals and single application of 100 g a.s. ha^{-1} to maize. The aerobic soil metabolites BF 500-6 and BF 500-7 were also considered. Further, their potential of accumulation in soil was assessed.

The predicted concentrations in soil are appropriate to be used for the subsequent risk assessment for soil organisms.

CP 9.2 Fate and behaviour in water and sediment

CP 9.2.1 Aerobic mineralisation in surface water

No studies were performed with BAS 500 06 F. The aerobic mineralisation in surface water is sufficiently addressed by information given in M-CA 7.2.2.2.

CP 9.2.2 Water/sediment study

No water/sediment studies were performed with BAS 500 06 F. Data for the active substance and its metabolites are covered by information given in M-CA 7.2.2.3.

CP 9.2.3 Irradiated water/sediment study

No irradiated water/sediment studies were performed with BAS 500 06 F. Data for the active substance and its metabolites are covered by information given in M-CA 7.2.2.4.

CP 9.2.4 Estimation of concentrations in groundwater

CP 9.2.4.1 Calculation of concentrations in groundwater

Predicted environmental concentrations in groundwater (PEC_{GW})

Report: CP 9.2.4.1/1
Kallweit W., 2014a
Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes
2014/1000685

Guidelines: FOCUS Groundwater (2000) Sanco/321/2000, FOCUS groundwater (2009): SANCO/13144/2010, FOCUS Groundwater (2012) Generic guidance for FOCUS groundwater scenarios v 2.1, FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011, FOCUS Surface Water Report SANCO/4802/2001 rev. 2, FOCUS Surface Water (2012) Generic guidance for FOCUS surface water scenarios v 1.1, FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 139 pp., FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 169 pp.

GLP: no

Executive Summary

Predicted environmental concentrations in groundwater (PEC_{gw}) of pyraclostrobin and its aerobic soil metabolites BF 500-6 and BF 500-7, as well as its anaerobic soil metabolites BF 500-3, BF 500-4 and BF 500-5 following application of BAS 500 06 F to cereals and maize were calculated in accordance with the guidance of the FOCUS groundwater scenarios work group (2000, 2009, 2012). Although these anaerobic soil metabolites are degraded quickly under aerobic conditions, they were considered (according to Regulation 1107/2009) for the assessment in groundwater.

Tier 1 PEC_{gw} of pyraclostrobin and its soil metabolites were calculated with the models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3 for all available FOCUS scenarios of cereals (twofold application of 250 g a.s. ha^{-1}, BBCH 25, 21 day interval, 50 / 70% interception) and maize (single application of 200 g a.s. ha^{-1}, BBCH 30, 50% interception) with an annual application pattern (continuous cropping over a period of 26 years).

The maximum PEC_{gw} for pyraclostrobin and its soil metabolites were < 0.001 µg L^{-1} and thus clearly below the 0.1 µg L^{-1} threshold value.

I. MATERIAL AND METHODS

The leaching assessment for pyraclostrobin and its metabolites was conducted at Tier 1 of the tiered assessment scheme proposed by the FOCUS groundwater higher tier working group. The aerobic soil metabolites BF 500-6 and BF 500-7 were considered in the assessment. In addition the anaerobic soil metabolites BF 500-3, BF 500-4 and BF 500-5 were considered even though they are degraded quickly under aerobic conditions.

The simulations were carried out using FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3 in combination with the FOCUS standard scenarios.

Applied procedures, methods and scenarios follow the recommendations of the following guidelines:

- *FOCUS (2009): Assessing Potential for Movement of Active Substances and their Metabolites to Groundwater in the EU Final Report of the Groundwater Workgroup of FOCUS, amending FOCUS (2000), EC Document Reference Sanco/13144/2010, 604 pp.*
- *FOCUS (2000): "FOCUS groundwater scenarios in the EU plant protection product review process" Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000, 197 pp.*
- *FOCUS (2012): Generic guidance for Tier 1 FOCUS Ground Water Assessments, v2.1, 64 pp.*

Application scenarios

PEC_{gw} values of pyraclostrobin were calculated for the same worst-case application scenarios as for the PEC_{soil} assessment (see M-CP 9.1.3). PEC_{gw} calculations were performed for all FOCUS groundwater scenarios that are parameterized for winter and spring cereals as well as maize.

Application dates were selected following FOCUS recommendations. The worst-case application scenarios and application dates for all relevant FOCUS scenarios are shown in Table 9.2.4.1-1 and Table 9.2.4.1-2 for cereals and maize, respectively.

Table 9.2.4.1-1 Worst-case application scenarios for PEC_{gw} calculations of pyraclostrobin applied to cereals

Crop	**Cereals**	
Growth stage at first application [BBCH]	25	
No. of applications [-]	2	
Interval [days]	21	
Application rate [g a.s. ha^{-1}]	250	
Interception [%]	50 / 70	
Amount reaching the soil surface [g a.s. ha^{-1}]	125 / 75	
Total yearly soil load [g a.s. ha^{-1}]	200	
Scenario	**Application dates – winter cereals**	
	1st application	**2nd application**
Châteaudun	15th March	5th April
Hamburg	15th March	5th April
Jokioinen	15th April	6th May
Kremsmünster	15th March	5th April
Okehampton	15th March	5th April
Piacenza	15th March	5th April
Porto	15th February	8th March
Sevilla	15th February	8th March
Thiva	15th February	8th March
Scenario	**Application dates – spring cereals**	
	1st application	**2nd application**
Châteaudun	7th April	28th April
Hamburg	29th April	20th May
Jokioinen	15th June	6th July
Kremsmünster	29th April	20th May
Okehampton	29th April	20th May
Porto	7th April	28th April

Table 9.2.4.1-2: Worst-case application scenario for PEC_{gw} calculations of pyraclostrobin applied to maize

Crop	Maize
Growth stage at first application [BBCH]	30
No. of applications [-]	1
Application rate [g a.s. ha^{-1}]	200
Interception [%]	50
Amount reaching the soil surface [g a.s. ha^{-1}]	100
Total yearly soil load [g a.s. ha^{-1}]	100
Scenario	**Application dates**
Châteaudun	15th May
Hamburg	19th May
Kremsmünster	19th May
Okehampton	8th June
Piacenza	29th May
Porto	15th May
Sevilla	21st March
Thiva	4th May

Environmental fate parameters

Degradation parameters of the parent

A total of 18 degradation endpoints for environmental fate modeling from different laboratory and field trials were reported (see M-CP 9.1.3) in four studies [*CA 7.1.2.1.1/3, Eickler B. – BASF DocID 2014/1093424; CA 7.1.2.1.1/1, Hassink J., Kuhnke G. – BASF DocID 2013/1337273; CA 7.1.2.2.1/1, Eickler B. – BASF DocID 2014/1093423; CA 7.1.2.2.1/4, Pape L. – BASF DocID 2014/1105764*]. As the endpoint in *Hassink J., Kuhnke G.* [*BASF DocID 2013/1337273*] was calculated for actual study conditions, it was further normalized to reference conditions (20°C, pF2). In all other studies normalized degradation endpoints were reported. In study *CA 7.1.2.2.1/1* [*Eickler B. – BASF DocID 2014/1093423*] the two Spanish field trials (ALO/01/98 and ALO/02/98) were considered to be not appropriate for derivation of modeling endpoints as the extreme climatic conditions during the trials were not representative for the EU.

An overview of individual DT_{50} values considered to select endpoints for PEC_{gw} calculations is given in Table 7.1.2.1.1-26 and/or Table 7.1.2.1.1-27 (laboratory studies) and in Table 7.1.2.2.1-24 (field studies).

For derivation of an appropriate degradation endpoint for PEC_{gw} modeling the EFSA endpoint selector was used [*EFSA $DegT_{50}$ and Sorption Endpoint Selector. Download from: http://www.efsa.europa.eu/en/130725a/docs/130725aax1.xls, date of access: 15.04.2014*]. Accordingly, the bias-corrected geometric mean $DegT_{50}$ value of 28.3 days based on the results of the field studies was used as modeling input.

Sorption of pyraclostrobin to soil

The sorption behavior of pyraclostrobin was investigated in six soils by batch-equilibrium experiments [*old EU Dossier, A II M 7.1.2/1, Ziegler G. - BASF DocID 1998/10650*]. An overview of individual $K_{f,oc}$ and 1/n values considered to select endpoints for PEC_{gw} calculations is given in M-CA 7.1.3.1.1. For the PEC_{gw} calculations of pyraclostrobin, an arithmetic mean $K_{f,oc}$ of 9304 mL g^{-1} (equivalent to a $K_{f,om}$ value of 5397 mL g^{-1}) and an arithmetic mean 1/n of 0.95 were used.

Formation and degradation of the metabolites in soil

BF 500-3
In laboratory experiments under aerobic conditions and in field dissipation trials with pyraclostrobin, the metabolite BF 500-3 was either not detected or formed in amounts far below 5% of the amount of applied parent (see M-CA 7.1.1). Under anaerobic conditions it was formed in high amounts by a very fast de-methoxylation of pyraclostrobin (see M-CA 7.1.1). This reaction is the first step also in the aerobic soil degradation process. However, further reaction to successive metabolites and bound residues is too fast to detect this short-lived transient intermediate.

To account for the rapid formation and degradation of BF 500-3 under aerobic conditions, a conservative default DT_{50} of 1 day and a formation fraction of 1 from pyraclostrobin were considered for PEC_{gw} calculations.

BF 500-4
The metabolite BF 500-4 was never found in soil under aerobic conditions. Under anaerobic conditions it was observed with up to 11.1% TAR [*old EU Dossier, A II M 7.1.1.2.1/5, Kellner O. - BASF DocID 1999/10079; old EU Dossier, A II M 7.1.1.2.1/6, Kellner O. - BASF DocID 1999/11103*]. The aerobic degradation of BF 500-4 under laboratory conditions was investigated in three soils [*CA 7.1.2.1.2/3, Ebert D., Dalkmann P. – BASF DocID 2013/1294779*]. The corresponding DT_{50} values were normalized to reference conditions (20°C, pF2), see Table 7.1.2.1.2-24 at the end of study *CA 7.1.2.1.2/3*. The geometric mean of the normalized DT_{50} values of 2.82 days was used as input parameter for BF 500-4 for PEC_{gw} calculations.

In the kinetic re-evaluation of the anaerobic soil degradation studies of pyraclostrobin formation fractions of BF 500-4 from BF 500-3 of 1 and 0.692 were reported [*CA 7.1.2.1.3/1, Pape L. – BASF DocID 2014/1000701*]. Therefore, a formation fraction of 1 was used as worst-case assumption for PEC_{gw} calculations.

BF 500-5
Under aerobic conditions, the metabolite BF 500-5 occurred only in one soil with 2.8% TAR. Under anaerobic conditions it was found with up to 7.7% TAR [*Kellner O. - BASF DocID 1999/11103*]. The aerobic degradation of BF 500-5 under laboratory conditions was investigated in three soils [*CA 7.1.2.1.2/4, Schoof S., Possienke M. – BASF DocID 2013/1294780*]. The corresponding DT_{50} were normalized to reference conditions (20°C, pF2), see Table 7.1.2.1.2-34 at the end of study *CA 7.1.2.1.2/4*. As all DT_{50} values were below 1 day a default DT_{50} value of 1 day was used for PEC_{gw} calculations as worst-case assumption.

In the kinetic re-evaluation of the anaerobic soil degradation studies of pyraclostrobin a formation fraction of BF 500-5 from BF 500-4 of 0.409 was reported and was considered for PEC_{gw} calculations [*CA 7.1.2.1.3/1*, *Pape L. – BASF DocID 2014/1000701*].

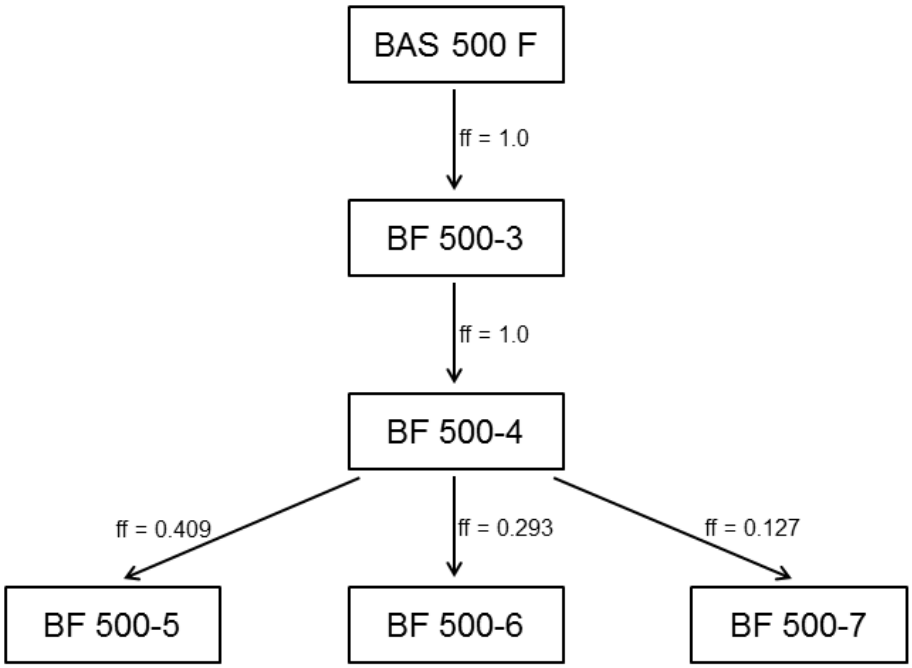
BF 500-6 and BF 500-7
The metabolites BF 500-6 and BF 500-7 were found in aerobic soil degradation studies as well as in field dissipation studies with pyraclostrobin. Degradation endpoints for modeling are available from kinetic evaluations of aerobic laboratory degradation studies with pyraclostrobin and aerobic laboratory degradation studies with the metabolites (see table at the end of study *CA 7.1.2.1.2/4*) as well as with the new field dissipation study with pyraclostrobin (see table at the end of study *CA 7.1.2.2.1/4*) [*Hassink J., Kuhnke G. – BASF DocID 2013/1337273; Eickler B. – BASF DocID 2014/1093424; Pape L. – BASF DocID 2014/1105764; CA 7.1.2.1.2/1, Tornisielo A., Sacchi R.R. – BASF DocID 2011/1142307; CA 7.1.2.1.2/2, Tornisielo A., Sacchi R.R. – BASF DocID 2011/1142308*].

Where necessary, the reported DT_{50} values were normalized to reference conditions (20°C, pF2), see tables at the end of study *CA 7.1.2.1.2/1* (BF 500-6) and at the end of study *CA 7.1.2.1.2/2* (BF 500-7). For derivation of appropriate degradation endpoints for PEC_{gw} modeling the EFSA endpoint selector was used. Accordingly, the bias-corrected geometric mean of pooled laboratory and field DT_{50} values of 321.0 days for BF 500-6 and 312.0 days for BF 500-7 was used for the PEC_{gw} calculations.

Formation fractions of BF 500-6 and BF 500-7 were reported in the kinetic evaluations of laboratory and field studies with pyraclostrobin either directly from the parent or from BF 500-3 (see table at the end of study *CA 7.1.2.1.2/4* and table at the end of study *CA 7.1.2.2.1/4*).

In the aerobic degradation study with BF 500-4, the two metabolites occurred as reaction products of BF 500-4 [*CA 7.1.2.1.2/3, Ebert D., Dalkmann P. – BASF DocID 2013/1294779*]. Consequently, this pathway was also assumed for PEC_{gw} calculations. For the metabolites BF 500-3 and BF 500-4, formation fractions of 1 were considered, respectively (see above). Thus, for BF 500-6 and BF 500-7 the reported formation fractions from pyraclostrobin or from BF 500-3 can be directly considered. As such the arithmetic mean values derived from pooled field and laboratory data (following the EFSA approach for degradation rates) of 0.293 (BF 500-6) and 0.127 (BF 500-7) were used.

The complete transformation scheme considered for the PEC_{gw} calculations is shown in Figure 9.2.4.1-1.



ff formation fraction

Figure 9.2.4.1-1: Transformation scheme of pyraclostrobin in soil used for PEC_{gw} simulations

Sorption of the metabolites to soil

An overview of individual K_{oc} values for the metabolites of pyraclostrobin considered to select endpoints for PEC_{gw} calculations is given in M-CA 7.1.3.1.2 and summarized in the tables below. For BF 500-3 and BF 500-4 arithmetic mean K_{oc} of 9315 mL g^{-1} (n = 6) and 9819 mL g^{-1} (n = 5) as well as a worst-case default 1/n of 1 were used for PEC_{gw} calculations, respectively. For BF 500-5, an arithmetic mean $K_{f,oc}$ of 705 mL g^{-1} (n = 5) and the corresponding arithmetic mean 1/n of 0.85 were considered, while for BF 500-6 and BF 500-7, the median of 11 K_{oc} values of 107301 mL g^{-1} and 149900 mL g^{-1} and a default 1/n of 1 were used.

Input parameters for pyraclostrobin and its metabolites

A summary of the substance parameters used for the Tier 1 PEC_{gw} calculations is given in Table 9.2.4.1-3 to Table 9.2.4.1-8.

Table 9.2.4.1-3: Overview of input parameters for pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	Value	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	387.8	
Water solubility	[mg L^{-1}]	1.9 (20°C)	
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	2.6 x 10^{-8} (20°C)	
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	28.3 (20°C, pF2)	Bias-corrected geometric mean of field studies (n=8)
Degradation rate to metabolite (PELMO)	[d^{-1}]	0.0245 (to BF 500-3)	=ln2/DT_{50}*formation fraction (ff = 1)
Q_{10} value (PELMO)	[-]	2.58	EFSA opinion
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	5397	Arithmetic mean (n=6)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	9304	
Freundlich exponent 1/n	[-]	0.95	
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

Table 9.2.4.1-4: Overview of input parameters for metabolite BF 500-3 of pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	BF 500-3	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	357.8	
Water solubility	[mg L^{-1}]	0.03 (20°C)	
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	1 x 10^{-10} (20°C)	Worst-case assumption
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	1	Worst-case assumption
Transformation rate (PELMO)	[d^{-1}]	0.6931 (to BF 500-4)	=ln2/DT_{50}*formation fraction (ff = 1)
Formation fraction (PEARL)	[-]	1 (from parent)	Worst-case assumption
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	5403	Arithmetic mean (n=6)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	9315	
Freundlich exponent 1/n	[-]	1	Worst-case assumption
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

Table 9.2.4.1-5: Overview of input parameters for metabolite BF 500-4 of pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	BF 500-4	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	299.8	
Water solubility	[mg L^{-1}]	1000	Worst-case assumption
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	1 x 10^{-10} (20°C)	Worst-case assumption
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	2.82	Geometric mean from laboratory study (n=3)
Transformation rate (PELMO)	[d^{-1}]	0.1008 (to BF 500-5) 0.0720 (to BF 500-6) 0.0312 (to BF-500-7) 0.0418 (to SINK)	=ln2/DT_{50}*formation fraction (ff = 0.409) (ff = 0.293) (ff = 0.127)
Formation fraction (PEARL)	[-]	1 (from BF 500-3)	Worst-case assumption
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	5695	Arithmetic mean (n=5)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	9819	
Freundlich exponent 1/n	[-]	1	Worst-case assumption
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

Table 9.2.4.1-6: Overview of input parameters for metabolite BF 500-5 of pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	BF 500-5	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	194.6	
Water solubility	[mg L^{-1}]	21.8 (20°C)	
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	1 x 10^{-10} (20°C)	Worst-case assumption
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	1	Worst-case assumption
Transformation rate (PELMO)	[d^{-1}]	0.6931 (to SINK)	$=ln2/DT_{50}$
Formation fraction (PEARL)	[-]	0.409 (from BF 500-4)	
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	409	Arithmetic mean (n=5)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	705	
Freundlich exponent 1/n	[-]	0.85	
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

Table 9.2.4.1-7: Overview of input parameters for metabolite BF 500-6 of pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	BF 500-6	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	305.8	0.5 of molar mass as metabolite is a dimer
Water solubility	[mg L^{-1}]	0.003 (20°C)	
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	1 x 10^{-10} (20°C)	Worst-case assumption
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	321.0	Bias-corrected geometric mean of lab- and field-DT_{50}
Transformation rate (PELMO)	[d^{-1}]	0.0019 (to SINK)	=ln2/DT_{50}
Formation fraction (PEARL)	[-]	0.293 (from BF 500-4)	
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	62240	Median (n=11)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	107301	
Freundlich exponent 1/n	[-]	1	Worst-case assumption
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

Table 9.2.4.1-8: Overview of input parameters for metabolite BF 500-7 of pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	BF 500-7	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	297.8	0.5 of molar mass as metabolite is a dimer
Water solubility	[mg L^{-1}]	0.005 (20°C)	
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	1 x 10^{-10} (20°C)	Worst-case assumption
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	312.0	Bias-corrected geometric mean of lab- and field-DT_{50}
Transformation rate (PELMO)	[d^{-1}]	0.0022 (to SINK)	=ln2/DT_{50}
Formation fraction (PEARL)	[-]	0.127 (from BF 500-4)	
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	86949	Median (n=11)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	149900	
Freundlich exponent 1/n	[-]	1	Worst-case assumption
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

II. RESULTS AND DISCUSSION

The 80th percentiles of the predicted annual leachate concentrations in groundwater after application to winter and spring cereals as well as maize were below 0.001 µg L^{-1} for pyraclostrobin and its metabolites BF 500-3, BF 500-4, BF 500-5, BF 500-6 and BF 500-7 for all scenarios. Consequently, the leaching of unacceptable amounts of pyraclostrobin after application to cereals and maize is highly unlikely.

III. CONCLUSION

A tiered approach was considered to address the risk for groundwater following the guidelines of FOCUS groundwater. No risk was identified for the parent compound, nor for its soil metabolites at Tier 1 (PEC_{gw} < 0.001 µg L^{-1}).

CP 9.2.4.2 Additional field tests

No additional field tests were performed with BAS 500 06 F. Data for the active substance and its metabolites are covered by information given in M-CA 7.

CP 9.2.5 Estimation of concentrations in surface water and sediment

Predicted environmental concentrations in surface water (PEC_{SW}) and sediment (PEC_{SED})

Report: CP 9.2.5/1
Kallweit W., 2014a
Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes
2014/1000685

Guidelines: FOCUS Groundwater (2000) Sanco/321/2000, FOCUS groundwater (2009): SANCO/13144/2010, FOCUS Groundwater (2012) Generic guidance for FOCUS groundwater scenarios v 2.1, FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011, FOCUS Surface Water Report SANCO/4802/2001 rev. 2, FOCUS Surface Water (2012) Generic guidance for FOCUS surface water scenarios v 1.1, FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 139 pp., FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 169 pp.

GLP: no

Executive Summary

Predicted environmental concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) were calculated for pyraclostrobin after single and twofold spray application of 250 g a.s. ha^{-1} to cereals and single spray application of 200 g a.s. ha^{-1} to maize considering the entry pathways spray drift, drainage and runoff.

The metabolites of pyraclostrobin which were observed in major amounts in water (BF 500-5, BF 500-11, BF 500-13, BF 500-14) and in sediment (BF 500-3, BF 500-6, BF 500-7) were also considered for the assessment in the respective environmental compartments, taking into account their formation after entry of the parent substance.

Additionally, for the metabolites BF 500-6 and BF 500-7 occurring in major amounts in soil under aerobic conditions the entry pathways runoff and drainage of the metabolites into surface water were taken into account. Since both metabolites have very low water solubilities (2 - 3 µg L^{-1}) and extremely high sorption values (K_{oc}>10^5 mL g^{-1}), they will reach surface water bodies via runoff or drainage in a soil particle-bound form. They then sink to the sediment surface, i.e. they are extremely unlikely to occur freely dissolved in molecular form in the water phase. Consequently BF 500-6 and BF 500-7 were only considered for PEC calculations in the sediment compartment.

For the metabolites BF 500-3 and BF 500-5 runoff and drainage of the metabolites are not relevant as entry pathways into surface water as the metabolites did not occur in relevant amounts in soil under aerobic conditions. The metabolite BF 500-4 was not found either in water/sediment studies or in soil under aerobic conditions and was therefore not considered in the assessment.

The calculations were performed according to the FOCUS surface water guidance documents [*FOCUS (2001, 2012)*].

Calculations for pyraclostrobin were carried out at Step 1 to Step 4. A tiered approach was considered for the parent substance, with endpoints derived from a dark water/sediment study being used at Tier 1 and endpoints from an irradiated water/sediment study being used at Tier 2. PEC values for the metabolites were calculated for FOCUS Step 1 and 2 level. Input parameters for the metabolites were derived from the study in which they occurred, i.e. in dark or irradiated water/sediment systems.

The software packages STEPS1-2 in FOCUS version 2.1, FOCUS-PRZM version 1.5.6, FOCUS-MACRO version 4.4.2 and FOCUS-TOXSWA version 3.3.1 for Step 3 were used for the calculations. In addition, the pre- and post-processing software tool PEC Robot v1.4 was used to prepare Step 4 simulations. At Step 2 of the assessment, the regions ‘North Europe’ and ‘South Europe’ were taken into account. At Step 3 and 4, all FOCUS scenarios available for winter and spring cereals as well as maize were considered.

A summary of the maximum PEC_{sw} of pyraclostrobin and its metabolites calculated for each step of the assessment is given in the tables below.

Pyraclostrobin

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-1: Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 250 g a.s. ha^{-1} to winter cereals (Tier 1)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	17.031	-[a]	-[a]	1160
Step 2	-	2.299	-[a]	-[a]	187.230
Step 3	-	1.589 D2 d, single	1.509 D2 d, single	1.437 D2 d, single	10.583 R4 s, twofold
Step 4	50N	0.794 D2 d, single	0.754 D2 d, single	0.718 D2 d, single	(not reported)
	75N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	90N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	5mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.467 D2 s, single	
	5mD50N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	5mD75N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD50N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD+R	0.283 R3 s, single	0.260 D2 s, single	0.247 D2 s, single	
	15mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	20mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	20mD+R	0.147 R3 s, single	0.135 D2 s, single	0.129 D2 s, single	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application, twofold = twofold application
[a] only maximum values for Step 1 and 2 are reported

Table 9.2.5-2: Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 250 g a.s. ha^{-1} to spring cereals (Tier 1)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	17.031	-[a]	-[a]	1160
Step 2	-	2.299	-[a]	-[a]	187.230
Step 3	-	1.759 D1 d, twofold	1.680 D1 d, twofold	1.611 D1 d, twofold	9.836 D1 d, twofold
Step 4	50N	0.877 D1 d, twofold	0.837 D1 d, twofold	0.803 D1 d, twofold	(not reported)
	75N	0.437 D1 d, twofold	0.417 D1 d, twofold	0.400 D1 d, twofold	
	90N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	5mD	0.507 D1 s, single	0.433 D1 d, twofold	0.415 D1 d, twofold	
	5mD50N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	5mD75N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	10mD	0.269 D1 s, single	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	10mD50N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	10mD+R	0.269 D1 s, single	0.224 D1 d, twofold	0.215 D1 d, twofold	
	15mD	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	20mD	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	20mD+R	0.140 D1 s, single	0.113 D1 d, twofold	0.109 D1 d, twofold	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application, twofold = twofold application
[a] only maximum values for Step 1 and 2 are reported

Table 9.2.5-3: Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 200 g a.s. ha^{-1} to <u>maize</u> (Tier 1)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	6.813	-[a]	-[a]	462.701
Step 2	-	1.839	-[a]	-[a]	95.014
Step 3	-	1.037 D3 d, single	0.797 D3 d, single	0.508 D3 d, single	6.572 R4 s, single
Step 4	50N	0.519 D3 d, single	0.398 D3 d, single	0.254 D3 d, single	(not reported)
	75N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	90N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	5mD	0.425 R3 s, single	0.264 R4 s, single	0.219 R4 s, single	
	5mD50N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	10mD	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	10mD+R	0.225 R3 s, single	0.138 D3 d, single	0.100 R4 s, single	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application
[a] only maximum values for Step 1 and 2 are reported

Tier 2 (parameters from irradiated water/sediment study)

Table 9.2.5-4: Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 250 g a.s. ha^{-1} to winter cereals (Tier 2)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	17.031	-[a]	-[a]	1160
Step 2	-	2.299	-[a]	-[a]	177.447
Step 3	-	1.589 D2 d, single	1.491 D2 d, single	1.406 D2 d, single	9.724 R4 s, twofold
Step 4	90N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	(not reported)
	5mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.456 D2 s, single	
	5mD50N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	5mD75N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD50N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD+R	0.283 R3 s, single	0.257 D2 s, single	0.242 D2 s, single	
	15mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	20mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	20mD+R	0.147 R3 s, single	0.133 D2 s, single	0.126 D2 s, single	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application, twofold = twofold application
[a] only maximum values for Step 1 and 2 are reported

Table 9.2.5-5: Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 250 g a.s. ha^{-1} to spring cereals (Tier 2)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	17.031	-[a]	-[a]	1160
Step 2	-	2.299	-[a]	-[a]	177.447
Step 3	-	1.588 D1 d, single	1.474 D1 d, single	1.375 D1 d, single	5.314 R4 s, single
Step 4	50N	0.794 D1 d, single	0.737 D1 d, single	0.687 D1 d, single	(not reported)
	75N	0.397 D1 d, single	0.368 D1 d, single	0.344 D1 d, single	
	90N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	5mD	0.507 D1 s, single	0.399 D1 d, single	0.373 D1 d, single	
	5mD50N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	5mD75N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	10mD	0.269 D1 s, single	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	10m50N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	10mD+R	0.269 D1 s, single	0.212 D1 d, single	0.198 D1 d, single	
	15mD	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	20mD	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	20mD+R	0.140 D1 s, single	0.110 D1 d, single	0.103 D1 d, single	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application, twofold = twofold application
[a] only maximum values for Step 1 and 2 are reported

Table 9.2.5-6: Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 200 g a.s. ha^{-1} to <u>maize</u> (Tier 2)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	6.813	-[a]	-[a]	462.701
Step 2	-	1.839	-[a]	-[a]	91.939
Step 3	-	1.037 D3 d, single	0.776 D3 d, single	0.489 D3 d, single	6.010 R4 s, single
Step 4	50N	0.519 D3 d, single	0.388 D3 d, single	0.244 D3 d, single	(not reported)
	75N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	90N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	5mD	0.425 R3 s, single	0.264 R4 s, single	0.219 R4 s, single	
	5mD50N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	10mD	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	10mD+R	0.225 R3 s, single	0.135 D3 d, single	0.100 R4 s, single	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application
[a] only maximum values for Step 1 and 2 are reported

Metabolites of pyraclostrobin

Metabolites observed in dark water/sediment study: BF 500-3, BF 500-5, BF 500-6, BF 500-7

Parameters from dark water/sediment study

Table 9.2.5-7: Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 250 g a.s. ha^{-1} to cereals (dark water/sediment study)

Cereals				
Compound	SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]		SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]	
	Step 1	Step 2	Step 1	Step 2
BF 500-3	not relevant [1]		20.030	17.430
BF 500-6	not relevant [2]		304.937	60.344
BF 500-7			182.976	36.588
BF 500-5	0.256	0.179	not relevant [3]	

[1] BF 500-3 did not occur in soil or water phase in significant amounts
[2] BF 500-6 and BF 500-7 will not occur free in water phase due to low water solubility and high sorption values
[3] BF 500-5 did not occur in soil or sediment phase in significant amounts

Table 9.2.5-8: Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 200 g a.s. ha^{-1} to maize (dark water/sediment study)

Maize				
Compound	SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]		SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]	
	Step 1	Step 2	Step 1	Step 2
BF 500-3	not relevant [1]		8.012	7.948
BF 500-6	not relevant [2]		121.975	24.752
BF 500-7			73.190	15.040
BF 500-5	0.102	0.101	not relevant [3]	

[1] BF 500-3 did not occur in soil or water phase in significant amounts
[2] BF 500-6 and BF 500-7 will not occur free in water phase due to low water solubility and high sorption values
[3] BF 500-5 did not occur in soil or sediment phase in significant amounts

Metabolites observed in irradiated water/sediment study: BF 500-3, BF 500-11, BF 500-13, BF 500-14

Parameters from irradiated water/sediment study

Table 9.2.5-9: Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 250 g a.s. ha^{-1} to cereals (irradiated water/sediment study)

Cereals				
Compound	**SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]**		**SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]**	
	Step 1	**Step 2**	**Step 1**	**Step 2**
BF 500-3	not relevant [1]		6.507	5.090
BF 500-11	0.407	0.266	not relevant [2]	
BF 500-13	0.533	0.413		
BF 500-14	0.573	0.352		

[1] BF 500-3 did not occur in soil or water phase in significant amounts
[2] BF 500-11, BF 500-13 and BF 500-14 did not occur in soil or sediment phase in significant amounts

Table 9.2.5-10: Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 200 g a.s. ha^{-1} to maize (irradiated water/sediment study)

Maize				
Compound	**SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]**		**SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]**	
	Step 1	**Step 2**	**Step 1**	**Step 2**
BF 500-3	not relevant [1]		2.603	2.485
BF 500-11	0.163	0.158	not relevant [2]	
BF 500-13	0.213	0.209		
BF 500-14	0.229	0.223		

[1] BF 500-3 did not occur in soil or water phase in significant amounts
[2] BF 500-11, BF 500-13 and BF 500-14 did not occur in soil or sediment phase in significant amounts

I. MATERIAL AND METHODS

Predicted environmental concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) were calculated for pyraclostrobin after single and twofold spray application of 250 g a.s. ha^{-1} to cereals and single spray application of 200 g a.s. ha^{-1} to maize considering the entry pathways spray drift, drainage and runoff.

The metabolites of pyraclostrobin, which were observed in major amounts in water (BF 500-5, BF 500-11, BF 500-13, BF 500-14) and in sediment (BF 500-3, BF 500-6, BF 500-7), were also considered for the assessment in the respective environmental compartments, taking into account their formation after entry of the parent substance. Additionally, for the metabolites occurring in major amounts in soil under aerobic conditions (BF 500-6, BF 500-7) the entry pathways runoff and drainage of the metabolites into surface water were taken into account.

Since both metabolites have very low water solubilities (2 - 3 µg L^{-1}) and extremely high sorption values (K_{oc}>10^5 mL g^{-1}), they will reach surface water bodies via runoff or drainage in a soil particle-bound form. They then sink to the sediment surface, i.e. they are extremely unlikely to occur freely dissolved in molecular form in the water phase. Consequently BF 500-6 and BF 500-7 were only considered for PEC calculations in the sediment compartment.

For the metabolites BF 500-3 and BF 500-5 runoff and drainage of the metabolites are not relevant as entry pathways into surface water as the metabolites did not occur in relevant amounts in soil under aerobic conditions. Metabolite BF 500-4 was neither found in water/sediment studies nor in soil under aerobic conditions and was therefore not considered in the assessment.

The calculations were performed according to the FOCUS surface water guidance documents [*FOCUS (2001): FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC Report of the FOCUS Working Group on Surface Water Scenarios. EC Document Reference SANCO/4802/2001-rev.2. final. 238 pp); FOCUS (2012): "FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC" Report of the FOCUS Working Group on Surface Water Scenarios. EC Document Reference SANCO/4802/2001 version 1.2. 357 pp.*].

Application scenarios

The worst-case application scenarios of pyraclostrobin considered for PEC_{sw} and PEC_{sed} calculations are given in Table 9.2.5-11.

Table 9.2.5-11: Application scenarios of pyraclostrobin applied to various crops considered for PEC_{sw} and PEC_{sed} calculations

$FOCUS_{sw}$ crop	Winter cereals	Spring cereals	Maize
Growth stage [BBCH]	25-69		30-65
Application rate [g a.s. ha^{-1}]	250		200
Max. no. of applications [-]	1 / 2		1
Interval [d]	- / 21		-
Total yearly application rate [g a.s. ha^{-1}]	250 / 500		200

Environmental fate parameters

Fate and behavior in soil

Degradation and sorption of pyraclostrobin

The degradation behavior of pyraclostrobin in soil has been investigated under both laboratory and field conditions. An overview of individual DT_{50} considered to select endpoints for PEC calculations is given in the tables at the end of study *CA 7.1.2.1.1/3* and study *CA 7.1.2.2.1/4*. The sorption behavior of pyraclostrobin is described in M-CP 9.2.4 (chapter on PEC_{gw}).

The normalized bias-corrected geometric mean half-life of 28.3 days (at 20°C and pF2) from the eight relevant field trials, an arithmetic mean $K_{f,oc}$ of 9304 mL g^{-1} and the corresponding 1/n of 0.95 were used for the PEC_{sw} calculations.

Degradation and sorption of the metabolites of pyraclostrobin

The degradation behavior of the metabolites of pyraclostrobin in soil has been investigated under laboratory (BF 500-3, BF 500-5, BF 500-6, BF 500-7) and field conditions (BF 500-6, BF 500-7). An overview of individual DT_{50} considered to select endpoints for PEC calculations is given in the tables at the end of study *CA 7.1.2.1.2/4* and study *CA 7.1.2.2.1/4*. The sorption behavior of the metabolites of pyraclostrobin is described in M-CP 9.2.4 (section on PEC_{gw}).

As the metabolites BF 500-3 and BF 500-5 did not occur in relevant amounts in soil under aerobic conditions, a maximum occurrence in soil of 0.01% and a DT_{50} of 1 day were assumed as worst-case default input parameters. The arithmetic mean K_{oc} values of 9315 mL g^{-1} and 705 mL g^{-1} for BF 500-3 and BF 500-5, respectively, were considered for the calculations.

For the metabolite BF 500-6 a maximum occurrence in soil of 31% TAR observed in the laboratory, a bias-corrected geometric mean DT_{50} of 321.0 days and a median K_{oc} value of 107301 mL g^{-1} were used as modeling input.

For the metabolite BF 500-7 a maximum occurrence in soil of 19% TAR observed in field degradation studies, a bias-corrected geometric mean DT_{50} of 312.0 days and a median K_{oc} value of 149900 mL g^{-1} were used as modeling input.

The metabolites BF 500-11, BF 500-13 and BF 500-14 occur only in water. As there are no data on soil degradation and sorption available, a maximum occurrence in soil of 0.01%, a DT_{50} of 1 day and a K_{oc} of 1 x 10^{-10} mL g^{-1} were assumed as worst-case default input parameters.

Fate and behavior in aquatic systems

Pyraclostrobin

Degradation and partitioning of pyraclostrobin in aquatic systems have been investigated in a range of studies with different test designs and an increasing level of environmental relevance: dark and irradiated water/sediment systems and outdoor mesocosm [*old EU Dossier, A II M 7.2.1.3.2/1, Staudenmaier H. - BASF DocID 1999/11241*; *old EU Dossier, A II M 7.2.1.3.2/2, Ebert D. - BASF DocID 1999/11791*; *CA 7.2.2.4/2, Ebert D. – BASF DocID 2011/1101715; old EU Dossier, A III M 10.2.2/1, Dohmen G. P. - BASF DocID 2000/1000011*]. In order to reflect the different test designs in the model calculations a tiered approach was considered.

At **Tier 1** the assessment was based on the results of the water/sediment study conducted under dark laboratory conditions [*Staudenmaier H. - BASF DocID 1999/11241*]. The degradation kinetics were re-evaluated in a separate study [*CA 7.2.2.3/1, Wiedemann G. – BASF DocID 2012/1165029*]. At Level P-I, for System A whole system DT_{50} values of 23.3 and 26.8 days were reported for chlorophenyl- and tolyl-labeled pyraclostrobin, respectively, while for System B no reliable endpoints could be derived. The kinetic evaluation at Level P-II did not result in reliable endpoints. According to FOCUS kinetics a case-by-case decision should be made if reliable endpoints (P-I, System B) cannot be derived with the recommended standard procedures. For System B a $DegT_{90}$ in the whole system of approximately 30 days can be estimated visually, which is considerably shorter than the endpoint calculated for System A. Hence, the results of the kinetic evaluation of System A represent the worst-case of the two w/s systems investigated. Consequently, only the results of the kinetic evaluation of System A are to be considered as worst-case for further usage. In accordance with FOCUS degradation kinetics [*FOCUS (2006)*] the geometric mean DT_{50} in the total system of 25.0 days was considered for the total system at Step 1. Due to the high sorption tendency of pyraclostrobin the total system DT_{50} value of 25.0 days was used at Step 2 and 3 for the sediment phase, while the conservative default value of 1000 days was used for the water phase.

At **Tier 2** the assessment was based on the results of the water/sediment-studies conducted under irradiated laboratory conditions [*Ebert D. - BASF DocID 1999/11791*; *Ebert D. – BASF DocID 2011/1101715*]. While in *Ebert D.* [*BASF DocID 2011/1101715*] degradation endpoints could be derived only at Level P-I, the kinetic re-evaluation of *Ebert D.* [*BASF DocID 1999/11791*] resulted in appropriate degradation endpoints at Level P-I and Level P-II [*CA 7.2.2.4/1, Miles B. - BASF DocID 2012/1021122*]. The reported endpoints (dissipation half-life in water of 4.47 days) are considered as a realistic worst-case scenario compared to the outdoor mesocosm study, where a dissipation half-life in water of 3.5 days was reported. Consequently, the respective Level P-II degradation half-lives of 7.50 days in water and 6.48 days in sediment were used as input for modeling at Step 2 and 3 while for Step 1 calculations a $DegT_{50}$ in the total system calculated at Level P-I of 7.2 days was considered in accordance with FOCUS kinetics [*FOCUS (2006)*].

Metabolites of pyraclostrobin

In the dark water/sediment study the metabolites BF 500-3, BF 500-6 and BF 500-7 occurred as major metabolites [*Staudenmaier H. - BASF DocID 1999/11241*]. Additionally, BF 500-3, BF 500-11, BF 500-13 and BF 500-14 occurred in relevant amounts in the irradiated water/sediment study [*Ebert D. - BASF DocID 1999/11791*], and BF 500-5 was detected as major metabolite in the study on aerobic mineralization in surface water [*CA 7.2.2.2/1, Ebert D., Possienke M. – BASF DocID 2013/1002741*]. An overview of the DT_{50} values for the metabolites of pyraclostrobin in water/sediment systems is shown at the end of M-CA 7.2.2.

BF 500-3 was observed at amounts >5% TAR in the sediment compartment of the water/sediment studies under dark as well as under irradiated conditions, whereas in the water compartment no residues above 5% TAR were detected [*Staudenmaier H. - BASF DocID 1999/11241; Ebert D. - BASF DocID 1999/11791; Ebert D. – BASF DocID 2011/1101715*]. Therefore, PEC_{sed} calculations were conducted considering the results from the dark and the irradiated studies separately. Regarding the calculations representing the dark conditions, the maximum occurrence in the total system (mean of two labels) of 67.7% TAR [*Staudenmaier H. - BASF DocID 1999/11241*] and a conservative default DT_{50} of 1000 days were used, while for irradiated conditions the maximum occurrence in the total system of 21.9% TAR and a DT_{50} in the total system of 92.5 days from the kinetic re-evaluation were considered [*Ebert D. - BASF DocID 1999/11791; CA 7.2.2.4/1, Miles B. - BASF DocID 2012/1021122*].

BF 500-5 was observed as major metabolite in the water phase of the study on aerobic mineralization in surface water, whereas it did not occur in relevant amounts in the sediment phase [*CA 7.2.2.2/1, Ebert D., Possienke M. – BASF DocID 2013/1002741*]. Therefore, PEC_{sw} calculations were conducted considering the maximum occurrence of 10.9% TAR observed in the pelagic test and a conservative default DT_{50} of 1000 days.

BF 500-6 and BF 500-7 were observed at amounts >5% TAR in the sediment compartment of the water/sediment study under dark conditions, whereas in the water compartment no residues above 5% TAR were detected [*Staudenmaier H. - BASF DocID 1999/11241*]. Therefore, PEC_{sed} calculations were conducted considering the maximum occurrences of 6.5% TAR and 6.3% TAR (mean of two labels) for BF 500-6 and BF 500-7, respectively, and a conservative default DT_{50} of 1000 days.

BF 500-11, BF 500-13 and BF 500-14 were observed as major metabolites under irradiated conditions in the water compartment, whereas they did not occur in relevant amounts in the sediment compartment [*Ebert D. - BASF DocID 1999/11791*]. Therefore, PEC_{sw} calculations were conducted considering the sum of the maximum occurrences in water and sediment of 12.0% TAR, 17.8% TAR and 12.1% TAR for BF 500-11, BF 500-13 and BF 500-14, respectively. For BF 500-11 and BF 500-14 the DT_{50} values in the total system of 22.6 days and 17.3 days from the kinetic re-evaluation were considered, respectively [*CA 7.2.2.4/1, Miles B. - BASF DocID 2012/1021122*]. For BF 500-13 a reliable half-life could not be estimated from the data of the irradiated water/sediment study, because the maximum occurrence of the metabolite was at the last sampling date [*Ebert D. - BASF DocID 1999/11791*]. However, results of the aqueous photolysis study show a significant degradation of BF 500-13 in water with a calculated half-life of 30.7 days (rounded value) under continuous irradiation [*old EU Dossier, A II M 7.2.1.2/2, Scharf J. - BASF DocID 1999/11286*]. In order to represent a realistic radiation scenario (12 h radiation followed by 12 h darkness) the reported half-life was multiplied with a factor of 2 resulting in a DT_{50} of 61.3 days, which was used as input parameter for PEC_{sed} calculations.

Surface water assessment according to FOCUS

The calculations were performed in a stepwise approach according to the recommendations of the FOCUS working group on surface water scenarios (2001, 2012).

For all calculations, the following model versions were used: STEPS1-2 in FOCUS version 2.1, FOCUS-PRZM version 1.5.6, FOCUS-MACRO version 4.4.2 and FOCUS-TOXSWA version 3.3.1. The modeling runs were set up with SWASH version 3.1 and PEC Robot v1.4.

Setup of FOCUS surface water runs

Pyraclostrobin

FOCUS Step 1 to Step 4 calculations were carried out for PEC_{sw} and PEC_{sed} for pyraclostrobin considering the entry routes spray drift, drainage and runoff for spray application to winter and spring cereals as well as maize.

STEP 1 and Step 2 scenario settings

At Step 1 and 2, appropriate application periods and parameters for crop interception according to the actual GAP for cereals and maize were considered as shown in Table 9.2.5-12. The regions 'North Europe' and 'South Europe' as implemented in FOCUS STEPS1-2 were considered.

Table 9.2.5-12: Steps 1-2 FOCUS scenarios for the selected FOCUS crops

$FOCUS_{sw}$ crop	Interception class	Application period	Region
Winter cereals	Average crop cover	Mar – May	North and South Europe
Spring cereals			
Maize			

Step 3 scenario settings

At Step 3, all FOCUS scenarios parameterized for winter cereals (D1 - D6, R1, R3, R4), spring cereals (D1, D3 - D5, R4) and maize (D3 - D6, R1 - R4) were selected for the simulations. In the SWASH shell the application method was set to 'ground spray'. The chemical application method (CAM) was set to option '2' (application to foliage). The length of the application window, that is required for the Pesticide Application Tool (PAT) to determine actual application dates, was chosen to cover the whole application window as specified in the GAP. For single application (as for maize) a minimum application window of 30 days is required. For multiple application to cereals, considering the minimum application interval of 21 days, a minimum length of the application window of 51 days is required. The actual dates for field application differ according to the crop development in the different European regions and from year to year.

For winter cereals appropriate dates for the first treatment indicating growth stage 25 were selected for Northern Europe (15th April), Middle Europe (15th March) and Southern Europe (15th February) and were assigned to the ten FOCUS scenarios. The start date of the application window for application to spring cereals was assumed 28 days after application. For both, winter and spring cereals, the last application was assumed 35 days prior to harvest (derived from the pre-harvest interval (PHI) provided in the GAP).

For application to maize, the first application was scheduled at BBCH stage 30, which was assumed to take place 14 days after emergence. The time interval between BBCH growth stages 30 and 65 (first and last application, respectively) was set to 110 days.

The detailed application timing used for the simulations is shown in Table 9.2.5-13 to Table 9.2.5-15.

Table 9.2.5-13: Application timing for pyraclostrobin in winter cereals in the relevant scenarios (Step 3 and 4)

Scenario	Water body	Application window[a]	Application dates according to PAT[b]
Winter cereals			
D1 - Lanna	ditch	15th April - 22nd July	25th April / 16th May
D1 - Lanna	stream	15th April - 22nd July	25th April / 16th May
D2 - Brimstone	ditch	15th March - 26th May	15th March / 7th May
D2 - Brimstone	stream	15th March - 26th May	15th March / 7th May
D3 - Vredepeel	ditch	15th March - 11th July	16th March / 6th April
D4 - Skousbo	pond	15th March - 17th July	19th March / 18th April
D4 - Skousbo	stream	15th March - 17th July	19th March / 18th April
D5 - La Jailliere	pond	15th March - 10th June	8th April / 11th May
D5 - La Jailliere	stream	15th March - 10th June	8th April / 11th May
D6 - Thiva	ditch	15th February - 26th May	27th February / 9th April
R1 - Weiherbach	pond	15th March - 26th June	17th March / 26th April
R1 - Weiherbach	stream	15th March - 26th June	17th March / 26th April
R3 - Bologna	stream	15th February - 27th May	19th February / 20th March
R4 - Roujan	stream	15th February - 10th June	2nd March / 4th April

[a] calculations for single and multiple applications were conducted in separate runs
[b] for calculation of single application the first date was considered

Table 9.2.5-14: Application timing for pyraclostrobin in spring cereals in the relevant scenarios (Step 3 and 4)

Scenario	Water body	Application window[a]	Application dates according to PAT[b]
Spring cereals			
D1 - Lanna	ditch	2nd June - 31st July	17th June / 8th July
D1 - Lanna	stream	2nd June - 31st July	17th June / 8th July
D3 - Vredepeel	ditch	29th April - 16th July	4th May / 27th May
D4 - Skousbo	pond	24th May - 22nd July	30th May / 4th July
D4 - Skousbo	stream	24th May - 22nd July	30th May / 4th July
D5 - La Jailliere	pond	12th April - 15th June	14th April / 11th May
D5 - La Jailliere	stream	12th April - 15th June	14th April / 11th May
R4 - Roujan	stream	12th April - 15th June	4th May / 27th May

[a] calculations for single and multiple applications were conducted in separate runs
[b] for calculation of single application the first date was considered

Table 9.2.5-15: Application timing for pyraclostrobin in maize in the relevant scenarios (Step 3 and 4)

Scenario	Water body	Application window	Application dates according to PAT
Maize			
D3 - Vredepeel	ditch	19th May - 6th September	18th May
D4 - Skousbo	pond	24th May - 11th September	30th May
D4 - Skousbo	stream	24th May - 11th September	30th May
D5 - La Jailliere	pond	24th May - 11th September	27th May
D5 - La Jailliere	stream	24th May - 11th September	27th May
D6 - Thiva	ditch	4th May - 22nd August	14th May
R1 - Weiherbach	pond	17th May - 4th September	13th June
R1 - Weiherbach	stream	17th May - 4th September	13th June
R2 - Porto	stream	15th May - 2nd September	20th May
R3 - Bologna	stream	15th May - 2nd September	18th May
R4 - Roujan	stream	24th April - 12th August	4th May

Mitigation measures at Step 4

At Step 4, drift mitigation of pyraclostrobin due to no-spray buffer zones was taken into account for all FOCUS scenarios parameterized in Step 3. Buffer zones of 5 m up to 20 m for winter and spring cereals and 5 m to 10 m for maize were selected directly from the spray drift mitigation page of PEC Robot. For all crops, drift reduction of 50%, 75% and 90% due to drift reducing nozzles was additionally taken into account. Furthermore, mitigation of the runoff/erosion entry into the surface water bodies by a 10 m or 20 m vegetated filter strip was considered.

The runoff/erosion reduction factors recommended by the FOCUS working group on landscape and mitigation factors in ecological risk assessments [*FOCUS (2007): "Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations". Report of the FOCUS Working Group on Landscape and Mitigation Factors in Ecological Risk Assessment, EC Document Reference SANCO/10422/2005 v2.0. 169 pp., FOCUS (2007a): "Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 2. Detailed Technical Reviews". Report of the FOCUS Working Group on Landscape and Mitigation Factors in Ecological Risk Assessment, EC Document Reference SANCO/10422/2005 v2.0. 436 pp.*] were considered. Table 9.2.5-16 gives an overview on the runoff/erosion reduction factors used in the assessment.

Table 9.2.5-16: Filtration capacity (factor for the reduction of runoff volume, sediment mass and corresponding pesticide loadings) by vegetated filter strips of 10 m and 20 m for Step 4 calculations

Width of planted buffer strips [m]	Reduction in runoff flux and volume [%]	Reduction in erosion flux and sediment mass [%]	Reference
10	60	85	*FOCUS (2007)*
20	80	95	

Metabolites of pyraclostrobin

For the metabolites of pyraclostrobin, PEC values were calculated at FOCUS Step 1 and 2. The results of the calculations are reported for those compartments where the respective metabolites had been detected as major metabolites. The simulations were carried out according to the approach and application scenarios for the parent compound.

Overview of the FOCUS levels used for the simulations

Table 9.2.5-17 gives an overview of the FOCUS levels, at which the PEC values of the respective compounds were simulated.

Table 9.2.5-17: Summary of the selection of the FOCUS levels for each compound

Compound	PEC_{sw} calculations conducted with FOCUS Step	PEC_{sed} calculations conducted with FOCUS Step	Reported PEC_{sw}	Reported PEC_{sed}
Pyraclostrobin	1, 2, 3, 4	1, 2, 3	max, act, twa	max
BF 500-3	-[1]	1, 2	-[1]	max
BF 500-5	1, 2	-[2]	max	-[2]
BF 500-6	-[3]	1, 2	-[3]	max
BF 500-7	-[3]	1, 2	-[3]	max
BF 500-11	1, 2	-[4]	max	-[4]
BF 500-13	1, 2	-[4]	max	-[4]
BF 500-14	1, 2	-[4]	max	-[4]

[1] BF 500-3 did not occur in soil or water phase in significant amounts
[2] BF 500-5 did not occur in soil or sediment phase in significant amounts
[3] BF 500-6 and BF 500-7 will not occur free in water phase due to low water solubility and high sorption values
[4] BF 500-11, BF 500-13 and BF 500-14 did not occur in soil or sediment phase in significant amounts

Pyraclostrobin

For a summary of the environmental fate parameters of pyraclostrobin used for modeling at Step 1 to Step 4 of the assessment see Table 9.2.5-18. The input parameters for the metabolites are summarized in Table 9.2.5-19 to Table 9.2.5-25. Some default assumptions were made as the measured data could not cover all required model input, e.g. solubility in water, maximum occurrence in soil, DT_{50} in soil and K_{oc} of the aquatic metabolites BF 500-11, BF 500-13 and BF 500-14.

Table 9.2.5-18: Summary of FOCUS input parameters for pyraclostrobin

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	
Molecular weight [g mol^{-1}]	387.8	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	1.9	Phys.-chem. properties
Vapor pressure at 20°C [Pa]	2.6×10^{-8}	Phys.-chem. properties
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	28.3	Bias-corrected geometric mean of field study
Temperature correction function Reference temperature [°C] MACRO temp. exponent [K^{-1}] PRZM Q_{10} [-]	 20 0.095 2.58	EFSA opinion
Moisture correction function Reference moisture [-] PRZM / MACRO moisture exponent [-]	 pF 2 0.7	FOCUS recommendation
SORPTION TO SOIL		
$K_{f,oc}$ [mL g^{-1}]	9304	Arithmetic mean (n=6)
1/n [-]	0.95	Arithmetic mean (n=6)
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1) - Tier 1 - Tier 2	 25.0 7.2	 System $DegT_{50}$ level P-I (dark study) System $DegT_{50}$ level P-I (irradiated study)
DT_{50} water [d] (Step 2, Step 3, Step 4) - Tier 1 - Tier 2	 1000 7.5	 Conservative assumption Water $DegT_{50}$ level P-II (irradiated study)
DT_{50} sediment [d] (Step 2, Step 3, Step 4) - Tier 1 - Tier 2	 25.0 6.5	 System $DegT_{50}$ level P-I (dark study) Sediment $DegT_{50}$ level P-II (irradiated study)
DT_{50} crop [d] (Step 3, Step 4)	10	FOCUS recommendation
Temperature correction function Reference temperature [°C] TOXSWA: activation energy [J mol^{-1}]	 20 65400	EFSA opinion
MANAGEMENT RELATED PARAMETERS		
Crop uptake factor [-]	0	
Wash off coefficient PRZM: [cm^{-1}] MACRO: [mm^{-1}]	 0.5 0.05	FOCUS recommendation

Table 9.2.5-19: Summary of FOCUS input parameters for BF 500-3

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	357.8	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	0.03	Phys.-chem. properties
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	1	Worst-case default value
Max. observed occurrence in soil [%]	0.01	Worst-case default value
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	9315	Arithmetic mean (n=6)
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1) - parameters from dark study - parameters from irradiated study	 1000 92.5	 Worst-case default value System DT_{50} level M-I (irradiated study)
DT_{50} water [d] (Step 2) - parameters from dark study - parameters from irradiated study	 1000 92.5	 Worst-case default value System DT_{50} level M-I (irradiated study)
DT_{50} sediment [d] (Step 2) - parameters from dark study - parameters from irradiated study	 1000 92.5	 Worst-case default value System DT_{50} level M-I (irradiated study)
Sum of max. observed occurrence in water/sediment [%] - parameters from dark study - parameters from irradiated study	 67.7 21.9	 Dark water/sediment study Irradiated water/sediment study

Table 9.2.5-20: Summary of FOCUS input parameters for BF 500-5

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	194.6	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	21.8	
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	1	Worst-case default value
Max. observed occurrence in soil [%]	0.01	Worst-case default value
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	705	Arithmetic mean (n=5)
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	1000	Worst-case default value
DT_{50} water [d] (Step 2)	1000	Worst-case default value
DT_{50} sediment [d] (Step 2)	1000	Worst-case default value
Sum of max. observed occurrence in water/sediment [%]	10.9	Study on aerobic mineralization in surface water

Table 9.2.5-21: Summary of FOCUS input parameters for BF 500-6

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	305.8	0.5 of molar mass as metabolite is a dimer
Water solubility at 20°C [mg L^{-1}]	0.003	Phys.-chem. properties
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	321.0	Bias-corrected geometric mean from pooled laboratory and field studies
Max. observed occurrence in soil [%]	31.0	Maximum in laboratory study
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	107301	Median (n=11)
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	1000	Worst-case default value
DT_{50} water [d] (Step 2)	1000	Worst-case default value
DT_{50} sediment [d] (Step 2)	1000	Worst-case default value
Sum of max. observed occurrence in water/sediment [%]	6.5	Dark water/sediment study

Table 9.2.5-22: Summary of FOCUS input parameters for BF 500-7

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	297.8	0.5 of molar mass as metabolite is a dimer
Water solubility at 20°C [mg L^{-1}]	0.005	Phys.-chem. properties
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	312.0	Bias-corrected geometric mean from pooled laboratory and field studies
Max. observed occurrence in soil [%]	19.0	Maximum in field degradation study
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	149900	Median (n=11)
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	1000	Worst-case default value
DT_{50} water [d] (Step 2)	1000	Worst-case default value
DT_{50} sediment [d] (Step 2)	1000	Worst-case default value
Sum of max. observed occurrence in water/sediment [%]	6.3	Dark water/sediment study

Table 9.2.5-23: Summary of FOCUS input parameters for BF 500-11

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	277.3	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	1000	Worst-case default value
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	1	Worst-case default value
Max. observed occurrence in soil [%]	0.01	Worst-case default value
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	1×10^{-10}	Worst-case default value
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	22.6	System DT_{50} level M-I, irradiated water/sediment study
DT_{50} water [d] (Step 2)	22.6	System DT_{50} level M-I, irradiated water/sediment study
DT_{50} sediment [d] (Step 2)	22.6	System DT_{50} level M-I, irradiated water/sediment study
Sum of max. observed occurrence in water/sediment [%]	12.0	Irradiated water/sediment study

Table 9.2.5-24: Summary of FOCUS input parameters for BF 500-13

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	247.3	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	1000	Worst-case default value
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	1	Worst-case default value
Max. observed occurrence in soil [%]	0.01	Worst-case default value
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	1 x 10^{-10}	Worst-case default value
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	1000	Worst-case default value
DT_{50} water [d] (Step 2)	61.3	Aqueous photolysis study
DT_{50} sediment [d] (Step 2)	1000	Worst-case default value
Sum of max. observed occurrence in water/sediment [%]	17.8	Irradiated water/sediment study

Table 9.2.5-25: Summary of FOCUS input parameters for BF 500-14

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	387.8	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	1000	Worst-case default value
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	1	Worst-case default value
Max. observed occurrence in soil [%]	0.01	Worst-case default value
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	1 x 10^{-10}	Worst-case default value
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	17.3	System DT_{50} level M-I, irradiated water/sediment study
DT_{50} water [d] (Step 2)	17.3	System DT_{50} level M-I, irradiated water/sediment study
DT_{50} sediment [d] (Step 2)	17.3	System DT_{50} level M-I, irradiated water/sediment study
Sum of max. observed occurrence in water/sediment [%]	12.1	Irradiated water/sediment study

II. RESULTS AND DISCUSSION

All calculated PEC_{sw} and PEC_{sed} values are available in the study report. For the sake of compactness, only the maximum concentrations of pyraclostrobin and its metabolites in surface water (PEC_{sw}) and in sediment (PEC_{sed}) are reported below. Actual and time-weighted average concentrations of pyraclostrobin in surface water are presented exemplarily for application to winter cereals at Tier 1 as it provides the worst-case PECs.

SURFACE WATER

Global maximum concentrations – pyraclostrobin

Step 1 and 2

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-26: Steps 1-2: $PEC_{sw,max}$ of pyraclostrobin following application to various crops (Tier 1)

$FOCUS_{sw}$ crop	Pyraclostrobin $PEC_{sw,max}$ [µg L^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	8.516	17.031	2.299	2.168	2.299	2.168
Maize	6.813	-[a]	1.839	-[a]	1.839	-[a]

[a] Only single application calculated for maize

Tier 2 (parameters from irradiated water/sediment study)

Table 9.2.5-27: Steps 1-2: $PEC_{sw,max}$ of pyraclostrobin following application to various crops (Tier 2)

$FOCUS_{sw}$ crop	Pyraclostrobin $PEC_{sw,max}$ [µg L^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	8.516	17.031	2.299	2.056	2.299	2.056
Maize	6.813	-[a]	1.839	-[a]	1.839	-[a]

[a] Only single application calculated for maize

Step 3 and 4

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-28: Step 3 and 4: $PEC_{sw,max}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4 (Tier 1)

			Winter cereals												
			Single application												
Location	Water body		Step 3	Step 4											
			Edge-of-Field	50N	75N	90N	5mD	5mD 50N	5mD 75N	10mD	10mD 50N	10mD +R	15mD	20mD	20mD +R
D1	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.584 Drift	0.792 Drift	0.396 Drift	0.158 Drift	0.429 Drift	0.214 Drift	0.107 Drift	0.228 Drift	0.114 Drift	0.228 Drift	0.155 Drift	0.118 Drift	0.118 Drift
D1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.346 Drift	0.673 Drift	0.336 Drift	0.134 Drift	0.491 Drift	0.246 Drift	0.123 Drift	0.260 Drift	0.130 Drift	0.260 Drift	0.178 Drift	0.135 Drift	0.135 Drift
D2	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.589 Drift	0.794 Drift	0.397 Drift	0.159 Drift	0.431 Drift	0.215 Drift	0.108 Drift	0.228 Drift	0.114 Drift	0.228 Drift	0.156 Drift	0.119 Drift	0.119 Drift
D2	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.414 Drift	0.707 Drift	0.353 Drift	0.141 Drift	0.516 Drift	0.258 Drift	0.129 Drift	0.274 Drift	0.137 Drift	0.274 Drift	0.187 Drift	0.142 Drift	0.142 Drift
D3	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.567 Drift	0.783 Drift	0.392 Drift	0.157 Drift	0.424 Drift	0.212 Drift	0.106 Drift	0.225 Drift	0.112 Drift	0.225 Drift	0.154 Drift	0.117 Drift	0.117 Drift
D4	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.054 Drift	0.027 Drift	0.014 Drift	0.005 Drift	0.047 Drift	0.023 Drift	0.012 Drift	0.034 Drift	0.017 Drift	0.034 Drift	0.027 Drift	0.022 Drift	0.022 Drift
D4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.180 Drift	0.590 Drift	0.295 Drift	0.118 Drift	0.431 Drift	0.215 Drift	0.108 Drift	0.228 Drift	0.114 Drift	0.228 Drift	0.156 Drift	0.119 Drift	0.119 Drift
D5	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.054 Drift	0.027 Drift	0.014 Drift	0.005 Drift	0.047 Drift	0.023 Drift	0.012 Drift	0.034 Drift	0.017 Drift	0.034 Drift	0.027 Drift	0.022 Drift	0.022 Drift
D5	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.263 Drift	0.631 Drift	0.316 Drift	0.126 Drift	0.461 Drift	0.231 Drift	0.115 Drift	0.244 Drift	0.122 Drift	0.244 Drift	0.167 Drift	0.127 Drift	0.127 Drift
D6	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.547 Drift	0.773 Drift	0.387 Drift	0.155 Drift	0.419 Drift	0.210 Drift	0.105 Drift	0.222 Drift	0.111 Drift	0.222 Drift	0.152 Drift	0.115 Drift	0.115 Drift
R1	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.054 Drift	0.027 Drift	0.016 Runoff	0.014 Runoff	0.047 Drift	0.023 Drift	0.015 Runoff	0.034 Drift	0.017 Drift	0.034 Drift	0.027 Drift	0.022 Drift	0.022 Drift
R1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.037 Drift	0.518 Drift	0.259 Drift	0.159 Runoff	0.379 Drift	0.189 Drift	0.159 Runoff	0.201 Drift	0.159 Runoff	0.201 Drift	0.159 Runoff	0.159 Runoff	0.104 Drift

			Winter cereals												
			Single application												
Location	Water body		Step 3	Step 4											
			Edge-of-Field	50N	75N	90N	5mD	5mD 50N	5mD 75N	10mD	10mD 50N	10mD +R	15mD	20mD	20mD +R
R3	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.463 Drift	0.731 Drift	0.365 Drift	0.163 Runoff	0.534 Drift	0.267 Drift	0.163 Runoff	0.283 Drift	0.163 Runoff	0.283 Drift	0.193 Drift	0.163 Runoff	0.147 Drift
R4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.038 Drift	0.519 Drift	0.296 Runoff	0.296 Runoff	0.379 Drift	0.296 Runoff	0.296 Runoff	0.296 Runoff	0.296 Runoff	0.201 Drift	0.296 Runoff	0.296 Runoff	0.104 Drift

N = Drift mitigation by nozzle reduction, D = Drift mitigation by no-spray buffer zones, R = Runoff mitigation by vegetated filter strips

Table 9.2.5-29: Step 3 and 4: $PEC_{sw,max}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4 (Tier 1)

			Winter cereals												
			Twofold application												
Location	Water body		Step 3	Step 4											
			Edge-of-Field	50N	75N	90N	5mD	5mD 50N	5mD 75N	10mD	10mD 50N	10mD +R	15mD	20mD	20mD +R
D1	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.504 Drift	0.751 Drift	0.375 Drift	0.150 Drift	0.389 Drift	0.194 Drift	0.097 Drift	0.202 Drift	0.101 Drift	0.202 Drift	0.136 Drift	0.102 Drift	0.102 Drift
D1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.201 Drift	0.600 Drift	0.300 Drift	0.120 Drift	0.424 Drift	0.212 Drift	0.106 Drift	0.220 Drift	0.110 Drift	0.220 Drift	0.148 Drift	0.112 Drift	0.112 Drift
D2	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.396 Drift	0.698 Drift	0.349 Drift	0.140 Drift	0.362 Drift	0.181 Drift	0.091 Drift	0.188 Drift	0.094 Drift	0.188 Drift	0.127 Drift	0.095 Drift	0.095 Drift
D2	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.225 Drift	0.612 Drift	0.306 Drift	0.122 Drift	0.432 Drift	0.216 Drift	0.108 Drift	0.225 Drift	0.112 Drift	0.225 Drift	0.151 Drift	0.114 Drift	0.114 Drift
D3	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.370 Drift	0.685 Drift	0.342 Drift	0.137 Drift	0.355 Drift	0.178 Drift	0.089 Drift	0.184 Drift	0.092 Drift	0.184 Drift	0.125 Drift	0.094 Drift	0.094 Drift
D4	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.065 Drift	0.033 Drift	0.016 Drift	0.007 Drift	0.056 Drift	0.028 Drift	0.014 Drift	0.040 Drift	0.020 Drift	0.040 Drift	0.031 Drift	0.026 Drift	0.026 Drift

			Winter cereals											
			Twofold application											
Location	Water body		Step 3	Step 4										
			Edge-of-Field	50N	75N	90N	5mD	5mD 50N	5mD 75N	10mD	10mD 50N	10mD +R	15mD	20mD	20mD +R
D4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.076 Drift	0.538 Drift	0.269 Drift	0.107 Drift	0.380 Drift	0.190 Drift	0.095 Drift	0.197 Drift	0.099 Drift	0.197 Drift	0.133 Drift	0.100 Drift	0.100 Drift
D5	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.066 Drift	0.033 Drift	0.017 Drift	0.007 Drift	0.057 Drift	0.029 Drift	0.014 Drift	0.040 Drift	0.020 Drift	0.040 Drift	0.032 Drift	0.027 Drift	0.027 Drift
D5	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.251 Drift	0.625 Drift	0.312 Drift	0.125 Drift	0.442 Drift	0.221 Drift	0.110 Drift	0.229 Drift	0.114 Drift	0.229 Drift	0.155 Drift	0.116 Drift	0.116 Drift
D6	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.381 Drift	0.691 Drift	0.345 Drift	0.138 Drift	0.358 Drift	0.179 Drift	0.090 Drift	0.186 Drift	0.093 Drift	0.186 Drift	0.126 Drift	0.094 Drift	0.094 Drift
R1	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.065 Drift	0.042 Runoff	0.035 Runoff	0.031 Runoff	0.056 Drift	0.040 Runoff	0.034 Runoff	0.046 Runoff	0.036 Runoff	0.039 Drift	0.042 Runoff	0.039 Runoff	0.026 Drift
R1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.897 Drift	0.448 Drift	0.240 Runoff	0.240 Runoff	0.317 Drift	0.240 Runoff	0.240 Runoff	0.240 Runoff	0.240 Runoff	0.164 Drift	0.240 Runoff	0.240 Runoff	0.084 Drift
R3	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.265 Drift	0.632 Drift	0.316 Drift	0.163 Runoff	0.447 Drift	0.223 Drift	0.163 Runoff	0.232 Drift	0.163 Runoff	0.232 Drift	0.163 Runoff	0.163 Runoff	0.118 Drift
R4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.898 Drift	0.543 Runoff	0.543 Runoff	0.543 Runoff	0.543 Runoff	0.543 Runoff	0.543 Runoff	0.543 Runoff	0.543 Runoff	0.248 Runoff	0.543 Runoff	0.543 Runoff	0.130 Runoff

N = Drift mitigation by nozzle reduction, D = Drift mitigation by no-spray buffer zones, R = Runoff mitigation by vegetated filter strips

Table 9.2.5-30: Step 3 and 4: $PEC_{sw,max}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to spring cereals, considering drift and runoff mitigation at Step 4 (Tier 1)

			Spring cereals												
			Single application												
Location	Water body		Step 3	Step 4											
			Edge-of-Field	50N	75N	90N	5mD	5mD 50N	5mD 75N	10mD	10mD 50N	10mD +R	15mD	20mD	20mD +R
D1	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.588 Drift	0.794 Drift	0.397 Drift	0.159 Drift	0.430 Drift	0.215 Drift	0.108 Drift	0.228 Drift	0.114 Drift	0.228 Drift	0.156 Drift	0.118 Drift	0.118 Drift
D1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.389 Drift	0.694 Drift	0.347 Drift	0.139 Drift	0.507 Drift	0.254 Drift	0.127 Drift	0.269 Drift	0.134 Drift	0.269 Drift	0.184 Drift	0.140 Drift	0.140 Drift
D3	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.569 Drift	0.784 Drift	0.392 Drift	0.157 Drift	0.425 Drift	0.212 Drift	0.106 Drift	0.225 Drift	0.113 Drift	0.225 Drift	0.154 Drift	0.117 Drift	0.117 Drift
D4	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.054 Drift	0.027 Drift	0.014 Drift	0.005 Drift	0.047 Drift	0.023 Drift	0.012 Drift	0.034 Drift	0.017 Drift	0.034 Drift	0.027 Drift	0.022 Drift	0.022 Drift
D4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.300 Drift	0.650 Drift	0.325 Drift	0.130 Drift	0.474 Drift	0.237 Drift	0.119 Drift	0.252 Drift	0.126 Drift	0.252 Drift	0.172 Drift	0.131 Drift	0.131 Drift
D5	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.054 Drift	0.027 Drift	0.014 Drift	0.005 Drift	0.047 Drift	0.023 Drift	0.012 Drift	0.034 Drift	0.017 Drift	0.034 Drift	0.027 Drift	0.022 Drift	0.022 Drift
D5	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.319 Drift	0.659 Drift	0.329 Drift	0.132 Drift	0.481 Drift	0.241 Drift	0.120 Drift	0.255 Drift	0.128 Drift	0.255 Drift	0.174 Drift	0.133 Drift	0.133 Drift
R4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.038 Drift	0.519 Drift	0.267 Runoff	0.267 Runoff	0.379 Drift	0.267 Runoff	0.267 Runoff	0.267 Runoff	0.267 Runoff	0.201 Drift	0.267 Runoff	0.267 Runoff	0.104 Drift

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips

Table 9.2.5-31: Step 3 and 4: $PEC_{sw,max}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to spring cereals, considering drift and runoff mitigation at Step 4 (Tier 1)

			Spring cereals												
			Twofold application												
Location	Water body		Step 3	Step 4											
			Edge-of-Field	50N	75N	90N	5mD	5mD 50N	5mD 75N	10mD	10mD 50N	10mD +R	15mD	20mD	20mD +R
D1	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.759 Drift	0.877 Drift	0.437 Drift	0.174 Drift	0.453 Drift	0.226 Drift	0.113 Drift	0.235 Drift	0.117 Drift	0.235 Drift	0.158 Drift	0.119 Drift	0.119 Drift
D1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.201 Drift	0.601 Drift	0.300 Drift	0.120 Drift	0.424 Drift	0.212 Drift	0.106 Drift	0.220 Drift	0.110 Drift	0.220 Drift	0.148 Drift	0.112 Drift	0.112 Drift
D3	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.374 Drift	0.687 Drift	0.343 Drift	0.137 Drift	0.356 Drift	0.178 Drift	0.089 Drift	0.185 Drift	0.093 Drift	0.185 Drift	0.125 Drift	0.094 Drift	0.094 Drift
D4	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.065 Drift	0.033 Drift	0.016 Drift	0.006 Drift	0.056 Drift	0.028 Drift	0.014 Drift	0.040 Drift	0.020 Drift	0.040 Drift	0.031 Drift	0.026 Drift	0.026 Drift
D4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.171 Drift	0.586 Drift	0.293 Drift	0.117 Drift	0.414 Drift	0.207 Drift	0.103 Drift	0.215 Drift	0.107 Drift	0.215 Drift	0.145 Drift	0.109 Drift	0.109 Drift
D5	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.068 Drift	0.034 Drift	0.017 Drift	0.007 Drift	0.058 Drift	0.029 Drift	0.015 Drift	0.041 Drift	0.021 Drift	0.041 Drift	0.033 Drift	0.027 Drift	0.027 Drift
D5	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.184 Drift	0.592 Drift	0.296 Drift	0.118 Drift	0.418 Drift	0.209 Drift	0.104 Drift	0.217 Drift	0.108 Drift	0.217 Drift	0.146 Drift	0.110 Drift	0.110 Drift
R4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.898 Drift	0.449 Drift	0.267 Runoff	0.267 Runoff	0.318 Drift	0.267 Runoff	0.267 Runoff	0.267 Runoff	0.267 Runoff	0.165 Drift	0.267 Runoff	0.267 Runoff	0.084 Drift

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips

Table 9.2.5-32: Step 3 and 4: $PEC_{sw,max}$ of pyraclostrobin following single application of 200 g a.s. ha^{-1} to maize, considering drift and runoff mitigation at Step 4 (Tier 1)

			Maize							
			Single application							
Location	Water body		Step 3	Step 4						
			Edge-of-Field	50N	75N	90N	5mD	5mD50N	10mD	10mD+R
D3	ditch	$PEC_{sw,max}$ [µg L^{-1}]	1.037	0.519	0.259	0.104	0.340	0.170	0.180	0.180
		main entry route	Drift	Drift	Drift	Drift	Drift	Drift	Drift	Drift
D4	pond	$PEC_{sw,max}$ [µg L^{-1}]	0.042	0.021	0.010	0.004	0.037	0.019	0.027	0.027
		main entry route	Drift	Drift	Drift	Drift	Drift	Drift	Drift	Drift
D4	stream	$PEC_{sw,max}$ [µg L^{-1}]	0.895	0.448	0.224	0.089	0.377	0.188	0.200	0.200
		main entry route	Drift	Drift	Drift	Drift	Drift	Drift	Drift	Drift
D5	pond	$PEC_{sw,max}$ [µg L^{-1}]	0.042	0.021	0.010	0.004	0.037	0.019	0.027	0.027
		main entry route	Drift	Drift	Drift	Drift	Drift	Drift	Drift	Drift
D5	stream	$PEC_{sw,max}$ [µg L^{-1}]	0.888	0.444	0.222	0.089	0.374	0.187	0.198	0.198
		main entry route	Drift	Drift	Drift	Drift	Drift	Drift	Drift	Drift
D6	ditch	$PEC_{sw,max}$ [µg L^{-1}]	1.029	0.514	0.257	0.103	0.337	0.168	0.179	0.179
		main entry route	Drift	Drift	Drift	Drift	Drift	Drift	Drift	Drift
R1	pond	$PEC_{sw,max}$ [µg L^{-1}]	0.042	0.026	0.018	0.013	0.038	0.024	0.030	0.027
		main entry route	Drift	Runoff	Runoff	Runoff	Runoff	Runoff	Runoff	Drift
R1	stream	$PEC_{sw,max}$ [µg L^{-1}]	0.720	0.360	0.180	0.153	0.303	0.153	0.161	0.161
		main entry route	Drift	Drift	Drift	Runoff	Drift	Runoff	Drift	Drift
R2	stream	$PEC_{sw,max}$ [µg L^{-1}]	0.963	0.482	0.241	0.096	0.405	0.203	0.215	0.215
		main entry route	Drift	Drift	Drift	Drift	Drift	Drift	Drift	Drift
R3	stream	$PEC_{sw,max}$ [µg L^{-1}]	1.011	0.505	0.253	0.193	0.425	0.213	0.225	0.225
		main entry route	Drift	Drift	Drift	Runoff	Drift	Drift	Drift	Drift
R4	stream	$PEC_{sw,max}$ [µg L^{-1}]	0.703	0.351	0.301	0.301	0.301	0.301	0.301	0.157
		main entry route	Drift	Drift	Runoff	Runoff	Runoff	Runoff	Runoff	Drift

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips

Tier 2 (parameters from irradiated water/sediment study)

For the single application pattern, $PEC_{sw,max}$ at Tier 2 are identical to the $PEC_{sw,max}$ at Tier 1. Consequently, at Tier 2 only $PEC_{sw,max}$ following multiple application to winter and spring cereals are reported here.
$PEC_{sw,max}$ for multiple application as well as $PEC_{sw,twa}$ are very similar for Tier 1 and Tier 2. Although degradation rates in the water compartment between Tier 1 and Tier 2 differ largely, this behavior can be explained by the predominance of the strong sorption behavior of pyraclostrobin to sediment particles against the degradation within the water compartment.

Table 9.2.5-33: Step 3 and 4: $PEC_{sw,max}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4 (Tier 2)

			Winter cereals										
			Twofold application										
Location	Water body		Step 3	Step 4									
			Edge-of-Field	90N	5mD	5mD 50N	5mD 75N	10mD	10mD 50N	10mD+ R	15mD	20mD	20mD+ R
D1	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.451 Drift	0.145 Drift	0.376 Drift	0.188 Drift	0.094 Drift	0.195 Drift	0.098 Drift	0.195 Drift	0.132 Drift	0.099 Drift	0.099 Drift
D1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.201 Drift	0.120 Drift	0.424 Drift	0.212 Drift	0.106 Drift	0.220 Drift	0.110 Drift	0.220 Drift	0.148 Drift	0.112 Drift	0.112 Drift
D2	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.391 Drift	0.139 Drift	0.361 Drift	0.180 Drift	0.090 Drift	0.187 Drift	0.094 Drift	0.187 Drift	0.126 Drift	0.095 Drift	0.095 Drift
D2	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.223 Drift	0.122 Drift	0.432 Drift	0.216 Drift	0.108 Drift	0.224 Drift	0.112 Drift	0.224 Drift	0.151 Drift	0.114 Drift	0.114 Drift
D3	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.370 Drift	0.137 Drift	0.355 Drift	0.178 Drift	0.089 Drift	0.184 Drift	0.092 Drift	0.184 Drift	0.125 Drift	0.094 Drift	0.094 Drift
D4	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.056 Drift	0.006 Drift	0.048 Drift	0.024 Drift	0.012 Drift	0.034 Drift	0.017 Drift	0.034 Drift	0.027 Drift	0.022 Drift	0.022 Drift
D4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.076 Drift	0.107 Drift	0.380 Drift	0.190 Drift	0.095 Drift	0.197 Drift	0.099 Drift	0.197 Drift	0.133 Drift	0.100 Drift	0.100 Drift
D5	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.051 Drift	0.005 Drift	0.044 Drift	0.022 Drift	0.011 Drift	0.031 Drift	0.016 Drift	0.031 Drift	0.024 Drift	0.020 Drift	0.020 Drift

Location	Water body		Winter cereals										
			Twofold application										
			Step 3	Step 4									
			Edge-of-Field	90N	5mD	5mD 50N	5mD 75N	10mD	10mD 50N	10mD+ R	15mD	20mD	20mD+ R
D5	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.251 Drift	0.125 Drift	0.442 Drift	0.221 Drift	0.110 Drift	0.229 Drift	0.114 Drift	0.229 Drift	0.155 Drift	0.116 Drift	0.116 Drift
D6	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.381 Drift	0.138 Drift	0.358 Drift	0.179 Drift	0.089 Drift	0.186 Drift	0.093 Drift	0.186 Drift	0.126 Drift	0.094 Drift	0.094 Drift
R1	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.052 Drift	0.023 Runoff	0.044 Drift	0.025 Runoff	0.023 Runoff	0.032 Drift	0.024 Runoff	0.031 Drift	0.025 Drift	0.024 Runoff	0.021 Drift
R1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.897 Drift	0.240 Runoff	0.317 Drift	0.240 Runoff	0.240 Runoff	0.240 Runoff	0.240 Runoff	0.164 Drift	0.240 Runoff	0.240 Runoff	0.084 Drift
R3	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.265 Drift	0.163 Runoff	0.447 Drift	0.223 Drift	0.163 Runoff	0.232 Drift	0.163 Runoff	0.232 Drift	0.163 Runoff	0.163 Runoff	0.118 Drift
R4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.898 Drift	0.543 Runoff	0.543 Runoff	0.543 Runoff	0.543 Runoff	0.543 Runoff	0.543 Runoff	0.248 Runoff	0.543 Runoff	0.543 Runoff	0.130 Runoff

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips

Table 9.2.5-34: Step 3 and 4: $PEC_{sw,max}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to spring cereals, considering drift and runoff mitigation at Step 4 (Tier 2)

Location	Water body		Spring cereals												
			Twofold application												
			Step 3	Step 4											
			Edge-of-Field	50N	75N	90N	5mD	5mD 50N	5mD 75N	10mD	10m 50N	10mD +R	15mD	20mD	20mD +R
D1	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.511 Drift	0.754 Drift	0.376 Drift	0.150 Drift	0.391 Drift	0.195 Drift	0.097 Drift	0.203 Drift	0.101 Drift	0.203 Drift	0.137 Drift	0.103 Drift	0.103 Drift
D1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.201 Drift	0.601 Drift	0.300 Drift	0.120 Drift	0.424 Drift	0.212 Drift	0.106 Drift	0.220 Drift	0.110 Drift	0.220 Drift	0.148 Drift	0.112 Drift	0.112 Drift
D3	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.374 Drift	0.687 Drift	0.343 Drift	0.137 Drift	0.356 Drift	0.178 Drift	0.089 Drift	0.185 Drift	0.093 Drift	0.185 Drift	0.125 Drift	0.094 Drift	0.094 Drift
D4	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.047 Drift	0.024 Drift	0.012 Drift	0.005 Drift	0.041 Drift	0.020 Drift	0.010 Drift	0.029 Drift	0.015 Drift	0.029 Drift	0.023 Drift	0.019 Drift	0.019 Drift
D4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.171 Drift	0.586 Drift	0.293 Drift	0.117 Drift	0.414 Drift	0.207 Drift	0.103 Drift	0.215 Drift	0.107 Drift	0.215 Drift	0.145 Drift	0.109 Drift	0.109 Drift
D5	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.053 Drift	0.026 Drift	0.013 Drift	0.005 Drift	0.045 Drift	0.023 Drift	0.011 Drift	0.032 Drift	0.016 Drift	0.032 Drift	0.025 Drift	0.021 Drift	0.021 Drift
D5	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	1.184 Drift	0.592 Drift	0.296 Drift	0.118 Drift	0.418 Drift	0.209 Drift	0.104 Drift	0.217 Drift	0.108 Drift	0.217 Drift	0.146 Drift	0.110 Drift	0.110 Drift
R4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.898 Drift	0.449 Drift	0.267 Runoff	0.267 Runoff	0.317 Drift	0.267 Runoff	0.267 Runoff	0.267 Runoff	0.267 Runoff	0.165 Drift	0.267 Runoff	0.267 Runoff	0.084 Drift

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips

Global maximum concentrations – metabolites of pyraclostrobin

Table 9.2.5-35: Steps 1-2: $PEC_{sw,max}$ of BF 500-5 following application of pyraclostrobin to cereals and maize

$FOCUS_{sw}$ crop	BF 500-5 $PEC_{sw,max}$ [µg L^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	0.128	0.256	0.126	0.179	0.126	0.179
Maize	0.102	-[a]	0.101	-[a]	0.101	-[a]

[a] Only single application calculated for maize

Table 9.2.5-36: Steps 1-2: $PEC_{sw,max}$ of BF 500-11 following application of pyraclostrobin to cereals and maize

$FOCUS_{sw}$ crop	BF 500-11 $PEC_{sw,max}$ [µg L^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	0.203	0.407	0.197	0.266	0.197	0.266
Maize	0.163	-[a]	0.158	-[a]	0.158	-[a]

[a] Only single application calculated for maize

Table 9.2.5-37: Steps 1-2: $PEC_{sw,max}$ of BF 500-13 following application of pyraclostrobin to cereals and maize

$FOCUS_{sw}$ crop	BF 500-13 $PEC_{sw,max}$ [µg L^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	0.266	0.533	0.261	0.413	0.261	0.413
Maize	0.213	-[a]	0.209	-[a]	0.209	-[a]

[a] Only single application calculated for maize

Table 9.2.5-38: Steps 1-2: $PEC_{sw,max}$ of BF 500-14 following application of pyraclostrobin to cereals and maize

$FOCUS_{sw}$ crop	BF 500-14 $PEC_{sw,max}$ [µg L^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	0.287	0.573	0.278	0.352	0.278	0.352
Maize	0.229	-[a]	0.223	-[a]	0.223	-[a]

[a] Only single application calculated for maize

Actual and time-weighted average concentrations – pyraclostrobin

Step 1 and 2

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-39: Step 1: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following application of 250 g a.s. ha^{-1} to cereals (Tier 1)

Time[a] [d]	Step 1			
	Cereals			
	Single application		Multiple application	
	PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
	Actual	TWA	Actual	TWA
0	8.516	-	17.031	-
1	6.213	7.364	12.427	14.729
2	6.043	6.746	12.087	13.492
4	5.717	6.313	11.435	12.625
7	5.261	5.958	10.522	11.917
14	4.333	5.370	8.666	10.740
21	3.569	4.893	7.137	9.786
28	2.939	4.481	5.878	8.961
42	1.994	3.799	3.987	7.598
50	1.597	3.477	3.194	6.955
100	0.399	2.171	0.799	4.341

[a] Time: days following maximum concentration (Actual) or time interval (TWA)

Table 9.2.5-40: Step 2: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following application of 250 g a.s. ha^{-1} to cereals (Tier 1)

Time[a] [d]	Cereals							
	Step 2							
	North Europe (Mar-May)				South Europe (Mar-May)			
	Single application		Multiple application		Single application		Multiple application	
	PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
0	2.299	-	2.168	-	2.299	-	2.168	-
1	0.880	1.590	0.911	1.540	0.880	1.590	0.911	1.540
2	0.440	1.125	0.519	1.127	0.440	1.125	0.519	1.127
4	0.817	0.795	1.248	0.882	1.381	0.865	2.149	0.995
7	0.684	0.764	1.065	0.986	1.219	1.042	1.920	1.429
14	0.572	0.695	0.890	0.980	1.019	1.079	1.604	1.593
21	0.478	0.638	0.743	0.925	0.851	1.030	1.340	1.552
28	0.399	0.588	0.621	0.864	0.711	0.967	1.120	1.470
42	0.278	0.504	0.434	0.750	0.496	0.844	0.781	1.294
50	0.227	0.463	0.353	0.693	0.404	0.781	0.636	1.200
100	0.063	0.295	0.098	0.446	0.112	0.504	0.176	0.779

[a]Time: days following maximum concentration (Actual) or time interval (TWA)

Tier 2 (parameters from irradiated water/sediment study)

Table 9.2.5-41: Step 1: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following application of 250 g a.s. ha^{-1} to cereals (Tier 2)

Time[a] [d]	Step 1			
	Cereals			
	Single application		Multiple application	
	PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
	Actual	TWA	Actual	TWA
0	8.516	-	17.031	-
1	5.802	7.159	11.603	14.317
2	5.269	6.345	10.538	12.690
4	4.346	5.569	8.693	11.138
7	3.256	4.800	6.512	9.600
14	1.660	3.585	3.319	7.169
21	0.846	2.792	1.692	5.584
28	0.431	2.248	0.862	4.496
42	0.112	1.578	0.224	3.155
50	0.052	1.338	0.104	2.675
100	<0.001	0.674	<0.001	1.348

[a]Time: days following maximum concentration (Actual) or time interval (TWA)

Table 9.2.5-42: Step 2: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following application of 250 g a.s. ha^{-1} to cereals (Tier 2)

Time[a] [d]	Cereals							
	Step 2							
	North Europe (Mar-May)				South Europe (Mar-May)			
	Single application		Multiple application		Single application		Multiple application	
	PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
0	2.299	-	2.056	-	2.299	-	2.056	-
1	0.803	1.551	0.732	1.394	0.803	1.551	0.732	1.394
2	0.367	1.068	0.345	0.966	0.367	1.068	0.345	0.966
4	0.743	0.731	1.076	0.716	1.307	0.801	1.976	0.829
7	0.500	0.674	0.747	0.790	0.916	0.923	1.412	1.189
14	0.239	0.514	0.357	0.659	0.437	0.786	0.674	1.094
21	0.114	0.399	0.170	0.524	0.209	0.627	0.322	0.888
28	0.054	0.319	0.081	0.423	0.100	0.507	0.154	0.723
42	0.012	0.222	0.019	0.296	0.023	0.356	0.035	0.509
50	0.005	0.188	0.008	0.251	0.010	0.301	0.015	0.431
100	<0.001	0.095	<0.001	0.126	<0.001	0.152	<0.001	0.217

[a]Time: days following maximum concentration (Actual) or time interval (TWA)

Step 3 and 4

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-43: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Location	Time[a] [d]	Winter cereals							
		Step 3		Step 4: Buffer zones and mitigation					
		Edge-of-field		50N		75N		90N	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D1, ditch	0	1.584	-	0.792	-	0.396	-	0.158	-
	1	1.389	1.481	0.694	0.740	0.347	0.370	0.139	0.148
	2	1.238	1.395	0.618	0.697	0.309	0.349	0.124	0.139
	4	1.025	1.258	0.512	0.629	0.256	0.314	0.102	0.126
	7	0.814	1.110	0.405	0.554	0.202	0.277	0.080	0.111
	14	0.085	0.796	0.042	0.397	0.020	0.198	0.008	0.079
	21	0.134	0.568	0.066	0.283	0.033	0.141	0.013	0.056
	28	0.136	0.460	0.068	0.229	0.033	0.114	0.013	0.045
	42	0.105	0.347	0.052	0.173	0.026	0.086	0.010	0.034
	50	0.086	0.307	0.043	0.153	0.021	0.076	0.008	0.030
	100	0.023	0.178	0.011	0.089	0.006	0.044	0.002	0.018

Table 9.2.5-43: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals									
Location	Time[a] [d]	Step 3 Edge-of-field		Step 4: Buffer zones and mitigation					
				50N		75N		90N	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D1, stream	0	1.346	-	0.673	-	0.336	-	0.134	-
	1	<0.001	0.315	<0.001	0.157	<0.001	0.079	<0.001	0.031
	2	<0.001	0.157	<0.001	0.079	<0.001	0.039	<0.001	0.016
	4	0.001	0.079	<0.001	0.039	<0.001	0.020	<0.001	0.008
	7	<0.001	0.045	<0.001	0.023	<0.001	0.011	<0.001	0.005
	14	<0.001	0.023	<0.001	0.011	<0.001	0.006	<0.001	0.002
	21	<0.001	0.015	<0.001	0.008	<0.001	0.004	<0.001	0.002
	28	<0.001	0.012	<0.001	0.006	<0.001	0.003	<0.001	0.001
	42	<0.001	0.008	<0.001	0.004	<0.001	0.002	<0.001	0.001
	50	<0.001	0.006	<0.001	0.003	<0.001	0.002	<0.001	0.001
	100	<0.001	0.003	<0.001	0.002	<0.001	0.001	<0.001	<0.001
D2, ditch	0	1.589	-	0.794	-	0.397	-	0.159	-
	1	1.435	1.509	0.717	0.754	0.359	0.377	0.143	0.151
	2	1.303	1.437	0.651	0.718	0.325	0.359	0.130	0.144
	4	0.398	1.208	0.198	0.604	0.099	0.302	0.040	0.121
	7	0.026	0.752	0.013	0.376	0.006	0.188	0.002	0.075
	14	0.002	0.382	0.001	0.191	0.001	0.095	<0.001	0.038
	21	0.018	0.258	0.009	0.129	0.005	0.064	0.002	0.026
	28	0.010	0.196	0.005	0.098	0.003	0.049	0.001	0.020
	42	0.004	0.131	0.002	0.066	0.001	0.033	<0.001	0.013
	50	0.008	0.111	0.004	0.056	0.002	0.028	0.001	0.011
	100	0.005	0.058	0.002	0.029	0.001	0.015	<0.001	0.006
D2, stream	0	1.414	-	0.707	-	0.353	-	0.141	-
	1	1.277	1.342	0.638	0.671	0.319	0.335	0.128	0.134
	2	1.160	1.279	0.580	0.639	0.290	0.320	0.116	0.128
	4	0.001	0.867	<0.001	0.433	<0.001	0.216	<0.001	0.087
	7	<0.001	0.496	<0.001	0.248	<0.001	0.124	<0.001	0.050
	14	<0.001	0.248	<0.001	0.124	<0.001	0.062	<0.001	0.025
	21	0.001	0.165	<0.001	0.083	<0.001	0.041	<0.001	0.017
	28	<0.001	0.124	<0.001	0.062	<0.001	0.031	<0.001	0.012
	42	<0.001	0.083	<0.001	0.041	<0.001	0.021	<0.001	0.008
	50	0.001	0.070	<0.001	0.035	<0.001	0.017	<0.001	0.007
	100	0.002	0.036	0.001	0.018	0.001	0.009	<0.001	0.004

Table 9.2.5-43: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals									
Location	Time[a] [d]	Step 3 Edge-of-field		Step 4: Buffer zones and mitigation					
				50N		75N		90N	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D3, ditch	0	1.567	-	0.783	-	0.392	-	0.157	-
	1	0.633	1.172	0.316	0.586	0.158	0.293	0.063	0.117
	2	0.058	0.722	0.029	0.361	0.014	0.181	0.006	0.072
	4	0.004	0.368	0.002	0.184	0.001	0.092	<0.001	0.037
	7	0.003	0.212	0.001	0.106	0.001	0.053	<0.001	0.021
	14	0.002	0.107	0.001	0.054	<0.001	0.027	<0.001	0.011
	21	0.001	0.072	0.001	0.036	<0.001	0.018	<0.001	0.007
	28	0.001	0.054	<0.001	0.027	<0.001	0.014	<0.001	0.005
	42	<0.001	0.036	<0.001	0.018	<0.001	0.009	<0.001	0.004
	50	<0.001	0.031	<0.001	0.015	<0.001	0.008	<0.001	0.003
	100	<0.001	0.015	<0.001	0.008	<0.001	0.004	<0.001	0.002
D4, pond	0	0.054	-	0.027	-	0.014	-	0.005	-
	1	0.052	0.053	0.026	0.027	0.013	0.013	0.005	0.005
	2	0.050	0.052	0.025	0.026	0.013	0.013	0.005	0.005
	4	0.047	0.050	0.023	0.025	0.012	0.013	0.005	0.005
	7	0.043	0.048	0.021	0.024	0.011	0.012	0.004	0.005
	14	0.035	0.043	0.018	0.022	0.009	0.011	0.003	0.004
	21	0.030	0.040	0.015	0.020	0.007	0.010	0.003	0.004
	28	0.027	0.037	0.013	0.018	0.007	0.009	0.003	0.004
	42	0.022	0.033	0.011	0.016	0.005	0.008	0.002	0.003
	50	0.020	0.031	0.010	0.015	0.005	0.008	0.002	0.003
	100	0.011	0.023	0.006	0.011	0.003	0.006	0.001	0.002
D4, stream	0	1.180	-	0.590	-	0.295	-	0.118	-
	1	<0.001	0.054	<0.001	0.027	<0.001	0.014	<0.001	0.005
	2	<0.001	0.027	<0.001	0.014	<0.001	0.007	<0.001	0.003
	4	<0.001	0.014	<0.001	0.007	<0.001	0.003	<0.001	0.001
	7	<0.001	0.008	<0.001	0.004	<0.001	0.002	<0.001	0.001
	14	<0.001	0.004	<0.001	0.002	<0.001	0.001	<0.001	<0.001
	21	<0.001	0.003	<0.001	0.001	<0.001	0.001	<0.001	<0.001
	28	<0.001	0.002	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
	42	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
	50	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
	100	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 9.2.5-43: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals									
Location	Time[a] [d]	Step 3 Edge-of-field		Step 4: Buffer zones and mitigation					
				50N		75N		90N	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D5, pond	0	0.054	-	0.027	-	0.014	-	0.005	-
	1	0.052	0.053	0.026	0.027	0.013	0.013	0.005	0.005
	2	0.050	0.052	0.025	0.026	0.013	0.013	0.005	0.005
	4	0.047	0.050	0.024	0.025	0.012	0.013	0.005	0.005
	7	0.043	0.048	0.022	0.024	0.011	0.012	0.004	0.005
	14	0.037	0.044	0.018	0.022	0.009	0.011	0.004	0.004
	21	0.032	0.041	0.016	0.020	0.008	0.010	0.003	0.004
	28	0.029	0.038	0.014	0.019	0.007	0.010	0.003	0.004
	42	0.024	0.034	0.012	0.017	0.006	0.009	0.002	0.003
	50	0.022	0.032	0.011	0.016	0.005	0.008	0.002	0.003
	100	0.012	0.024	0.006	0.012	0.003	0.006	0.001	0.002
D5, stream	0	1.263	-	0.631	-	0.316	-	0.126	-
	1	<0.001	0.055	<0.001	0.027	<0.001	0.014	<0.001	0.005
	2	<0.001	0.027	<0.001	0.014	<0.001	0.007	<0.001	0.003
	4	<0.001	0.014	<0.001	0.007	<0.001	0.003	<0.001	0.001
	7	<0.001	0.008	<0.001	0.004	<0.001	0.002	<0.001	0.001
	14	<0.001	0.004	<0.001	0.002	<0.001	0.001	<0.001	<0.001
	21	<0.001	0.003	<0.001	0.001	<0.001	0.001	<0.001	<0.001
	28	<0.001	0.002	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
	42	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
	50	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
	100	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
D6, ditch	0	1.547	-	0.773	-	0.387	-	0.155	-
	1	0.024	0.611	0.012	0.305	0.006	0.153	0.002	0.061
	2	0.001	0.308	<0.001	0.154	<0.001	0.077	<0.001	0.031
	4	0.001	0.155	<0.001	0.077	<0.001	0.039	<0.001	0.015
	7	0.001	0.089	0.001	0.044	<0.001	0.022	<0.001	0.009
	14	0.001	0.045	0.001	0.023	<0.001	0.011	<0.001	0.004
	21	0.001	0.030	<0.001	0.015	<0.001	0.008	<0.001	0.003
	28	0.001	0.023	0.001	0.012	<0.001	0.006	<0.001	0.002
	42	<0.001	0.016	<0.001	0.008	<0.001	0.004	<0.001	0.002
	50	0.001	0.013	<0.001	0.007	<0.001	0.003	<0.001	0.001
	100	<0.001	0.007	<0.001	0.003	<0.001	0.002	<0.001	0.001

Table 9.2.5-43: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals									
Location	Time[a] [d]	Step 3 Edge-of-field		Step 4: Buffer zones and mitigation					
				50N		75N		90N	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
R1, pond	0	0.054	-	0.027	-	0.016	-	0.014	-
	1	0.052	0.053	0.026	0.027	0.015	0.015	0.014	0.014
	2	0.050	0.052	0.025	0.026	0.015	0.015	0.013	0.014
	4	0.047	0.050	0.024	0.025	0.014	0.015	0.013	0.013
	7	0.043	0.048	0.022	0.024	0.013	0.014	0.012	0.013
	14	0.036	0.044	0.018	0.022	0.012	0.013	0.010	0.012
	21	0.034	0.041	0.018	0.021	0.010	0.013	0.009	0.011
	28	0.030	0.039	0.016	0.020	0.014	0.013	0.008	0.011
	42	0.024	0.035	0.013	0.018	0.010	0.013	0.007	0.011
	50	0.022	0.033	0.012	0.017	0.009	0.012	0.006	0.011
	100	0.022	0.027	0.017	0.016	0.005	0.010	0.004	0.009
R1, stream	0	1.037	-	0.518	-	0.259	-	0.159	-
	1	<0.001	0.216	<0.001	0.108	<0.001	0.080	<0.001	0.080
	2	<0.001	0.108	<0.001	0.054	<0.001	0.040	<0.001	0.040
	4	<0.001	0.054	<0.001	0.027	<0.001	0.020	<0.001	0.020
	7	<0.001	0.031	<0.001	0.016	<0.001	0.013	<0.001	0.013
	14	<0.001	0.016	<0.001	0.010	<0.001	0.010	<0.001	0.010
	21	<0.001	0.014	<0.001	0.009	<0.001	0.008	<0.001	0.008
	28	<0.001	0.011	<0.001	0.007	<0.001	0.007	<0.001	0.007
	42	<0.001	0.007	<0.001	0.005	<0.001	0.005	<0.001	0.005
	50	<0.001	0.006	<0.001	0.005	<0.001	0.005	<0.001	0.005
	100	<0.001	0.005	<0.001	0.004	<0.001	0.004	<0.001	0.003
R3, stream	0	1.463	-	0.731	-	0.365	-	0.163	-
	1	0.005	0.538	0.002	0.269	0.001	0.134	<0.001	0.078
	2	0.001	0.270	<0.001	0.135	<0.001	0.067	<0.001	0.039
	4	<0.001	0.135	<0.001	0.068	<0.001	0.034	<0.001	0.020
	7	<0.001	0.077	<0.001	0.039	<0.001	0.019	<0.001	0.011
	14	<0.001	0.044	<0.001	0.025	<0.001	0.015	<0.001	0.009
	21	<0.001	0.030	<0.001	0.017	<0.001	0.010	<0.001	0.006
	28	<0.001	0.022	<0.001	0.013	<0.001	0.008	<0.001	0.005
	42	<0.001	0.015	<0.001	0.008	<0.001	0.005	<0.001	0.003
	50	<0.001	0.013	<0.001	0.007	<0.001	0.004	<0.001	0.003
	100	<0.001	0.007	<0.001	0.004	<0.001	0.003	<0.001	0.002

Table 9.2.5-43: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals									
Location	Time[a] [d]	Step 3 Edge-of-field		Step 4: Buffer zones and mitigation					
				50N		75N		90N	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
R4, stream	0	1.038	-	0.519	-	0.296	-	0.296	-
	1	<0.001	0.221	<0.001	0.218	0.001	0.218	0.001	0.218
	2	<0.001	0.160	<0.001	0.160	<0.001	0.160	<0.001	0.160
	4	<0.001	0.080	<0.001	0.080	<0.001	0.080	<0.001	0.080
	7	<0.001	0.046	<0.001	0.046	<0.001	0.046	<0.001	0.046
	14	<0.001	0.023	<0.001	0.023	<0.001	0.023	<0.001	0.023
	21	<0.001	0.026	<0.001	0.020	<0.001	0.018	<0.001	0.016
	28	<0.001	0.019	<0.001	0.019	0.202	0.019	0.202	0.019
	42	<0.001	0.015	<0.001	0.015	<0.001	0.015	<0.001	0.015
	50	<0.001	0.017	<0.001	0.015	<0.001	0.014	<0.001	0.013
	100	<0.001	0.010	<0.001	0.009	<0.001	0.008	<0.001	0.008

N = Drift mitigation by nozzle reduction
[a] Time: days after maximum concentration (Actual) or time interval (TWA)

Table 9.2.5-44: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation									
				5mD		5mD50N		5mD75N		10mD		10mD50N	
				PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D1, ditch	0	1.584	-	0.429	-	0.214	-	0.107	-	0.228	-	0.114	-
	1	1.389	1.481	0.376	0.401	0.188	0.200	0.094	0.100	0.200	0.213	0.100	0.106
	2	1.238	1.395	0.335	0.378	0.167	0.189	0.084	0.095	0.178	0.200	0.089	0.100
	4	1.025	1.258	0.277	0.341	0.138	0.170	0.069	0.085	0.147	0.181	0.073	0.090
	7	0.814	1.110	0.219	0.300	0.109	0.150	0.054	0.075	0.116	0.159	0.058	0.079
	14	0.085	0.796	0.022	0.215	0.011	0.107	0.005	0.053	0.012	0.114	0.006	0.057
	21	0.134	0.568	0.035	0.153	0.017	0.076	0.009	0.038	0.019	0.081	0.009	0.040
	28	0.136	0.460	0.036	0.124	0.018	0.062	0.009	0.031	0.019	0.065	0.009	0.033
	42	0.105	0.347	0.028	0.093	0.014	0.046	0.007	0.023	0.015	0.049	0.007	0.025
	50	0.086	0.307	0.023	0.083	0.012	0.041	0.006	0.020	0.012	0.044	0.006	0.022
	100	0.023	0.178	0.006	0.048	0.003	0.024	0.002	0.012	0.003	0.025	0.002	0.013
D1, stream	0	1.346	-	0.491	-	0.246	-	0.123	-	0.260	-	0.130	-
	1	<0.001	0.315	<0.001	0.115	<0.001	0.057	<0.001	0.029	<0.001	0.061	<0.001	0.031
	2	<0.001	0.157	<0.001	0.058	<0.001	0.029	<0.001	0.014	<0.001	0.031	<0.001	0.015
	4	0.001	0.079	<0.001	0.029	<0.001	0.014	<0.001	0.007	<0.001	0.015	<0.001	0.008
	7	<0.001	0.045	<0.001	0.017	<0.001	0.008	<0.001	0.004	<0.001	0.009	<0.001	0.004
	14	<0.001	0.023	<0.001	0.008	<0.001	0.004	<0.001	0.002	<0.001	0.004	<0.001	0.002
	21	<0.001	0.015	<0.001	0.006	<0.001	0.003	<0.001	0.001	<0.001	0.003	<0.001	0.001
	28	<0.001	0.012	<0.001	0.004	<0.001	0.002	<0.001	0.001	<0.001	0.002	<0.001	0.001
	42	<0.001	0.008	<0.001	0.003	<0.001	0.001	<0.001	0.001	<0.001	0.001	<0.001	0.001
	50	<0.001	0.006	<0.001	0.002	<0.001	0.001	<0.001	0.001	<0.001	0.001	<0.001	0.001
	100	<0.001	0.003	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001

Table 9.2.5-44: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation									
				5mD		5mD50N		5mD75N		10mD		10mD50N	
				PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D2, ditch	0	1.589	-	0.431	-	0.215	-	0.108	-	0.228	-	0.114	-
	1	1.435	1.509	0.389	0.409	0.194	0.204	0.097	0.102	0.206	0.217	0.103	0.108
	2	1.303	1.437	0.353	0.389	0.176	0.195	0.088	0.097	0.187	0.206	0.093	0.103
	4	0.398	1.208	0.107	0.327	0.054	0.163	0.027	0.082	0.057	0.173	0.028	0.087
	7	0.026	0.752	0.007	0.204	0.003	0.102	0.002	0.051	0.004	0.108	0.002	0.054
	14	0.002	0.382	0.001	0.103	<0.001	0.052	<0.001	0.026	<0.001	0.055	<0.001	0.027
	21	0.018	0.258	0.005	0.070	0.002	0.035	0.001	0.017	0.003	0.037	0.001	0.019
	28	0.010	0.196	0.003	0.053	0.001	0.026	0.001	0.013	0.002	0.028	0.001	0.014
	42	0.004	0.131	0.001	0.036	0.001	0.018	<0.001	0.009	0.001	0.019	<0.001	0.009
	50	0.008	0.111	0.002	0.030	0.001	0.015	0.001	0.008	0.001	0.016	0.001	0.008
	100	0.005	0.058	0.001	0.016	0.001	0.008	<0.001	0.004	0.001	0.008	<0.001	0.004
D2, stream	0	1.414	-	0.516	-	0.258	-	0.129	-	0.274	-	0.137	-
	1	1.277	1.342	0.466	0.490	0.233	0.245	0.116	0.122	0.247	0.260	0.124	0.130
	2	1.160	1.279	0.423	0.467	0.212	0.233	0.106	0.117	0.224	0.247	0.112	0.124
	4	0.001	0.867	<0.001	0.316	<0.001	0.158	<0.001	0.079	<0.001	0.168	<0.001	0.084
	7	<0.001	0.496	<0.001	0.181	<0.001	0.090	<0.001	0.045	<0.001	0.096	<0.001	0.048
	14	<0.001	0.248	<0.001	0.091	<0.001	0.045	<0.001	0.023	<0.001	0.048	<0.001	0.024
	21	0.001	0.165	<0.001	0.060	<0.001	0.030	<0.001	0.015	<0.001	0.032	<0.001	0.016
	28	<0.001	0.124	<0.001	0.045	<0.001	0.023	<0.001	0.011	<0.001	0.024	<0.001	0.012
	42	<0.001	0.083	<0.001	0.030	<0.001	0.015	<0.001	0.008	<0.001	0.016	<0.001	0.008
	50	0.001	0.070	<0.001	0.025	<0.001	0.013	<0.001	0.006	<0.001	0.014	<0.001	0.007
	100	0.002	0.036	0.001	0.013	<0.001	0.007	<0.001	0.003	<0.001	0.007	<0.001	0.003

Table 9.2.5-44: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation									
				5mD		5mD50N		5mD75N		10mD		10mD50N	
				PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D3, ditch	0	1.567	-	0.424	-	0.212	-	0.106	-	0.225	-	0.112	-
	1	0.633	1.172	0.171	0.318	0.086	0.159	0.043	0.079	0.091	0.168	0.045	0.084
	2	0.058	0.722	0.016	0.196	0.008	0.098	0.004	0.049	0.008	0.104	0.004	0.052
	4	0.004	0.368	0.001	0.100	<0.001	0.050	<0.001	0.025	<0.001	0.053	<0.001	0.026
	7	0.003	0.212	0.001	0.057	<0.001	0.029	<0.001	0.014	<0.001	0.030	<0.001	0.015
	14	0.002	0.107	<0.001	0.029	<0.001	0.015	<0.001	0.007	<0.001	0.015	<0.001	0.008
	21	0.001	0.072	<0.001	0.019	<0.001	0.010	<0.001	0.005	<0.001	0.010	<0.001	0.005
	28	0.001	0.054	<0.001	0.015	<0.001	0.007	<0.001	0.004	<0.001	0.008	<0.001	0.004
	42	<0.001	0.036	<0.001	0.010	<0.001	0.005	<0.001	0.002	<0.001	0.005	<0.001	0.003
	50	<0.001	0.031	<0.001	0.008	<0.001	0.004	<0.001	0.002	<0.001	0.004	<0.001	0.002
	100	<0.001	0.015	<0.001	0.004	<0.001	0.002	<0.001	0.001	<0.001	0.002	<0.001	0.001
D4, pond	0	0.054	-	0.047	-	0.023	-	0.012	-	0.034	-	0.017	-
	1	0.052	0.053	0.045	0.046	0.023	0.023	0.011	0.011	0.032	0.033	0.016	0.016
	2	0.050	0.052	0.043	0.045	0.022	0.023	0.011	0.011	0.031	0.032	0.016	0.016
	4	0.047	0.050	0.041	0.044	0.020	0.022	0.010	0.011	0.029	0.031	0.015	0.016
	7	0.043	0.048	0.037	0.041	0.018	0.021	0.009	0.010	0.027	0.030	0.013	0.015
	14	0.035	0.043	0.030	0.037	0.015	0.019	0.007	0.009	0.022	0.027	0.011	0.013
	21	0.030	0.040	0.026	0.034	0.013	0.017	0.006	0.008	0.019	0.025	0.009	0.012
	28	0.027	0.037	0.023	0.032	0.011	0.016	0.006	0.008	0.017	0.023	0.008	0.011
	42	0.022	0.033	0.019	0.028	0.009	0.014	0.005	0.007	0.014	0.020	0.007	0.010
	50	0.020	0.031	0.017	0.027	0.009	0.013	0.004	0.007	0.012	0.019	0.006	0.009
	100	0.011	0.023	0.010	0.020	0.005	0.010	0.002	0.005	0.007	0.014	0.003	0.007

Table 9.2.5-44: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation									
				5mD		5mD50N		5mD75N		10mD		10mD50N	
				PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D4, stream	0	1.180	-	0.431	-	0.215	-	0.108	-	0.228	-	0.114	-
	1	<0.001	0.054	<0.001	0.020	<0.001	0.010	<0.001	0.005	<0.001	0.011	<0.001	0.005
	2	<0.001	0.027	<0.001	0.010	<0.001	0.005	<0.001	0.002	<0.001	0.005	<0.001	0.003
	4	<0.001	0.014	<0.001	0.005	<0.001	0.002	<0.001	0.001	<0.001	0.003	<0.001	0.001
	7	<0.001	0.008	<0.001	0.003	<0.001	0.001	<0.001	0.001	<0.001	0.002	<0.001	0.001
	14	<0.001	0.004	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
	21	<0.001	0.003	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	28	<0.001	0.002	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	42	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	50	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	100	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
D5, pond	0	0.054	-	0.047	-	0.023	-	0.012	-	0.034	-	0.017	-
	1	0.052	0.053	0.045	0.046	0.023	0.023	0.011	0.011	0.032	0.033	0.016	0.016
	2	0.050	0.052	0.044	0.045	0.022	0.023	0.011	0.011	0.031	0.032	0.016	0.016
	4	0.047	0.050	0.041	0.044	0.020	0.022	0.010	0.011	0.029	0.031	0.015	0.016
	7	0.043	0.048	0.037	0.042	0.019	0.021	0.009	0.010	0.027	0.030	0.013	0.015
	14	0.037	0.044	0.032	0.038	0.016	0.019	0.008	0.009	0.023	0.027	0.011	0.014
	21	0.032	0.041	0.028	0.035	0.014	0.018	0.007	0.009	0.020	0.025	0.010	0.013
	28	0.029	0.038	0.025	0.033	0.012	0.017	0.006	0.008	0.018	0.024	0.009	0.012
	42	0.024	0.034	0.021	0.030	0.010	0.015	0.005	0.007	0.015	0.021	0.007	0.011
	50	0.022	0.032	0.019	0.028	0.009	0.014	0.005	0.007	0.014	0.020	0.007	0.010
	100	0.012	0.024	0.010	0.021	0.005	0.011	0.002	0.005	0.007	0.015	0.004	0.007

Table 9.2.5-44: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 5mD PEC_{sw} [µg L^{-1}]		5mD50N PEC_{sw} [µg L^{-1}]		5mD75N PEC_{sw} [µg L^{-1}]		10mD PEC_{sw} [µg L^{-1}]		10mD50N PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D5, stream	0	1.263	-	0.461	-	0.231	-	0.115	-	0.244	-	0.122	-
	1	<0.001	0.055	<0.001	0.020	<0.001	0.010	<0.001	0.005	<0.001	0.011	<0.001	0.005
	2	<0.001	0.027	<0.001	0.010	<0.001	0.005	<0.001	0.002	<0.001	0.005	<0.001	0.003
	4	<0.001	0.014	<0.001	0.005	<0.001	0.002	<0.001	0.001	<0.001	0.003	<0.001	0.001
	7	<0.001	0.008	<0.001	0.003	<0.001	0.001	<0.001	0.001	<0.001	0.002	<0.001	0.001
	14	<0.001	0.004	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
	21	<0.001	0.003	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
	28	<0.001	0.002	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	42	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	50	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	100	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
D6, ditch	0	1.547	-	0.419	-	0.210	-	0.105	-	0.222	-	0.111	-
	1	0.024	0.611	0.006	0.165	0.003	0.083	0.002	0.041	0.003	0.088	0.002	0.044
	2	0.001	0.308	<0.001	0.084	<0.001	0.042	<0.001	0.021	<0.001	0.044	<0.001	0.022
	4	0.001	0.155	<0.001	0.042	<0.001	0.021	<0.001	0.011	<0.001	0.022	<0.001	0.011
	7	0.001	0.089	<0.001	0.024	<0.001	0.012	<0.001	0.006	<0.001	0.013	<0.001	0.006
	14	0.001	0.045	<0.001	0.012	<0.001	0.006	<0.001	0.003	<0.001	0.006	<0.001	0.003
	21	0.001	0.030	<0.001	0.008	<0.001	0.004	<0.001	0.002	<0.001	0.004	<0.001	0.002
	28	0.001	0.023	<0.001	0.006	<0.001	0.003	<0.001	0.002	<0.001	0.003	<0.001	0.002
	42	<0.001	0.016	<0.001	0.004	<0.001	0.002	<0.001	0.001	<0.001	0.002	<0.001	0.001
	50	0.001	0.013	<0.001	0.004	<0.001	0.002	<0.001	0.001	<0.001	0.002	<0.001	0.001
	100	<0.001	0.007	<0.001	0.002	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001

Table 9.2.5-44: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 5mD PEC_{sw} [µg L^{-1}]		5mD50N PEC_{sw} [µg L^{-1}]		5mD75N PEC_{sw} [µg L^{-1}]		10mD PEC_{sw} [µg L^{-1}]		10mD50N PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
R1, pond	0	0.054	-	0.047	-	0.023	-	0.015	-	0.034	-	0.017	-
	1	0.052	0.053	0.045	0.046	0.023	0.023	0.015	0.015	0.032	0.033	0.016	0.016
	2	0.050	0.052	0.044	0.045	0.022	0.023	0.014	0.015	0.031	0.032	0.016	0.016
	4	0.047	0.050	0.041	0.044	0.020	0.022	0.014	0.014	0.029	0.031	0.015	0.016
	7	0.043	0.048	0.037	0.042	0.019	0.021	0.013	0.014	0.027	0.030	0.013	0.015
	14	0.036	0.044	0.031	0.038	0.016	0.019	0.011	0.013	0.022	0.027	0.011	0.014
	21	0.034	0.041	0.030	0.036	0.016	0.018	0.010	0.012	0.022	0.026	0.013	0.013
	28	0.030	0.039	0.026	0.034	0.014	0.018	0.013	0.013	0.019	0.025	0.011	0.014
	42	0.024	0.035	0.021	0.030	0.011	0.016	0.010	0.012	0.016	0.022	0.009	0.013
	50	0.022	0.033	0.019	0.029	0.010	0.015	0.009	0.012	0.014	0.021	0.008	0.013
	100	0.022	0.027	0.020	0.024	0.016	0.014	0.005	0.010	0.018	0.018	0.015	0.011
R1, stream	0	1.037	-	0.379	-	0.189	-	0.159	-	0.201	-	0.159	-
	1	<0.001	0.216	<0.001	0.080	<0.001	0.080	<0.001	0.080	<0.001	0.080	<0.001	0.080
	2	<0.001	0.108	<0.001	0.040	<0.001	0.040	<0.001	0.040	<0.001	0.040	<0.001	0.040
	4	<0.001	0.054	<0.001	0.020	<0.001	0.020	<0.001	0.020	<0.001	0.020	<0.001	0.020
	7	<0.001	0.031	<0.001	0.013	<0.001	0.013	<0.001	0.013	<0.001	0.013	<0.001	0.013
	14	<0.001	0.016	<0.001	0.010	<0.001	0.010	<0.001	0.010	<0.001	0.010	<0.001	0.010
	21	<0.001	0.014	<0.001	0.008	<0.001	0.008	<0.001	0.008	<0.001	0.008	<0.001	0.008
	28	<0.001	0.011	<0.001	0.007	<0.001	0.007	<0.001	0.007	<0.001	0.007	<0.001	0.007
	42	<0.001	0.007	<0.001	0.005	<0.001	0.005	<0.001	0.005	<0.001	0.005	<0.001	0.005
	50	<0.001	0.006	<0.001	0.005	<0.001	0.005	<0.001	0.005	<0.001	0.005	<0.001	0.005
	100	<0.001	0.005	<0.001	0.004	<0.001	0.004	<0.001	0.003	<0.001	0.004	<0.001	0.003

Table 9.2.5-44: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 5mD PEC_{sw} [µg L^{-1}]		5mD50N PEC_{sw} [µg L^{-1}]		5mD75N PEC_{sw} [µg L^{-1}]		10mD PEC_{sw} [µg L^{-1}]		10mD50N PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
R3, stream	0	1.463	-	0.534	-	0.267	-	0.163	-	0.283	-	0.163	-
	1	0.005	0.538	0.002	0.196	0.001	0.098	<0.001	0.078	0.001	0.104	<0.001	0.078
	2	0.001	0.270	<0.001	0.098	<0.001	0.049	<0.001	0.039	<0.001	0.052	<0.001	0.039
	4	<0.001	0.135	<0.001	0.049	<0.001	0.025	<0.001	0.020	<0.001	0.026	<0.001	0.020
	7	<0.001	0.077	<0.001	0.028	<0.001	0.014	<0.001	0.011	<0.001	0.015	<0.001	0.011
	14	<0.001	0.044	<0.001	0.020	<0.001	0.013	<0.001	0.009	<0.001	0.013	<0.001	0.009
	21	<0.001	0.030	<0.001	0.013	<0.001	0.008	<0.001	0.006	<0.001	0.009	<0.001	0.006
	28	<0.001	0.022	<0.001	0.010	<0.001	0.006	<0.001	0.005	<0.001	0.007	<0.001	0.005
	42	<0.001	0.015	<0.001	0.007	<0.001	0.004	<0.001	0.003	<0.001	0.004	<0.001	0.003
	50	<0.001	0.013	<0.001	0.006	<0.001	0.004	<0.001	0.003	<0.001	0.004	<0.001	0.003
	100	<0.001	0.007	<0.001	0.004	<0.001	0.003	<0.001	0.002	<0.001	0.003	<0.001	0.002
R4, stream	0	1.038	-	0.379	-	0.296	-	0.296	-	0.296	-	0.296	-
	1	<0.001	0.221	<0.001	0.218	0.001	0.218	0.001	0.218	0.001	0.218	0.001	0.218
	2	<0.001	0.160	<0.001	0.160	<0.001	0.160	<0.001	0.160	<0.001	0.160	<0.001	0.160
	4	<0.001	0.080	<0.001	0.080	<0.001	0.080	<0.001	0.080	<0.001	0.080	<0.001	0.080
	7	<0.001	0.046	<0.001	0.046	<0.001	0.046	<0.001	0.046	<0.001	0.046	<0.001	0.046
	14	<0.001	0.023	<0.001	0.023	<0.001	0.023	<0.001	0.023	<0.001	0.023	<0.001	0.023
	21	<0.001	0.026	<0.001	0.019	<0.001	0.017	<0.001	0.016	<0.001	0.017	<0.001	0.016
	28	<0.001	0.019	<0.001	0.019	0.202	0.019	0.202	0.019	0.202	0.019	0.202	0.019
	42	<0.001	0.015	<0.001	0.015	<0.001	0.015	<0.001	0.015	<0.001	0.015	<0.001	0.015
	50	<0.001	0.017	<0.001	0.015	<0.001	0.014	<0.001	0.013	<0.001	0.014	<0.001	0.013
	100	<0.001	0.010	<0.001	0.008	<0.001	0.008	<0.001	0.008	<0.001	0.008	<0.001	0.008

D = Drift mitigation by no-spray buffer zones
N = Drift mitigation by nozzle reduction
[a] Time: days after maximum concentration (Actual) or time interval (TWA)

Table 9.2.5-45: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 10mD+R PEC_{sw} [µg L^{-1}]		15mD PEC_{sw} [µg L^{-1}]		20mD PEC_{sw} [µg L^{-1}]		20mD+R PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D1, ditch	0	1.584	-	0.228	-	0.155	-	0.118	-	0.118	-
	1	1.389	1.481	0.200	0.213	0.136	0.145	0.104	0.110	0.104	0.110
	2	1.238	1.395	0.178	0.200	0.121	0.137	0.092	0.104	0.092	0.104
	4	1.025	1.258	0.147	0.181	0.100	0.123	0.076	0.094	0.076	0.094
	7	0.814	1.110	0.116	0.159	0.079	0.108	0.060	0.083	0.060	0.083
	14	0.085	0.796	0.012	0.114	0.008	0.077	0.006	0.059	0.006	0.059
	21	0.134	0.568	0.019	0.081	0.013	0.055	0.009	0.042	0.009	0.042
	28	0.136	0.460	0.019	0.065	0.013	0.045	0.010	0.034	0.010	0.034
	42	0.105	0.347	0.015	0.049	0.010	0.034	0.008	0.026	0.008	0.026
	50	0.086	0.307	0.012	0.044	0.008	0.030	0.006	0.023	0.006	0.023
	100	0.023	0.178	0.003	0.025	0.002	0.017	0.002	0.013	0.002	0.013
D1, stream	0	1.346	-	0.260	-	0.178	-	0.135	-	0.135	-
	1	<0.001	0.315	<0.001	0.061	<0.001	0.042	<0.001	0.032	<0.001	0.032
	2	<0.001	0.157	<0.001	0.031	<0.001	0.021	<0.001	0.016	<0.001	0.016
	4	0.001	0.079	<0.001	0.015	<0.001	0.010	<0.001	0.008	<0.001	0.008
	7	<0.001	0.045	<0.001	0.009	<0.001	0.006	<0.001	0.005	<0.001	0.005
	14	<0.001	0.023	<0.001	0.004	<0.001	0.003	<0.001	0.002	<0.001	0.002
	21	<0.001	0.015	<0.001	0.003	<0.001	0.002	<0.001	0.002	<0.001	0.002
	28	<0.001	0.012	<0.001	0.002	<0.001	0.002	<0.001	0.001	<0.001	0.001
	42	<0.001	0.008	<0.001	0.001	<0.001	0.001	<0.001	0.001	<0.001	0.001
	50	<0.001	0.006	<0.001	0.001	<0.001	0.001	<0.001	0.001	<0.001	0.001
	100	<0.001	0.003	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 9.2.5-45: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 10mD+R PEC_{sw} [µg L^{-1}]		15mD PEC_{sw} [µg L^{-1}]		20mD PEC_{sw} [µg L^{-1}]		20mD+R PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D2, ditch	0	1.589	-	0.228	-	0.156	-	0.119	-	0.119	-
	1	1.435	1.509	0.206	0.217	0.141	0.148	0.107	0.113	0.107	0.113
	2	1.303	1.437	0.187	0.206	0.128	0.141	0.097	0.107	0.097	0.107
	4	0.398	1.208	0.057	0.173	0.039	0.118	0.030	0.090	0.030	0.090
	7	0.026	0.752	0.004	0.108	0.002	0.074	0.002	0.056	0.002	0.056
	14	0.002	0.382	<0.001	0.055	<0.001	0.037	<0.001	0.028	<0.001	0.028
	21	0.018	0.258	0.003	0.037	0.002	0.025	0.001	0.019	0.001	0.019
	28	0.010	0.196	0.002	0.028	0.001	0.019	0.001	0.015	0.001	0.015
	42	0.004	0.131	0.001	0.019	<0.001	0.013	<0.001	0.010	<0.001	0.010
	50	0.008	0.111	0.001	0.016	0.001	0.011	0.001	0.008	0.001	0.008
	100	0.005	0.058	0.001	0.008	<0.001	0.006	<0.001	0.004	<0.001	0.004
D2, stream	0	1.414	-	0.274	-	0.187	-	0.142	-	0.142	-
	1	1.277	1.342	0.247	0.260	0.169	0.178	0.128	0.135	0.128	0.135
	2	1.160	1.279	0.224	0.247	0.153	0.169	0.116	0.129	0.116	0.129
	4	0.001	0.867	<0.001	0.168	<0.001	0.115	<0.001	0.087	<0.001	0.087
	7	<0.001	0.496	<0.001	0.096	<0.001	0.066	<0.001	0.050	<0.001	0.050
	14	<0.001	0.248	<0.001	0.048	<0.001	0.033	<0.001	0.025	<0.001	0.025
	21	0.001	0.165	<0.001	0.032	<0.001	0.022	<0.001	0.017	<0.001	0.017
	28	<0.001	0.124	<0.001	0.024	<0.001	0.016	<0.001	0.013	<0.001	0.013
	42	<0.001	0.083	<0.001	0.016	<0.001	0.011	<0.001	0.008	<0.001	0.008
	50	0.001	0.070	<0.001	0.014	<0.001	0.009	<0.001	0.007	<0.001	0.007
	100	0.002	0.036	<0.001	0.007	<0.001	0.005	<0.001	0.004	<0.001	0.004

Table 9.2.5-45: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 10mD+R PEC_{sw} [µg L^{-1}]		15mD PEC_{sw} [µg L^{-1}]		20mD PEC_{sw} [µg L^{-1}]		20mD+R PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D3, ditch	0	1.567	-	0.225	-	0.154	-	0.117	-	0.117	-
	1	0.633	1.172	0.091	0.168	0.062	0.115	0.047	0.088	0.047	0.088
	2	0.058	0.722	0.008	0.104	0.006	0.071	0.004	0.054	0.004	0.054
	4	0.004	0.368	<0.001	0.053	<0.001	0.036	<0.001	0.028	<0.001	0.028
	7	0.003	0.212	<0.001	0.030	<0.001	0.021	<0.001	0.016	<0.001	0.016
	14	0.002	0.107	<0.001	0.015	<0.001	0.011	<0.001	0.008	<0.001	0.008
	21	0.001	0.072	<0.001	0.010	<0.001	0.007	<0.001	0.005	<0.001	0.005
	28	0.001	0.054	<0.001	0.008	<0.001	0.005	<0.001	0.004	<0.001	0.004
	42	<0.001	0.036	<0.001	0.005	<0.001	0.004	<0.001	0.003	<0.001	0.003
	50	<0.001	0.031	<0.001	0.004	<0.001	0.003	<0.001	0.002	<0.001	0.002
	100	<0.001	0.015	<0.001	0.002	<0.001	0.002	<0.001	0.001	<0.001	0.001
D4, pond	0	0.054	-	0.034	-	0.027	-	0.022	-	0.022	-
	1	0.052	0.053	0.032	0.033	0.026	0.026	0.022	0.022	0.022	0.022
	2	0.050	0.052	0.031	0.032	0.025	0.026	0.021	0.022	0.021	0.022
	4	0.047	0.050	0.029	0.031	0.023	0.025	0.019	0.021	0.019	0.021
	7	0.043	0.048	0.027	0.030	0.021	0.024	0.018	0.020	0.018	0.020
	14	0.035	0.043	0.022	0.027	0.017	0.022	0.015	0.018	0.015	0.018
	21	0.030	0.040	0.019	0.025	0.015	0.020	0.012	0.016	0.012	0.016
	28	0.027	0.037	0.017	0.023	0.013	0.018	0.011	0.015	0.011	0.015
	42	0.022	0.033	0.014	0.020	0.011	0.016	0.009	0.013	0.009	0.013
	50	0.020	0.031	0.012	0.019	0.010	0.015	0.008	0.013	0.008	0.013
	100	0.011	0.023	0.007	0.014	0.006	0.011	0.005	0.009	0.005	0.009

Table 9.2.5-45: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 10mD+R PEC_{sw} [µg L^{-1}]		15mD PEC_{sw} [µg L^{-1}]		20mD PEC_{sw} [µg L^{-1}]		20mD+R PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D4, stream	0	1.180	-	0.228	-	0.156	-	0.119	-	0.119	-
	1	<0.001	0.054	<0.001	0.011	<0.001	0.007	<0.001	0.005	<0.001	0.005
	2	<0.001	0.027	<0.001	0.005	<0.001	0.004	<0.001	0.003	<0.001	0.003
	4	<0.001	0.014	<0.001	0.003	<0.001	0.002	<0.001	0.001	<0.001	0.001
	7	<0.001	0.008	<0.001	0.002	<0.001	0.001	<0.001	0.001	<0.001	0.001
	14	<0.001	0.004	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
	21	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	28	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	42	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	50	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	100	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
D5, pond	0	0.054	-	0.034	-	0.027	-	0.022	-	0.022	-
	1	0.052	0.053	0.032	0.033	0.026	0.026	0.022	0.022	0.022	0.022
	2	0.050	0.052	0.031	0.032	0.025	0.026	0.021	0.022	0.021	0.022
	4	0.047	0.050	0.029	0.031	0.023	0.025	0.020	0.021	0.020	0.021
	7	0.043	0.048	0.027	0.030	0.022	0.024	0.018	0.020	0.018	0.020
	14	0.037	0.044	0.023	0.027	0.018	0.022	0.015	0.018	0.015	0.018
	21	0.032	0.041	0.020	0.025	0.016	0.020	0.013	0.017	0.013	0.017
	28	0.029	0.038	0.018	0.024	0.014	0.019	0.012	0.016	0.012	0.016
	42	0.024	0.034	0.015	0.021	0.012	0.017	0.010	0.014	0.010	0.014
	50	0.022	0.032	0.014	0.020	0.011	0.016	0.009	0.013	0.009	0.013
	100	0.012	0.024	0.007	0.015	0.006	0.012	0.005	0.010	0.005	0.010

Table 9.2.5-45: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 10mD+R PEC_{sw} [µg L^{-1}]		15mD PEC_{sw} [µg L^{-1}]		20mD PEC_{sw} [µg L^{-1}]		20mD+R PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D5, stream	0	1.263	-	0.244	-	0.167	-	0.127	-	0.127	-
	1	<0.001	0.055	<0.001	0.011	<0.001	0.007	<0.001	0.005	<0.001	0.005
	2	<0.001	0.027	<0.001	0.005	<0.001	0.004	<0.001	0.003	<0.001	0.003
	4	<0.001	0.014	<0.001	0.003	<0.001	0.002	<0.001	0.001	<0.001	0.001
	7	<0.001	0.008	<0.001	0.002	<0.001	0.001	<0.001	0.001	<0.001	0.001
	14	<0.001	0.004	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
	21	<0.001	0.003	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	28	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	42	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	50	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	100	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
D6, ditch	0	1.547	-	0.222	-	0.152	-	0.115	-	0.115	-
	1	0.024	0.611	0.003	0.088	0.002	0.060	0.002	0.046	0.002	0.046
	2	0.001	0.308	<0.001	0.044	<0.001	0.030	<0.001	0.023	<0.001	0.023
	4	0.001	0.155	<0.001	0.022	<0.001	0.015	<0.001	0.012	<0.001	0.012
	7	0.001	0.089	<0.001	0.013	<0.001	0.009	<0.001	0.007	<0.001	0.007
	14	0.001	0.045	<0.001	0.006	<0.001	0.004	<0.001	0.003	<0.001	0.003
	21	0.001	0.030	<0.001	0.004	<0.001	0.003	<0.001	0.002	<0.001	0.002
	28	0.001	0.023	<0.001	0.003	<0.001	0.002	<0.001	0.002	<0.001	0.002
	42	<0.001	0.016	<0.001	0.002	<0.001	0.002	<0.001	0.001	<0.001	0.001
	50	0.001	0.013	<0.001	0.002	<0.001	0.001	<0.001	0.001	<0.001	0.001
	100	<0.001	0.007	<0.001	0.001	<0.001	0.001	<0.001	0.001	<0.001	0.001

Table 9.2.5-45: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 10mD+R PEC_{sw} [µg L^{-1}]		15mD PEC_{sw} [µg L^{-1}]		20mD PEC_{sw} [µg L^{-1}]		20mD+R PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
R1, pond	0	0.054	-	0.034	-	0.027	-	0.022	-	0.022	-
	1	0.052	0.053	0.032	0.033	0.026	0.026	0.022	0.022	0.022	0.022
	2	0.050	0.052	0.031	0.032	0.025	0.026	0.021	0.022	0.021	0.022
	4	0.047	0.050	0.029	0.031	0.023	0.025	0.020	0.021	0.020	0.021
	7	0.043	0.048	0.027	0.030	0.021	0.024	0.018	0.020	0.018	0.020
	14	0.036	0.044	0.022	0.027	0.018	0.022	0.015	0.018	0.015	0.018
	21	0.034	0.041	0.020	0.025	0.018	0.021	0.016	0.018	0.013	0.017
	28	0.030	0.039	0.018	0.024	0.016	0.020	0.014	0.017	0.012	0.016
	42	0.024	0.035	0.015	0.021	0.013	0.018	0.011	0.015	0.009	0.014
	50	0.022	0.033	0.013	0.020	0.011	0.017	0.010	0.015	0.008	0.013
	100	0.022	0.027	0.011	0.016	0.017	0.016	0.016	0.014	0.006	0.010
R1, stream	0	1.037	-	0.201	-	0.159	-	0.159	-	0.104	-
	1	<0.001	0.216	<0.001	0.042	<0.001	0.080	<0.001	0.080	<0.001	0.022
	2	<0.001	0.108	<0.001	0.021	<0.001	0.040	<0.001	0.040	<0.001	0.011
	4	<0.001	0.054	<0.001	0.011	<0.001	0.020	<0.001	0.020	<0.001	0.005
	7	<0.001	0.031	<0.001	0.006	<0.001	0.013	<0.001	0.013	<0.001	0.003
	14	<0.001	0.016	<0.001	0.005	<0.001	0.010	<0.001	0.010	<0.001	0.002
	21	<0.001	0.014	<0.001	0.004	<0.001	0.008	<0.001	0.008	<0.001	0.002
	28	<0.001	0.011	<0.001	0.003	<0.001	0.007	<0.001	0.007	<0.001	0.002
	42	<0.001	0.007	<0.001	0.002	<0.001	0.005	<0.001	0.005	<0.001	0.001
	50	<0.001	0.006	<0.001	0.002	<0.001	0.005	<0.001	0.005	<0.001	0.001
	100	<0.001	0.005	<0.001	0.002	<0.001	0.004	<0.001	0.003	<0.001	0.001

Table 9.2.5-45: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following single application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 10mD+R PEC_{sw} [µg L^{-1}]		15mD PEC_{sw} [µg L^{-1}]		20mD PEC_{sw} [µg L^{-1}]		20mD+R PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
R3, stream	0	1.463	-	0.283	-	0.193	-	0.163	-	0.147	-
	1	0.005	0.538	0.001	0.104	0.001	0.078	<0.001	0.078	<0.001	0.054
	2	0.001	0.270	<0.001	0.052	<0.001	0.039	<0.001	0.039	<0.001	0.027
	4	<0.001	0.135	<0.001	0.026	<0.001	0.020	<0.001	0.020	<0.001	0.014
	7	<0.001	0.077	<0.001	0.015	<0.001	0.011	<0.001	0.011	<0.001	0.008
	14	<0.001	0.044	<0.001	0.010	<0.001	0.011	<0.001	0.010	<0.001	0.005
	21	<0.001	0.030	<0.001	0.007	<0.001	0.007	<0.001	0.006	<0.001	0.003
	28	<0.001	0.022	<0.001	0.005	<0.001	0.005	<0.001	0.005	<0.001	0.003
	42	<0.001	0.015	<0.001	0.003	<0.001	0.004	<0.001	0.003	<0.001	0.002
	50	<0.001	0.013	<0.001	0.003	<0.001	0.003	<0.001	0.003	<0.001	0.001
	100	<0.001	0.007	<0.001	0.002	<0.001	0.002	<0.001	0.002	<0.001	0.001
R4, stream	0	1.038	-	0.201	-	0.296	-	0.296	-	0.104	-
	1	<0.001	0.221	<0.001	0.099	0.001	0.218	0.001	0.218	<0.001	0.052
	2	<0.001	0.160	<0.001	0.073	<0.001	0.160	<0.001	0.160	<0.001	0.038
	4	<0.001	0.080	<0.001	0.037	<0.001	0.080	<0.001	0.080	<0.001	0.019
	7	<0.001	0.046	<0.001	0.021	<0.001	0.046	<0.001	0.046	<0.001	0.011
	14	<0.001	0.023	<0.001	0.011	<0.001	0.023	<0.001	0.023	<0.001	0.005
	21	<0.001	0.026	<0.001	0.009	<0.001	0.017	<0.001	0.016	<0.001	0.005
	28	<0.001	0.019	<0.001	0.009	0.202	0.019	0.202	0.019	<0.001	0.005
	42	<0.001	0.015	<0.001	0.007	<0.001	0.015	<0.001	0.015	<0.001	0.004
	50	<0.001	0.017	<0.001	0.007	<0.001	0.013	<0.001	0.013	<0.001	0.003
	100	<0.001	0.010	<0.001	0.004	<0.001	0.008	<0.001	0.008	<0.001	0.002

D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
[a] Time: days after maximum concentration (Actual) or time interval (TWA)

Table 9.2.5-46: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals									
Location	Time[a] [d]	Step 3 Edge-of-field		Step 4: Buffer zones and mitigation					
				50N		75N		90N	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D1, ditch	0	1.504	-	0.751	-	0.375	-	0.150	-
	1	1.372	1.435	0.685	0.716	0.342	0.358	0.137	0.143
	2	1.259	1.374	0.628	0.686	0.313	0.342	0.125	0.137
	4	1.077	1.268	0.537	0.633	0.268	0.316	0.107	0.126
	7	0.885	1.142	0.440	0.570	0.219	0.284	0.087	0.113
	14	0.626	0.942	0.310	0.469	0.153	0.233	0.061	0.093
	21	0.479	0.810	0.237	0.403	0.117	0.200	0.046	0.079
	28	0.381	0.715	0.188	0.355	0.093	0.176	0.036	0.070
	42	0.254	0.653	0.126	0.325	0.062	0.162	0.024	0.064
	50	0.204	0.616	0.101	0.306	0.050	0.152	0.020	0.060
	100	0.047	0.406	0.023	0.201	0.012	0.100	0.005	0.040
D1, stream	0	1.201	-	0.600	-	0.300	-	0.120	-
	1	0.372	0.925	0.186	0.463	0.093	0.231	0.037	0.092
	2	0.017	0.526	0.008	0.263	0.004	0.131	0.002	0.053
	4	0.002	0.265	0.001	0.133	0.001	0.066	<0.001	0.027
	7	0.002	0.152	0.001	0.076	<0.001	0.038	<0.001	0.015
	14	0.001	0.077	0.001	0.038	<0.001	0.019	<0.001	0.008
	21	0.001	0.052	<0.001	0.026	<0.001	0.013	<0.001	0.005
	28	<0.001	0.048	<0.001	0.024	<0.001	0.012	<0.001	0.005
	42	<0.001	0.032	<0.001	0.016	<0.001	0.008	<0.001	0.003
	50	<0.001	0.027	<0.001	0.014	<0.001	0.007	<0.001	0.003
	100	<0.001	0.014	<0.001	0.007	<0.001	0.003	<0.001	0.001
D2, ditch	0	1.396	-	0.698	-	0.349	-	0.140	-
	1	1.262	1.326	0.631	0.663	0.315	0.331	0.126	0.133
	2	1.147	1.264	0.573	0.632	0.286	0.316	0.115	0.126
	4	0.963	1.157	0.481	0.578	0.240	0.289	0.096	0.115
	7	0.010	1.024	0.005	0.511	0.003	0.255	0.001	0.102
	14	0.019	0.520	0.009	0.260	0.005	0.130	0.002	0.052
	21	0.061	0.360	0.030	0.180	0.015	0.090	0.006	0.036
	28	0.073	0.287	0.037	0.143	0.018	0.072	0.007	0.029
	42	0.064	0.215	0.032	0.107	0.016	0.054	0.006	0.021
	50	0.056	0.190	0.028	0.095	0.014	0.047	0.006	0.019
	100	0.022	0.142	0.011	0.071	0.005	0.035	0.002	0.014

Table 9.2.5-46: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals									
Location	Time[a] [d]	Step 3 Edge-of-field		Step 4: Buffer zones and mitigation					
				50N		75N		90N	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D2, stream	0	1.225	-	0.612	-	0.306	-	0.122	-
	1	1.106	1.163	0.553	0.581	0.276	0.291	0.110	0.116
	2	1.005	1.108	0.502	0.554	0.251	0.277	0.100	0.111
	4	0.843	1.014	0.421	0.507	0.210	0.253	0.084	0.101
	7	<0.001	0.888	<0.001	0.444	<0.001	0.222	<0.001	0.088
	14	0.001	0.445	<0.001	0.222	<0.001	0.111	<0.001	0.044
	21	0.025	0.299	0.012	0.149	0.006	0.075	0.002	0.030
	28	0.044	0.233	0.022	0.117	0.011	0.058	0.004	0.023
	42	0.041	0.170	0.021	0.085	0.010	0.043	0.004	0.017
	50	0.035	0.149	0.018	0.075	0.009	0.037	0.004	0.015
	100	0.011	0.104	0.005	0.052	0.003	0.026	0.001	0.010
D3, ditch	0	1.370	-	0.685	-	0.342	-	0.137	-
	1	0.559	1.026	0.279	0.513	0.140	0.256	0.056	0.103
	2	0.052	0.634	0.026	0.317	0.013	0.158	0.005	0.063
	4	0.004	0.324	0.002	0.162	0.001	0.081	<0.001	0.032
	7	0.003	0.186	0.002	0.093	0.001	0.047	<0.001	0.019
	14	0.002	0.095	0.001	0.047	<0.001	0.024	<0.001	0.009
	21	0.001	0.064	0.001	0.032	<0.001	0.016	<0.001	0.006
	28	0.001	0.094	<0.001	0.047	<0.001	0.023	<0.001	0.009
	42	<0.001	0.063	<0.001	0.032	<0.001	0.016	<0.001	0.006
	50	<0.001	0.053	<0.001	0.027	<0.001	0.013	<0.001	0.005
	100	<0.001	0.027	<0.001	0.013	<0.001	0.007	<0.001	0.003
D4, pond	0	0.065	-	0.033	-	0.016	-	0.007	-
	1	0.063	0.064	0.032	0.032	0.016	0.016	0.006	0.006
	2	0.062	0.063	0.031	0.032	0.015	0.016	0.006	0.006
	4	0.059	0.062	0.029	0.031	0.015	0.015	0.006	0.006
	7	0.055	0.060	0.027	0.030	0.014	0.015	0.005	0.006
	14	0.048	0.055	0.024	0.028	0.012	0.014	0.005	0.005
	21	0.042	0.052	0.021	0.026	0.011	0.013	0.004	0.005
	28	0.038	0.049	0.019	0.024	0.009	0.012	0.004	0.005
	42	0.032	0.044	0.016	0.022	0.008	0.011	0.003	0.004
	50	0.029	0.042	0.014	0.021	0.007	0.010	0.003	0.004
	100	0.016	0.035	0.008	0.017	0.004	0.009	0.002	0.003

Table 9.2.5-46: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals									
Location	Time[a] [d]	Step 3 Edge-of-field		Step 4: Buffer zones and mitigation					
				50N		75N		90N	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D4, stream	0	1.076	-	0.538	-	0.269	-	0.107	-
	1	<0.001	0.073	<0.001	0.036	<0.001	0.018	<0.001	0.007
	2	<0.001	0.036	<0.001	0.018	<0.001	0.009	<0.001	0.004
	4	<0.001	0.018	<0.001	0.009	<0.001	0.005	<0.001	0.002
	7	<0.001	0.010	<0.001	0.005	<0.001	0.003	<0.001	0.001
	14	<0.001	0.005	<0.001	0.003	<0.001	0.001	<0.001	0.001
	21	<0.001	0.003	<0.001	0.002	<0.001	0.001	<0.001	<0.001
	28	<0.001	0.003	<0.001	0.001	<0.001	0.001	<0.001	<0.001
	42	<0.001	0.003	<0.001	0.001	<0.001	0.001	<0.001	<0.001
	50	<0.001	0.002	<0.001	0.001	<0.001	0.001	<0.001	<0.001
	100	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
D5, pond	0	0.066	-	0.033	-	0.017	-	0.007	-
	1	0.065	0.065	0.032	0.033	0.016	0.016	0.006	0.007
	2	0.063	0.065	0.031	0.032	0.016	0.016	0.006	0.006
	4	0.060	0.063	0.030	0.031	0.015	0.016	0.006	0.006
	7	0.056	0.061	0.028	0.030	0.014	0.015	0.006	0.006
	14	0.049	0.057	0.025	0.028	0.012	0.014	0.005	0.006
	21	0.044	0.053	0.022	0.027	0.011	0.013	0.004	0.005
	28	0.040	0.051	0.020	0.025	0.010	0.013	0.004	0.005
	42	0.033	0.046	0.017	0.023	0.008	0.011	0.003	0.005
	50	0.030	0.044	0.015	0.022	0.007	0.011	0.003	0.004
	100	0.016	0.036	0.008	0.018	0.004	0.009	0.002	0.004
D5, stream	0	1.251	-	0.625	-	0.312	-	0.125	-
	1	<0.001	0.314	<0.001	0.157	<0.001	0.078	<0.001	0.031
	2	<0.001	0.157	<0.001	0.079	<0.001	0.039	<0.001	0.016
	4	<0.001	0.079	<0.001	0.039	<0.001	0.020	<0.001	0.008
	7	<0.001	0.045	<0.001	0.023	<0.001	0.011	<0.001	0.004
	14	<0.001	0.023	<0.001	0.011	<0.001	0.006	<0.001	0.002
	21	<0.001	0.015	<0.001	0.008	<0.001	0.004	<0.001	0.002
	28	<0.001	0.011	<0.001	0.006	<0.001	0.003	<0.001	0.001
	42	<0.001	0.009	<0.001	0.004	<0.001	0.002	<0.001	0.001
	50	<0.001	0.007	<0.001	0.004	<0.001	0.002	<0.001	0.001
	100	<0.001	0.004	<0.001	0.002	<0.001	0.001	<0.001	<0.001

Table 9.2.5-46: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals									
Location	Time[a] [d]	Step 3 Edge-of-field		Step 4: Buffer zones and mitigation					
				50N		75N		90N	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D6, ditch	0	1.381	-	0.691	-	0.345	-	0.138	-
	1	1.226	1.299	0.613	0.649	0.306	0.324	0.122	0.130
	2	1.102	1.230	0.551	0.615	0.275	0.307	0.110	0.123
	4	0.832	1.101	0.415	0.550	0.207	0.275	0.083	0.110
	7	0.402	0.890	0.200	0.444	0.100	0.222	0.040	0.089
	14	0.084	0.539	0.041	0.269	0.020	0.134	0.008	0.053
	21	0.044	0.379	0.022	0.189	0.011	0.094	0.004	0.038
	28	0.027	0.293	0.013	0.146	0.007	0.073	0.003	0.029
	42	0.011	0.201	0.005	0.100	0.003	0.050	0.001	0.020
	50	0.006	0.170	0.003	0.085	0.002	0.042	0.001	0.017
	100	<0.001	0.091	<0.001	0.046	<0.001	0.023	<0.001	0.009
R1, pond	0	0.065	-	0.042	-	0.035	-	0.031	-
	1	0.063	0.064	0.041	0.042	0.034	0.034	0.031	0.031
	2	0.061	0.063	0.040	0.041	0.033	0.034	0.030	0.031
	4	0.058	0.061	0.039	0.040	0.032	0.033	0.028	0.030
	7	0.054	0.059	0.036	0.039	0.030	0.032	0.027	0.029
	14	0.046	0.054	0.032	0.037	0.026	0.030	0.023	0.027
	21	0.041	0.051	0.028	0.035	0.023	0.028	0.020	0.025
	28	0.048	0.050	0.036	0.035	0.021	0.028	0.019	0.025
	42	0.050	0.050	0.028	0.034	0.018	0.028	0.016	0.024
	50	0.043	0.049	0.025	0.033	0.016	0.027	0.014	0.024
	100	0.026	0.043	0.013	0.029	0.010	0.022	0.010	0.019
R1, stream	0	0.897	-	0.448	-	0.240	-	0.240	-
	1	<0.001	0.187	<0.001	0.128	<0.001	0.128	<0.001	0.128
	2	<0.001	0.094	<0.001	0.064	<0.001	0.064	<0.001	0.064
	4	<0.001	0.047	<0.001	0.032	0.001	0.032	0.001	0.032
	7	<0.001	0.031	<0.001	0.031	<0.001	0.031	<0.001	0.031
	14	<0.001	0.024	<0.001	0.024	0.008	0.024	0.008	0.024
	21	<0.001	0.019	<0.001	0.019	<0.001	0.019	<0.001	0.019
	28	<0.001	0.016	<0.001	0.016	<0.001	0.016	<0.001	0.016
	42	<0.001	0.014	<0.001	0.012	<0.001	0.012	<0.001	0.012
	50	<0.001	0.012	<0.001	0.011	<0.001	0.011	<0.001	0.011
	100	<0.001	0.010	<0.001	0.008	<0.001	0.007	<0.001	0.007

Table 9.2.5-46: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals									
Location	Time[a] [d]	Step 3 Edge-of-field		Step 4: Buffer zones and mitigation					
				50N		75N		90N	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
R3, stream	0	1.265	-	0.632	-	0.316	-	0.163	-
	1	0.004	0.465	0.002	0.233	0.001	0.116	<0.001	0.094
	2	<0.001	0.233	<0.001	0.117	<0.001	0.058	<0.001	0.048
	4	<0.001	0.117	<0.001	0.058	<0.001	0.029	<0.001	0.024
	7	<0.001	0.067	<0.001	0.033	<0.001	0.017	<0.001	0.014
	14	<0.001	0.039	<0.001	0.022	<0.001	0.014	<0.001	0.012
	21	<0.001	0.026	<0.001	0.015	<0.001	0.009	<0.001	0.008
	28	<0.001	0.020	<0.001	0.011	<0.001	0.007	<0.001	0.006
	42	<0.001	0.023	<0.001	0.013	<0.001	0.007	<0.001	0.004
	50	<0.001	0.019	<0.001	0.011	<0.001	0.006	<0.001	0.003
	100	<0.001	0.011	<0.001	0.007	<0.001	0.005	<0.001	0.003
R4, stream	0	0.898	-	0.543	-	0.543	-	0.543	-
	1	<0.001	0.532	0.506	0.532	0.506	0.532	0.506	0.532
	2	<0.001	0.394	0.002	0.394	0.002	0.394	0.002	0.394
	4	<0.001	0.198	0.001	0.198	0.001	0.198	0.001	0.198
	7	<0.001	0.113	0.001	0.113	0.001	0.113	0.001	0.113
	14	0.001	0.070	0.001	0.063	0.001	0.060	0.001	0.058
	21	0.001	0.047	<0.001	0.043	<0.001	0.040	<0.001	0.039
	28	0.001	0.040	0.359	0.037	0.359	0.035	0.359	0.034
	42	<0.001	0.031	<0.001	0.029	<0.001	0.028	<0.001	0.027
	50	<0.001	0.030	<0.001	0.026	<0.001	0.024	<0.001	0.023
	100	<0.001	0.018	<0.001	0.016	<0.001	0.015	<0.001	0.014

N = Drift mitigation by nozzle reduction

[a] Time: days after maximum concentration (Actual) or time interval (TWA)

Table 9.2.5-47: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 5mD PEC_{sw} [µg L^{-1}]		5mD50N PEC_{sw} [µg L^{-1}]		5mD75N PEC_{sw} [µg L^{-1}]		10mD PEC_{sw} [µg L^{-1}]		10mD50N PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D1, ditch	0	1.504	-	0.389	-	0.194	-	0.097	-	0.202	-	0.101	-
	1	1.372	1.435	0.355	0.371	0.177	0.185	0.088	0.093	0.184	0.192	0.092	0.096
	2	1.259	1.374	0.325	0.355	0.162	0.177	0.081	0.089	0.169	0.184	0.084	0.092
	4	1.077	1.268	0.278	0.328	0.139	0.164	0.069	0.082	0.144	0.170	0.072	0.085
	7	0.885	1.142	0.227	0.295	0.113	0.147	0.056	0.073	0.118	0.153	0.059	0.076
	14	0.626	0.942	0.159	0.242	0.079	0.121	0.039	0.060	0.082	0.125	0.041	0.062
	21	0.479	0.810	0.121	0.208	0.060	0.103	0.030	0.051	0.062	0.107	0.031	0.053
	28	0.381	0.715	0.096	0.183	0.047	0.091	0.023	0.045	0.049	0.094	0.024	0.047
	42	0.254	0.653	0.064	0.168	0.032	0.083	0.016	0.042	0.033	0.087	0.016	0.043
	50	0.204	0.616	0.052	0.158	0.026	0.079	0.013	0.039	0.027	0.082	0.013	0.041
	100	0.047	0.406	0.012	0.104	0.006	0.051	0.003	0.026	0.006	0.053	0.003	0.027
D1, stream	0	1.201	-	0.424	-	0.212	-	0.106	-	0.220	-	0.110	-
	1	0.372	0.925	0.131	0.327	0.066	0.163	0.033	0.082	0.068	0.170	0.034	0.085
	2	0.017	0.526	0.006	0.186	0.003	0.093	0.001	0.046	0.003	0.096	0.001	0.048
	4	0.002	0.265	0.001	0.094	<0.001	0.047	<0.001	0.023	<0.001	0.049	<0.001	0.024
	7	0.002	0.152	0.001	0.054	<0.001	0.027	<0.001	0.013	<0.001	0.028	<0.001	0.014
	14	0.001	0.077	<0.001	0.027	<0.001	0.014	<0.001	0.007	<0.001	0.014	<0.001	0.007
	21	0.001	0.052	<0.001	0.018	<0.001	0.009	<0.001	0.005	<0.001	0.009	<0.001	0.005
	28	<0.001	0.048	<0.001	0.017	<0.001	0.008	<0.001	0.004	<0.001	0.009	<0.001	0.004
	42	<0.001	0.032	<0.001	0.011	<0.001	0.006	<0.001	0.003	<0.001	0.006	<0.001	0.003
	50	<0.001	0.027	<0.001	0.010	<0.001	0.005	<0.001	0.002	<0.001	0.005	<0.001	0.002
	100	<0.001	0.014	<0.001	0.005	<0.001	0.002	<0.001	0.001	<0.001	0.003	<0.001	0.001

Table 9.2.5-47: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 5mD PEC_{sw} [µg L^{-1}]		5mD50N PEC_{sw} [µg L^{-1}]		5mD75N PEC_{sw} [µg L^{-1}]		10mD PEC_{sw} [µg L^{-1}]		10mD50N PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D2, ditch	0	1.396	-	0.362	-	0.181	-	0.091	-	0.188	-	0.094	-
	1	1.262	1.326	0.327	0.344	0.164	0.172	0.082	0.086	0.170	0.178	0.085	0.089
	2	1.147	1.264	0.297	0.328	0.149	0.164	0.074	0.082	0.154	0.170	0.077	0.085
	4	0.963	1.157	0.249	0.300	0.124	0.150	0.062	0.075	0.129	0.156	0.065	0.078
	7	0.010	1.024	0.003	0.265	0.001	0.132	0.001	0.066	0.001	0.137	0.001	0.069
	14	0.019	0.520	0.005	0.134	0.002	0.067	0.001	0.034	0.002	0.070	0.001	0.035
	21	0.061	0.360	0.016	0.093	0.008	0.047	0.004	0.023	0.008	0.048	0.004	0.024
	28	0.073	0.287	0.019	0.074	0.009	0.037	0.005	0.019	0.010	0.039	0.005	0.019
	42	0.064	0.215	0.017	0.056	0.008	0.028	0.004	0.014	0.009	0.029	0.004	0.014
	50	0.056	0.190	0.015	0.049	0.007	0.025	0.004	0.012	0.007	0.026	0.004	0.013
	100	0.022	0.142	0.006	0.037	0.003	0.018	0.001	0.009	0.003	0.019	0.001	0.010
D2, stream	0	1.225	-	0.432	-	0.216	-	0.108	-	0.225	-	0.112	-
	1	1.106	1.163	0.391	0.411	0.195	0.205	0.098	0.103	0.203	0.213	0.101	0.106
	2	1.005	1.108	0.355	0.391	0.177	0.195	0.089	0.098	0.184	0.203	0.092	0.101
	4	0.843	1.014	0.297	0.358	0.148	0.179	0.074	0.089	0.154	0.186	0.077	0.093
	7	<0.001	0.888	<0.001	0.313	<0.001	0.156	<0.001	0.078	<0.001	0.162	<0.001	0.081
	14	0.001	0.445	<0.001	0.157	<0.001	0.078	<0.001	0.039	<0.001	0.081	<0.001	0.041
	21	0.025	0.299	0.009	0.105	0.004	0.053	0.002	0.026	0.005	0.055	0.002	0.027
	28	0.044	0.233	0.016	0.082	0.008	0.041	0.004	0.021	0.008	0.043	0.004	0.021
	42	0.041	0.170	0.015	0.060	0.007	0.030	0.004	0.015	0.008	0.031	0.004	0.016
	50	0.035	0.149	0.013	0.053	0.006	0.026	0.003	0.013	0.007	0.027	0.003	0.014
	100	0.011	0.104	0.004	0.037	0.002	0.018	0.001	0.009	0.002	0.019	0.001	0.009

Table 9.2.5-47: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 5mD PEC_{sw} [µg L^{-1}]		5mD50N PEC_{sw} [µg L^{-1}]		5mD75N PEC_{sw} [µg L^{-1}]		10mD PEC_{sw} [µg L^{-1}]		10mD50N PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D3, ditch	0	1.370	-	0.355	-	0.178	-	0.089	-	0.184	-	0.092	-
	1	0.559	1.026	0.145	0.266	0.072	0.133	0.036	0.067	0.075	0.138	0.038	0.069
	2	0.052	0.634	0.014	0.164	0.007	0.082	0.003	0.041	0.007	0.085	0.003	0.043
	4	0.004	0.324	0.001	0.084	<0.001	0.042	<0.001	0.021	0.001	0.044	<0.001	0.022
	7	0.003	0.186	0.001	0.048	<0.001	0.024	<0.001	0.012	<0.001	0.025	<0.001	0.013
	14	0.002	0.095	0.001	0.025	<0.001	0.012	<0.001	0.006	<0.001	0.013	<0.001	0.006
	21	0.001	0.064	<0.001	0.016	<0.001	0.008	<0.001	0.004	<0.001	0.009	<0.001	0.004
	28	0.001	0.094	<0.001	0.024	<0.001	0.012	<0.001	0.006	<0.001	0.013	<0.001	0.006
	42	<0.001	0.063	<0.001	0.016	<0.001	0.008	<0.001	0.004	<0.001	0.008	<0.001	0.004
	50	<0.001	0.053	<0.001	0.014	<0.001	0.007	<0.001	0.003	<0.001	0.007	<0.001	0.004
	100	<0.001	0.027	<0.001	0.007	<0.001	0.003	<0.001	0.002	<0.001	0.004	<0.001	0.002
D4, pond	0	0.065	-	0.056	-	0.028	-	0.014	-	0.040	-	0.020	-
	1	0.063	0.064	0.055	0.055	0.027	0.028	0.014	0.014	0.039	0.039	0.019	0.020
	2	0.062	0.063	0.053	0.055	0.027	0.027	0.013	0.014	0.038	0.039	0.019	0.019
	4	0.059	0.062	0.050	0.053	0.025	0.027	0.013	0.013	0.036	0.038	0.018	0.019
	7	0.055	0.060	0.047	0.051	0.023	0.026	0.012	0.013	0.033	0.036	0.017	0.018
	14	0.048	0.055	0.041	0.047	0.020	0.024	0.010	0.012	0.029	0.034	0.015	0.017
	21	0.042	0.052	0.036	0.045	0.018	0.022	0.009	0.011	0.026	0.032	0.013	0.016
	28	0.038	0.049	0.033	0.042	0.016	0.021	0.008	0.010	0.023	0.030	0.012	0.015
	42	0.032	0.044	0.028	0.038	0.014	0.019	0.007	0.009	0.019	0.027	0.010	0.013
	50	0.029	0.042	0.025	0.036	0.012	0.018	0.006	0.009	0.018	0.026	0.009	0.013
	100	0.016	0.035	0.014	0.030	0.007	0.015	0.003	0.007	0.010	0.021	0.005	0.011

Table 9.2.5-47: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 5mD PEC_{sw} [µg L^{-1}]		5mD50N PEC_{sw} [µg L^{-1}]		5mD75N PEC_{sw} [µg L^{-1}]		10mD PEC_{sw} [µg L^{-1}]		10mD50N PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D4, stream	0	1.076	-	0.380	-	0.190	-	0.095	-	0.197	-	0.099	-
	1	<0.001	0.073	<0.001	0.026	<0.001	0.013	<0.001	0.006	<0.001	0.013	<0.001	0.007
	2	<0.001	0.036	<0.001	0.013	<0.001	0.006	<0.001	0.003	<0.001	0.007	<0.001	0.003
	4	<0.001	0.018	<0.001	0.006	<0.001	0.003	<0.001	0.002	<0.001	0.003	<0.001	0.002
	7	<0.001	0.010	<0.001	0.004	<0.001	0.002	<0.001	0.001	<0.001	0.002	<0.001	0.001
	14	<0.001	0.005	<0.001	0.002	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
	21	<0.001	0.003	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
	28	<0.001	0.003	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	42	<0.001	0.003	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
	50	<0.001	0.002	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	100	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
D5, pond	0	0.066	-	0.057	-	0.029	-	0.014	-	0.040	-	0.020	-
	1	0.065	0.065	0.056	0.056	0.028	0.028	0.014	0.014	0.039	0.040	0.020	0.020
	2	0.063	0.065	0.054	0.056	0.027	0.028	0.013	0.014	0.038	0.039	0.019	0.020
	4	0.060	0.063	0.052	0.054	0.026	0.027	0.013	0.013	0.037	0.038	0.018	0.019
	7	0.056	0.061	0.048	0.052	0.024	0.026	0.012	0.013	0.034	0.037	0.017	0.019
	14	0.049	0.057	0.042	0.049	0.021	0.024	0.011	0.012	0.030	0.035	0.015	0.017
	21	0.044	0.053	0.038	0.046	0.019	0.023	0.009	0.011	0.027	0.032	0.013	0.016
	28	0.040	0.051	0.034	0.043	0.017	0.022	0.008	0.011	0.024	0.031	0.012	0.015
	42	0.033	0.046	0.029	0.039	0.014	0.020	0.007	0.010	0.020	0.028	0.010	0.014
	50	0.030	0.044	0.026	0.037	0.013	0.019	0.006	0.009	0.018	0.027	0.009	0.013
	100	0.016	0.036	0.014	0.031	0.007	0.016	0.003	0.008	0.010	0.022	0.005	0.011

Table 9.2.5-47: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation									
				5mD		5mD50N		5mD75N		10mD		10mD50N	
				PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D5, stream	0	1.251	-	0.442	-	0.221	-	0.110	-	0.229	-	0.114	-
	1	<0.001	0.314	<0.001	0.111	<0.001	0.055	<0.001	0.028	<0.001	0.058	<0.001	0.029
	2	<0.001	0.157	<0.001	0.055	<0.001	0.028	<0.001	0.014	<0.001	0.029	<0.001	0.014
	4	<0.001	0.079	<0.001	0.028	<0.001	0.014	<0.001	0.007	<0.001	0.014	<0.001	0.007
	7	<0.001	0.045	<0.001	0.016	<0.001	0.008	<0.001	0.004	<0.001	0.008	<0.001	0.004
	14	<0.001	0.023	<0.001	0.008	<0.001	0.004	<0.001	0.002	<0.001	0.004	<0.001	0.002
	21	<0.001	0.015	<0.001	0.005	<0.001	0.003	<0.001	0.001	<0.001	0.003	<0.001	0.001
	28	<0.001	0.011	<0.001	0.004	<0.001	0.002	<0.001	0.001	<0.001	0.002	<0.001	0.001
	42	<0.001	0.009	<0.001	0.003	<0.001	0.002	<0.001	0.001	<0.001	0.002	<0.001	0.001
	50	<0.001	0.007	<0.001	0.003	<0.001	0.001	<0.001	0.001	<0.001	0.001	<0.001	0.001
	100	<0.001	0.004	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001
D6, ditch	0	1.381	-	0.358	-	0.179	-	0.090	-	0.186	-	0.093	-
	1	1.226	1.299	0.318	0.337	0.159	0.168	0.079	0.084	0.165	0.175	0.083	0.088
	2	1.102	1.230	0.286	0.319	0.143	0.159	0.071	0.080	0.148	0.165	0.074	0.083
	4	0.832	1.101	0.215	0.285	0.107	0.143	0.054	0.071	0.112	0.148	0.056	0.074
	7	0.402	0.890	0.103	0.230	0.051	0.115	0.026	0.057	0.053	0.119	0.027	0.060
	14	0.084	0.539	0.021	0.139	0.010	0.069	0.005	0.035	0.011	0.072	0.005	0.036
	21	0.044	0.379	0.011	0.098	0.005	0.049	0.003	0.024	0.006	0.051	0.003	0.025
	28	0.027	0.293	0.007	0.075	0.003	0.038	0.002	0.019	0.004	0.039	0.002	0.020
	42	0.011	0.201	0.003	0.052	0.001	0.026	0.001	0.013	0.001	0.027	0.001	0.013
	50	0.006	0.170	0.002	0.044	0.001	0.022	<0.001	0.011	0.001	0.023	<0.001	0.011
	100	<0.001	0.091	<0.001	0.024	<0.001	0.012	<0.001	0.006	<0.001	0.012	<0.001	0.006

Table 9.2.5-47: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 5mD PEC_{sw} [µg L^{-1}]		5mD50N PEC_{sw} [µg L^{-1}]		5mD75N PEC_{sw} [µg L^{-1}]		10mD PEC_{sw} [µg L^{-1}]		10mD50N PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
R1, pond	0	0.065	-	0.056	-	0.040	-	0.034	-	0.046	-	0.036	-
	1	0.063	0.064	0.054	0.055	0.039	0.040	0.033	0.033	0.045	0.045	0.035	0.036
	2	0.061	0.063	0.053	0.054	0.038	0.039	0.032	0.033	0.044	0.045	0.034	0.035
	4	0.058	0.061	0.050	0.053	0.037	0.038	0.031	0.032	0.042	0.044	0.033	0.035
	7	0.054	0.059	0.046	0.051	0.034	0.037	0.029	0.031	0.039	0.043	0.031	0.033
	14	0.046	0.054	0.040	0.047	0.030	0.035	0.025	0.029	0.034	0.040	0.027	0.031
	21	0.041	0.051	0.036	0.046	0.026	0.033	0.022	0.027	0.031	0.038	0.024	0.029
	28	0.048	0.050	0.043	0.045	0.034	0.033	0.020	0.028	0.038	0.038	0.032	0.030
	42	0.050	0.050	0.046	0.044	0.026	0.032	0.018	0.027	0.029	0.037	0.024	0.029
	50	0.043	0.049	0.040	0.044	0.024	0.032	0.015	0.026	0.026	0.036	0.022	0.028
	100	0.026	0.043	0.024	0.039	0.012	0.027	0.010	0.021	0.014	0.032	0.012	0.024
R1, stream	0	0.897	-	0.317	-	0.240	-	0.240	-	0.240	-	0.240	-
	1	<0.001	0.187	<0.001	0.128	<0.001	0.128	<0.001	0.128	<0.001	0.128	<0.001	0.128
	2	<0.001	0.094	<0.001	0.064	<0.001	0.064	<0.001	0.064	<0.001	0.064	<0.001	0.064
	4	<0.001	0.047	<0.001	0.032	0.001	0.032	0.001	0.032	0.001	0.032	0.001	0.032
	7	<0.001	0.031	<0.001	0.031	<0.001	0.031	<0.001	0.031	<0.001	0.031	<0.001	0.031
	14	<0.001	0.024	<0.001	0.024	0.008	0.024	0.008	0.024	0.008	0.024	0.008	0.024
	21	<0.001	0.019	<0.001	0.019	<0.001	0.019	<0.001	0.019	<0.001	0.019	<0.001	0.019
	28	<0.001	0.016	<0.001	0.016	<0.001	0.016	<0.001	0.016	<0.001	0.016	<0.001	0.016
	42	<0.001	0.014	<0.001	0.012	<0.001	0.012	<0.001	0.012	<0.001	0.012	<0.001	0.012
	50	<0.001	0.012	<0.001	0.011	<0.001	0.011	<0.001	0.011	<0.001	0.011	<0.001	0.011
	100	<0.001	0.010	<0.001	0.008	<0.001	0.007	<0.001	0.007	<0.001	0.007	<0.001	0.007

Table 9.2.5-47: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift mitigation at Step 4

Winter cereals													
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 5mD PEC_{sw} [µg L^{-1}]		5mD50N PEC_{sw} [µg L^{-1}]		5mD75N PEC_{sw} [µg L^{-1}]		10mD PEC_{sw} [µg L^{-1}]		10mD50N PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
R3, stream	0	1.265	-	0.447	-	0.223	-	0.163	-	0.232	-	0.163	-
	1	0.004	0.465	0.002	0.164	0.001	0.094	<0.001	0.094	0.001	0.094	<0.001	0.094
	2	<0.001	0.233	<0.001	0.082	<0.001	0.048	<0.001	0.048	<0.001	0.048	<0.001	0.048
	4	<0.001	0.117	<0.001	0.041	<0.001	0.024	<0.001	0.024	<0.001	0.024	<0.001	0.024
	7	<0.001	0.067	<0.001	0.024	<0.001	0.014	<0.001	0.014	<0.001	0.014	<0.001	0.014
	14	<0.001	0.039	<0.001	0.018	<0.001	0.012	<0.001	0.012	<0.001	0.012	<0.001	0.012
	21	<0.001	0.026	<0.001	0.012	<0.001	0.008	<0.001	0.008	<0.001	0.008	<0.001	0.008
	28	<0.001	0.020	<0.001	0.009	<0.001	0.006	<0.001	0.006	<0.001	0.006	<0.001	0.006
	42	<0.001	0.023	<0.001	0.009	<0.001	0.006	<0.001	0.004	<0.001	0.006	<0.001	0.004
	50	<0.001	0.019	<0.001	0.008	<0.001	0.005	<0.001	0.003	<0.001	0.005	<0.001	0.003
	100	<0.001	0.011	<0.001	0.006	<0.001	0.004	<0.001	0.003	<0.001	0.004	<0.001	0.003
R4, stream	0	0.898	-	0.543	-	0.543	-	0.543	-	0.543	-	0.543	-
	1	<0.001	0.532	0.506	0.532	0.506	0.532	0.506	0.532	0.506	0.532	0.506	0.532
	2	<0.001	0.394	0.002	0.394	0.002	0.394	0.002	0.394	0.002	0.394	0.002	0.394
	4	<0.001	0.198	0.001	0.198	0.001	0.198	0.001	0.198	0.001	0.198	0.001	0.198
	7	<0.001	0.113	0.001	0.113	0.001	0.113	0.001	0.113	0.001	0.113	0.001	0.113
	14	0.001	0.070	0.001	0.061	0.001	0.059	0.001	0.058	0.001	0.059	0.001	0.058
	21	0.001	0.047	<0.001	0.041	<0.001	0.040	<0.001	0.039	<0.001	0.040	<0.001	0.039
	28	0.001	0.040	0.359	0.036	0.359	0.035	0.359	0.034	0.359	0.035	0.359	0.034
	42	<0.001	0.031	<0.001	0.028	<0.001	0.028	<0.001	0.027	<0.001	0.028	<0.001	0.027
	50	<0.001	0.030	<0.001	0.025	<0.001	0.024	<0.001	0.023	<0.001	0.024	<0.001	0.023
	100	<0.001	0.018	<0.001	0.015	<0.001	0.014	<0.001	0.014	<0.001	0.014	<0.001	0.014

D = Drift mitigation by no-spray buffer zones
N = Drift mitigation by nozzle reduction
[a] Time: days after maximum concentration (Actual) or time interval (TWA)

Table 9.2.5-48: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation 10mD+R PEC_{sw} [µg L^{-1}]		15mD PEC_{sw} [µg L^{-1}]		20mD PEC_{sw} [µg L^{-1}]		20mD+R PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D1, ditch	0	1.504	-	0.202	-	0.136	-	0.102	-	0.102	-
	1	1.372	1.435	0.184	0.192	0.124	0.130	0.093	0.098	0.093	0.098
	2	1.259	1.374	0.169	0.184	0.114	0.124	0.086	0.093	0.086	0.093
	4	1.077	1.268	0.144	0.170	0.097	0.115	0.073	0.086	0.073	0.086
	7	0.885	1.142	0.118	0.153	0.079	0.103	0.059	0.077	0.059	0.077
	14	0.626	0.942	0.082	0.125	0.055	0.084	0.041	0.063	0.041	0.063
	21	0.479	0.810	0.062	0.107	0.042	0.072	0.031	0.054	0.031	0.054
	28	0.381	0.715	0.049	0.094	0.033	0.063	0.025	0.048	0.025	0.048
	42	0.254	0.653	0.033	0.087	0.022	0.058	0.017	0.044	0.017	0.044
	50	0.204	0.616	0.027	0.082	0.018	0.055	0.013	0.041	0.013	0.041
	100	0.047	0.406	0.006	0.053	0.004	0.036	0.003	0.027	0.003	0.027
D1, stream	0	1.201	-	0.220	-	0.148	-	0.112	-	0.112	-
	1	0.372	0.925	0.068	0.170	0.046	0.114	0.035	0.086	0.035	0.086
	2	0.017	0.526	0.003	0.096	0.002	0.065	0.002	0.049	0.002	0.049
	4	0.002	0.265	<0.001	0.049	<0.001	0.033	<0.001	0.025	<0.001	0.025
	7	0.002	0.152	<0.001	0.028	<0.001	0.019	<0.001	0.014	<0.001	0.014
	14	0.001	0.077	<0.001	0.014	<0.001	0.009	<0.001	0.007	<0.001	0.007
	21	0.001	0.052	<0.001	0.009	<0.001	0.006	<0.001	0.005	<0.001	0.005
	28	<0.001	0.048	<0.001	0.009	<0.001	0.006	<0.001	0.004	<0.001	0.004
	42	<0.001	0.032	<0.001	0.006	<0.001	0.004	<0.001	0.003	<0.001	0.003
	50	<0.001	0.027	<0.001	0.005	<0.001	0.003	<0.001	0.003	<0.001	0.003
	100	<0.001	0.014	<0.001	0.003	<0.001	0.002	<0.001	0.001	<0.001	0.001

Table 9.2.5-48: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation							
				10mD+R		15mD		20mD		20mD+R	
				PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D2, ditch	0	1.396	-	0.188	-	0.127	-	0.095	-	0.095	-
	1	1.262	1.326	0.170	0.178	0.115	0.121	0.086	0.091	0.086	0.091
	2	1.147	1.264	0.154	0.170	0.104	0.115	0.078	0.086	0.078	0.086
	4	0.963	1.157	0.129	0.156	0.087	0.105	0.066	0.079	0.066	0.079
	7	0.010	1.024	0.001	0.137	0.001	0.093	0.001	0.070	0.001	0.070
	14	0.019	0.520	0.002	0.070	0.002	0.047	0.001	0.035	0.001	0.035
	21	0.061	0.360	0.008	0.048	0.005	0.033	0.004	0.025	0.004	0.025
	28	0.073	0.287	0.010	0.039	0.007	0.026	0.005	0.020	0.005	0.020
	42	0.064	0.215	0.009	0.029	0.006	0.019	0.004	0.015	0.004	0.015
	50	0.056	0.190	0.007	0.026	0.005	0.017	0.004	0.013	0.004	0.013
	100	0.022	0.142	0.003	0.019	0.002	0.013	0.001	0.010	0.001	0.010
D2, stream	0	1.225	-	0.225	-	0.151	-	0.114	-	0.114	-
	1	1.106	1.163	0.203	0.213	0.137	0.144	0.103	0.108	0.103	0.108
	2	1.005	1.108	0.184	0.203	0.124	0.137	0.094	0.103	0.094	0.103
	4	0.843	1.014	0.154	0.186	0.104	0.125	0.078	0.094	0.078	0.094
	7	<0.001	0.888	<0.001	0.162	<0.001	0.109	<0.001	0.083	<0.001	0.083
	14	0.001	0.445	<0.001	0.081	<0.001	0.055	<0.001	0.041	<0.001	0.041
	21	0.025	0.299	0.005	0.055	0.003	0.037	0.002	0.028	0.002	0.028
	28	0.044	0.233	0.008	0.043	0.005	0.029	0.004	0.022	0.004	0.022
	42	0.041	0.170	0.008	0.031	0.005	0.021	0.004	0.016	0.004	0.016
	50	0.035	0.149	0.007	0.027	0.004	0.018	0.003	0.014	0.003	0.014
	100	0.011	0.104	0.002	0.019	0.001	0.013	0.001	0.010	0.001	0.010

Table 9.2.5-48: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation							
				10mD+R		15mD		20mD		20mD+R	
				PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D3, ditch	0	1.370	-	0.184	-	0.125	-	0.094	-	0.094	-
	1	0.559	1.026	0.075	0.138	0.051	0.093	0.038	0.070	0.038	0.070
	2	0.052	0.634	0.007	0.085	0.005	0.058	0.004	0.043	0.004	0.043
	4	0.004	0.324	0.001	0.044	<0.001	0.029	<0.001	0.022	<0.001	0.022
	7	0.003	0.186	<0.001	0.025	<0.001	0.017	<0.001	0.013	<0.001	0.013
	14	0.002	0.095	<0.001	0.013	<0.001	0.009	<0.001	0.006	<0.001	0.006
	21	0.001	0.064	<0.001	0.009	<0.001	0.006	<0.001	0.004	<0.001	0.004
	28	0.001	0.094	<0.001	0.013	<0.001	0.009	<0.001	0.006	<0.001	0.006
	42	<0.001	0.063	<0.001	0.008	<0.001	0.006	<0.001	0.004	<0.001	0.004
	50	<0.001	0.053	<0.001	0.007	<0.001	0.005	<0.001	0.004	<0.001	0.004
	100	<0.001	0.027	<0.001	0.004	<0.001	0.002	<0.001	0.002	<0.001	0.002
D4, pond	0	0.065	-	0.040	-	0.031	-	0.026	-	0.026	-
	1	0.063	0.064	0.039	0.039	0.031	0.031	0.025	0.026	0.025	0.026
	2	0.062	0.063	0.038	0.039	0.030	0.031	0.025	0.025	0.025	0.025
	4	0.059	0.062	0.036	0.038	0.028	0.030	0.023	0.025	0.023	0.025
	7	0.055	0.060	0.033	0.036	0.026	0.029	0.022	0.024	0.022	0.024
	14	0.048	0.055	0.029	0.034	0.023	0.027	0.019	0.022	0.019	0.022
	21	0.042	0.052	0.026	0.032	0.020	0.025	0.017	0.021	0.017	0.021
	28	0.038	0.049	0.023	0.030	0.018	0.024	0.015	0.020	0.015	0.020
	42	0.032	0.044	0.019	0.027	0.015	0.021	0.013	0.018	0.013	0.018
	50	0.029	0.042	0.018	0.026	0.014	0.020	0.012	0.017	0.012	0.017
	100	0.016	0.035	0.010	0.021	0.008	0.017	0.006	0.014	0.006	0.014

Table 9.2.5-48: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation							
				10mD+R		15mD		20mD		20mD+R	
				PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D4, stream	0	1.076	-	0.197	-	0.133	-	0.100	-	0.100	-
	1	<0.001	0.073	<0.001	0.013	<0.001	0.009	<0.001	0.007	<0.001	0.007
	2	<0.001	0.036	<0.001	0.007	<0.001	0.004	<0.001	0.003	<0.001	0.003
	4	<0.001	0.018	<0.001	0.003	<0.001	0.002	<0.001	0.002	<0.001	0.002
	7	<0.001	0.010	<0.001	0.002	<0.001	0.001	<0.001	0.001	<0.001	0.001
	14	<0.001	0.005	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
	21	<0.001	0.003	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	28	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	42	<0.001	0.003	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	50	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	100	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
D5, pond	0	0.066	-	0.040	-	0.032	-	0.027	-	0.027	-
	1	0.065	0.065	0.039	0.040	0.031	0.032	0.026	0.026	0.026	0.026
	2	0.063	0.065	0.038	0.039	0.030	0.031	0.025	0.026	0.025	0.026
	4	0.060	0.063	0.037	0.038	0.029	0.030	0.024	0.025	0.024	0.025
	7	0.056	0.061	0.034	0.037	0.027	0.029	0.023	0.024	0.023	0.024
	14	0.049	0.057	0.030	0.035	0.024	0.027	0.020	0.023	0.020	0.023
	21	0.044	0.053	0.027	0.032	0.021	0.026	0.018	0.021	0.018	0.021
	28	0.040	0.051	0.024	0.031	0.019	0.024	0.016	0.020	0.016	0.020
	42	0.033	0.046	0.020	0.028	0.016	0.022	0.013	0.018	0.013	0.018
	50	0.030	0.044	0.018	0.027	0.014	0.021	0.012	0.017	0.012	0.017
	100	0.016	0.036	0.010	0.022	0.008	0.017	0.006	0.015	0.006	0.015

Table 9.2.5-48: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation							
				10mD+R		15mD		20mD		20mD+R	
				PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
D5, stream	0	1.251	-	0.229	-	0.155	-	0.116	-	0.116	-
	1	<0.001	0.314	<0.001	0.058	<0.001	0.039	<0.001	0.029	<0.001	0.029
	2	<0.001	0.157	<0.001	0.029	<0.001	0.019	<0.001	0.015	<0.001	0.015
	4	<0.001	0.079	<0.001	0.014	<0.001	0.010	<0.001	0.007	<0.001	0.007
	7	<0.001	0.045	<0.001	0.008	<0.001	0.006	<0.001	0.004	<0.001	0.004
	14	<0.001	0.023	<0.001	0.004	<0.001	0.003	<0.001	0.002	<0.001	0.002
	21	<0.001	0.015	<0.001	0.003	<0.001	0.002	<0.001	0.001	<0.001	0.001
	28	<0.001	0.011	<0.001	0.002	<0.001	0.001	<0.001	0.001	<0.001	0.001
	42	<0.001	0.009	<0.001	0.002	<0.001	0.001	<0.001	0.001	<0.001	0.001
	50	<0.001	0.007	<0.001	0.001	<0.001	0.001	<0.001	0.001	<0.001	0.001
	100	<0.001	0.004	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
D6, ditch	0	1.381	-	0.186	-	0.126	-	0.094	-	0.094	-
	1	1.226	1.299	0.165	0.175	0.111	0.118	0.084	0.089	0.084	0.089
	2	1.102	1.230	0.148	0.165	0.100	0.112	0.075	0.084	0.075	0.084
	4	0.832	1.101	0.112	0.148	0.075	0.100	0.057	0.075	0.057	0.075
	7	0.402	0.890	0.053	0.119	0.036	0.081	0.027	0.061	0.027	0.061
	14	0.084	0.539	0.011	0.072	0.007	0.049	0.005	0.036	0.005	0.036
	21	0.044	0.379	0.006	0.051	0.004	0.034	0.003	0.026	0.003	0.026
	28	0.027	0.293	0.004	0.039	0.002	0.026	0.002	0.020	0.002	0.020
	42	0.011	0.201	0.001	0.027	0.001	0.018	0.001	0.014	0.001	0.014
	50	0.006	0.170	0.001	0.023	0.001	0.015	<0.001	0.012	<0.001	0.012
	100	<0.001	0.091	<0.001	0.012	<0.001	0.008	<0.001	0.006	<0.001	0.006

Table 9.2.5-48: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation							
				10mD+R		15mD		20mD		20mD+R	
				PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
R1, pond	0	0.065	-	0.039	-	0.042	-	0.039	-	0.026	-
	1	0.063	0.064	0.038	0.038	0.041	0.041	0.038	0.039	0.025	0.025
	2	0.061	0.063	0.037	0.038	0.040	0.041	0.037	0.038	0.024	0.025
	4	0.058	0.061	0.035	0.037	0.038	0.040	0.036	0.037	0.023	0.024
	7	0.054	0.059	0.032	0.035	0.036	0.039	0.034	0.036	0.021	0.023
	14	0.046	0.054	0.028	0.033	0.031	0.036	0.029	0.034	0.018	0.021
	21	0.041	0.051	0.025	0.031	0.028	0.034	0.026	0.032	0.016	0.020
	28	0.048	0.050	0.026	0.029	0.035	0.034	0.034	0.032	0.017	0.019
	42	0.050	0.050	0.026	0.029	0.027	0.034	0.026	0.032	0.016	0.018
	50	0.043	0.049	0.022	0.028	0.024	0.033	0.023	0.031	0.014	0.018
	100	0.026	0.043	0.013	0.023	0.013	0.029	0.012	0.026	0.008	0.015
R1, stream	0	0.897	-	0.164	-	0.240	-	0.240	-	0.084	-
	1	<0.001	0.187	<0.001	0.058	<0.001	0.128	<0.001	0.128	<0.001	0.031
	2	<0.001	0.094	<0.001	0.029	<0.001	0.064	<0.001	0.064	<0.001	0.015
	4	<0.001	0.047	<0.001	0.015	0.001	0.032	0.001	0.032	<0.001	0.008
	7	<0.001	0.031	<0.001	0.014	<0.001	0.031	<0.001	0.031	<0.001	0.007
	14	<0.001	0.024	<0.001	0.011	0.008	0.024	0.008	0.024	<0.001	0.006
	21	<0.001	0.019	<0.001	0.009	<0.001	0.019	<0.001	0.019	<0.001	0.005
	28	<0.001	0.016	<0.001	0.007	<0.001	0.016	<0.001	0.016	<0.001	0.004
	42	<0.001	0.014	<0.001	0.006	<0.001	0.012	<0.001	0.012	<0.001	0.003
	50	<0.001	0.012	<0.001	0.005	<0.001	0.011	<0.001	0.011	<0.001	0.003
	100	<0.001	0.010	<0.001	0.003	<0.001	0.007	<0.001	0.007	<0.001	0.002

Table 9.2.5-48: Step 3 and 4: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following twofold application of 250 g a.s. ha^{-1} to winter cereals, considering drift and runoff mitigation at Step 4

Winter cereals											
Location	Time[a] [d]	Step 3 Edge-of-field PEC_{sw} [µg L^{-1}]		Step 4: Buffer zones and mitigation							
				10mD+R		15mD		20mD		20mD+R	
				PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
		Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
R3, stream	0	1.265	-	0.232	-	0.163	-	0.163	-	0.118	-
	1	0.004	0.465	0.001	0.085	<0.001	0.094	<0.001	0.094	<0.001	0.043
	2	<0.001	0.233	<0.001	0.043	<0.001	0.048	<0.001	0.048	<0.001	0.022
	4	<0.001	0.117	<0.001	0.021	<0.001	0.024	<0.001	0.024	<0.001	0.011
	7	<0.001	0.067	<0.001	0.012	<0.001	0.014	<0.001	0.014	<0.001	0.006
	14	<0.001	0.039	<0.001	0.009	<0.001	0.012	<0.001	0.012	<0.001	0.004
	21	<0.001	0.026	<0.001	0.006	<0.001	0.008	<0.001	0.008	<0.001	0.003
	28	<0.001	0.020	<0.001	0.004	<0.001	0.006	<0.001	0.006	<0.001	0.002
	42	<0.001	0.023	<0.001	0.005	<0.001	0.005	<0.001	0.004	<0.001	0.002
	50	<0.001	0.019	<0.001	0.004	<0.001	0.004	<0.001	0.003	<0.001	0.002
	100	<0.001	0.011	<0.001	0.003	<0.001	0.004	<0.001	0.003	<0.001	0.001
R4, stream	0	0.898	-	0.248	-	0.543	-	0.543	-	0.130	-
	1	<0.001	0.532	0.228	0.243	0.506	0.532	0.506	0.532	0.119	0.127
	2	<0.001	0.394	<0.001	0.179	0.002	0.394	0.002	0.394	<0.001	0.094
	4	<0.001	0.198	<0.001	0.090	0.001	0.198	0.001	0.198	<0.001	0.047
	7	<0.001	0.113	<0.001	0.051	0.001	0.113	0.001	0.113	<0.001	0.027
	14	0.001	0.070	<0.001	0.028	0.001	0.058	0.001	0.058	<0.001	0.015
	21	0.001	0.047	<0.001	0.019	<0.001	0.039	<0.001	0.039	<0.001	0.010
	28	0.001	0.040	0.163	0.017	0.359	0.034	0.359	0.034	0.085	0.009
	42	<0.001	0.031	<0.001	0.013	<0.001	0.027	<0.001	0.027	<0.001	0.007
	50	<0.001	0.030	<0.001	0.012	<0.001	0.023	<0.001	0.023	<0.001	0.006
	100	<0.001	0.018	<0.001	0.007	<0.001	0.014	<0.001	0.014	<0.001	0.004

D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
[a] Time: days after maximum concentration (Actual) or time interval (TWA)

SEDIMENT

Only global maximum values are reported, which can also be considered as worst-case estimates of short-term and long-term exposure.

Global maximum concentrations – pyraclostrobin

Step 1 and 2

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-49: Steps 1-2: $PEC_{sed,max}$ of pyraclostrobin following application to cereals and maize (Tier 1)

$FOCUS_{sw}$ crop	Pyraclostrobin $PEC_{sed,max}$ [µg kg^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	578.377	1160.000	66.328	103.437	118.768	187.230
Maize	462.701	-[a]	53.062	-[a]	95.014	-[a]

[a] Only single application calculated for maize

Tier 2 (parameters from irradiated water/sediment study)

Table 9.2.5-50: Steps 1-2: $PEC_{sed,max}$ of pyraclostrobin following application to cereals and maize (Tier 2)

$FOCUS_{sw}$ crop	Pyraclostrobin $PEC_{sed,max}$ [µg kg^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	578.377	1160.000	62.483	93.654	114.923	177.447
Maize	462.701	-[a]	49.987	-[a]	91.939	-[a]

[a] Only single application calculated for maize

Step 3

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-51: Step 3: $PEC_{sed,max}$ of pyraclostrobin following application to winter cereals (Tier 1)

Location	Water body	$PEC_{sed,max}$ [µg kg^{-1}]	
		Winter cereals	
		Single application	Twofold application
D1	ditch	5.850	8.901
D1	stream	0.235	0.836
D2	ditch	3.320	5.032
D2	stream	2.453	4.212
D3	ditch	1.034	1.289
D4	pond	0.459	0.698
D4	stream	0.039	0.065
D5	pond	0.429	0.649
D5	stream	0.039	0.242
D6	ditch	0.454	3.807
R1	pond	0.500	0.897
R1	stream	1.817	4.063
R3	stream	0.911	2.011
R4	stream	4.180	10.583

Table 9.2.5-52: Step 3: $PEC_{sed,max}$ of pyraclostrobin following application to spring cereals (Tier 1)

Location	Water body	$PEC_{sed,max}$ [µg kg^{-1}]	
		Spring cereals	
		Single application	Twofold application
D1	ditch	6.438	9.836
D1	stream	0.869	1.015
D3	ditch	1.076	1.496
D4	pond	0.404	0.586
D4	stream	0.109	0.257
D5	pond	0.420	0.646
D5	stream	0.057	0.093
R4	stream	5.553	5.544

Table 9.2.5-53: Step 3: $PEC_{sed,max}$ of pyraclostrobin following application to <u>maize</u> (Tier 1)

Location	Water body	$PEC_{sed,max}$ [µg kg^{-1}]
		Maize
		Single application
D3	ditch	0.719
D4	pond	0.312
D4	stream	0.067
D5	pond	0.304
D5	stream	0.030
D6	ditch	0.388
R1	pond	0.387
R1	stream	2.006
R2	stream	3.593
R3	stream	1.859
R4	stream	6.572

Tier 2 (parameters from irradiated water/sediment study)

Table 9.2.5-54: Step 3: $PEC_{sed,max}$ of pyraclostrobin following application to <u>winter cereals</u> (Tier 2)

Location	Water body	$PEC_{sed,max}$ [µg kg^{-1}]	
		Winter cereals	
		Single application	Twofold application
D1	ditch	4.425	5.203
D1	stream	0.232	0.765
D2	ditch	3.036	3.561
D2	stream	2.313	3.082
D3	ditch	0.988	1.079
D4	pond	0.271	0.372
D4	stream	0.039	0.060
D5	pond	0.225	0.210
D5	stream	0.039	0.231
D6	ditch	0.440	2.637
R1	pond	0.272	0.294
R1	stream	1.382	3.166
R3	stream	0.698	1.545
R4	stream	3.567	9.724

Table 9.2.5-55: Step 3: $PEC_{sed,max}$ of pyraclostrobin following application to <u>spring cereals</u> (Tier 2)

Location	Water body	$PEC_{sed,max}$ [µg kg^{-1}]	
		Spring cereals	
		Single application	Twofold application
D1	ditch	3.905	3.949
D1	stream	0.818	0.776
D3	ditch	0.972	1.135
D4	pond	0.168	0.147
D4	stream	0.108	0.233
D5	pond	0.226	0.221
D5	stream	0.057	0.082
R4	stream	5.314	5.310

Table 9.2.5-56: Step 3: $PEC_{sed,max}$ of pyraclostrobin following application to <u>maize</u> (Tier 2)

Location	Water body	$PEC_{sed,max}$ [µg kg^{-1}]
		Maize
		Single application
D3	ditch	0.649
D4	pond	0.131
D4	stream	0.066
D5	pond	0.123
D5	stream	0.030
D6	ditch	0.358
R1	pond	0.187
R1	stream	1.927
R2	stream	3.471
R3	stream	1.059
R4	stream	6.010

Global maximum concentrations – metabolites of pyraclostrobin

Table 9.2.5-57: Steps 1-2: $PEC_{sed,max}$ of BF 500-3 under dark conditions following application of pyraclostrobin to cereals and maize

$FOCUS_{sw}$ crop	BF 500-3 $PEC_{sed,max}$ [µg kg^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	10.015	20.030	9.934	17.430	9.935	17.430
Maize	8.012	-[a]	7.947	-[a]	7.948	-[a]

[a] Only single application calculated for maize

Table 9.2.5-58: Steps 1-2: $PEC_{sed,max}$ of BF 500-3 under irradiated conditions following application of pyraclostrobin to cereals and maize

$FOCUS_{sw}$ crop	BF 500-3 $PEC_{sed,max}$ [µg kg^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	3.254	6.507	3.106	5.090	3.107	5.090
Maize	2.603	-[a]	2.485	-[a]	2.485	-[a]

[a] Only single application calculated for maize

Table 9.2.5-59: Steps 1-2: $PEC_{sed,max}$ of BF 500-6 following application of pyraclostrobin to cereals and maize

$FOCUS_{sw}$ crop	BF 500-6 $PEC_{sed,max}$ [µg kg^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	152.468	304.937	15.903	30.933	30.940	60.344
Maize	121.975	-[a]	12.723	-[a]	24.752	-[a]

[a] Only single application calculated for maize

Table 9.2.5-60: Steps 1-2: $PEC_{sed,max}$ of BF 500-7 following application of pyraclostrobin to cereals and maize

FOCUS$_{sw}$ crop	BF 500-7 $PEC_{sed,max}$ [µg kg^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Cereals	91.488	182.976	9.813	19.014	18.799	36.588
Maize	73.190	-[a]	7.850	-[a]	15.040	-[a]

[a] Only single application calculated for maize

III. CONCLUSION

Predicted environmental concentrations (PEC) were calculated for pyraclostrobin and its metabolites in surface water (BF 500-5, BF 500-11, BF 500-13, BF 500-14) and in sediment (BF 500-3, BF 500-6, BF 500-7) following application of the formulated product BAS 500 06 F to cereals and maize under European conditions.

For the parent compound a tiered approach using endpoints from dark (Tier 1) and irradiated (Tier 2) water/sediment studies was considered for the calculations. While this had almost no effect on maximum PEC_{sw}, the maximum PEC_{sed} in Tier 2 decreased slightly (with approximately 9% for winter cereals, twofold application, R4 –stream). Time weighted average (TWA) values did not differ significantly between the two tiers. Despite the large discrepancy between the degradation rates in the water compartment between the two tiers, the results show that sorption from the water phase to sediment is the dominating process. This is in agreement with the behavior observed in aqueous metabolism studies. Although the selection of endpoints has no major effect on the PEC_{sw} values, it considerably affects the behavior in sediment. Since pyraclostrobin breakdown under irradiated conditions is a relevant pathway and degradation occurs rapidly these Tier 2 endpoints should appropriately be considered for further usage.

The predicted concentrations in surface water are appropriate to be used for the subsequent risk assessment for aquatic organisms.

CP 9.3 Fate and behaviour in air

CP 9.3.1 Route and rate of degradation in air and transport via air

No studies were performed with BAS 500 06 F. The route and rate of degradation in air as well as transport via air is sufficiently addressed by information given in M-CA 7.3.

Predicted environmental concentrations from airborne transport

No risk assessment needs to be performed due to the very low volatilisation potential of pyraclostrobin and its short DT_{50} in air. Consequently no PECs have been calculated.

CP 9.4 Estimation of concentrations for other routes of exposure

No other routes of exposure are relevant for the use of BAS 500 06 F in cereals and maize.

BASF
The Chemical Company

BAS 500 06 F

DOCUMENT M-CP, Section 10

ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-July-2014		BASF DocID 2014/1162287 (version 1)
09-June-2015	Two studies were moved from M-CP 10.7 to M-CP 10.4. Both chapters have been adapted accordingly. New or changed text is marked in yellow.	BASF DocID 2015/1107661 (version 2)
27-Feb-2017	inclusion of a new field effect study and of a quantitative higher tier risk assessment to address the small herbivorous "vole" mammal scenario in 10.1.2; inclusion of two new amphibian studies in 10.1.3; update of 10.3.1 reflecting the information of a new bumblebee study described in detail in M-CA 8; New or changed text is marked in blue.	BASF DocID 2017/1032190 (version 3)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 10 ECOTOXICOLOGICAL STUDIES ON PLANT PROTECTION PRODUCT

BAS 500 06 F is a new representative formulation, which has not been evaluated during the previous Annex I inclusion process.

This document reviews the ecotoxicological studies for BAS 500 06 F, an EC solo formulation containing 200 g/L pyraclostrobin. In addition, risk assessments are provided, which show that BAS 500 06 F is safe for aquatic and terrestrial non-target organisms.

CP 10.1 Effects on birds and other terrestrial vertebrates

The risk assessment on birds and mammals considers the principles given in the latest guidance document by EFSA (*Anonymous 2009: Guidance Document on risk assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. European Food Safety Authority*), hereafter cited as EFSA/2009/1438. EU agreed endpoints and endpoints from new studies are used for the risk assessments on birds and mammals together with data from new additional studies.

In addition, the potential risk to amphibians and reptiles will be addressed following the new requirement under Regulation 1107/2009.

Studies conducted for avian and mammalian risk assessments

An overview on data from new studies relevant for the avian or mammalian risk assessment is shown in Table 10.1-1, full summaries of the studies and documents afterwards. Note that most of the study summaries are included in M-CP 10.1 (irrespective of their use for either the avian or the mammalian risk assessment), because the formal document structure of M-CP 10.1.1 and M-CP 10.1.2 does not include a dedicated chapter for such studies.

Besides the generic studies listed in Table 10.1-1, the higher tier mammalian risk assessments will also rely on results from further higher tier studies and associated evaluations. However, these studies and reports are active substance specific and are hence summarized in Document M-CA at the beginning of chapter 8.1.

Table 10.1-1: New information used for the avian and mammalian risk assessment

Data point (all M-CP)	References (BASF DocID)	Year	Title
10.1/1	2011/1112612	2011	Field monitoring of hares and rabbits in cereal fields.
10.1/2	2012/1105899	2012	First amendment to final report. Field monitoring of hares and rabbits in cereal fields.
10.1/3	2004/1025715	2004	Population dynamics of common voles in winter cereal fields – field monitoring in south western Germany
10.1.1.2/1	2008/1078602	2008	BAS 500 06 F - Acute toxicity in the bobwhite quail (Colinus virginianus) after single oral administration (LD_{50}).

Report:	CP 10.1/1 Schroeer A., Grimm T., 2011b Field monitoring of hares and rabbits in cereal fields 2011/1112612
Guidelines:	EC 1107/2009 (14 June 2011)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Report:	CP 10.1/2 Schroeer A., Grimm T., 2012b First amendment to final report - Field monitoring of hares and rabbits in cereals fields 2012/1105899
Guidelines:	EC 1107/2009 (14 June 2011)
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The selection of focal species for the refined risk assessment is based on results of a generic monitoring study of hare and rabbits (BASF DocID 2011/1112612) conducted in cereal fields in Germany at two different locations (Lower Saxony and Schleswig-Holstein). The selected areas represented typical cereal fields in arable landscape with regard to size and structure of the surroundings. Monitoring of hares and rabbits was conducted via spotlight scanning by car at night.

The above study was carried out with the specific purpose of deriving generic data to conduct risk assessments for mammals following recommendations given by EFSA/2009/1438. The aim of the study was to assess the frequency of occurrence and abundance of brown hares (*Lepus europaeus*) and European rabbits (*Oryctolagus cuniculus*) in cereal fields (BBCH <30) and consequently their potential of being exposed to plant protection products applied.

In summary, both hares and rabbits use cereal fields at BBCH <30 as night time refuge. While hares were often found in the cereal fields, the number of rabbits was low. Density measures exhibit the same pattern with the hare being frequently abundant in cereal crops

I. MATERIAL AND METHODS

In order to assess the potential exposure risk of hares and rabbits to plant production products in cereal fields, a total number of 90 fields were monitored in two regions of Northern Germany. Monitoring of hares and rabbits was conducted via spotlight scanning at night. For this purpose, alongside of each study field, a transect was defined. Two persons drove slowly (mean speed 12.4 km/h) along these transects in a car and thereby the study field or a part of the study field was surveyed by means of a hand-held spotlight. In this illuminated area (approximate depth of 150 m) all observed hares and rabbits were recorded. Spotlight scanning was carried out at four different sets of study fields, which were monitored on two consecutive nights each, resulting in a total of eight night surveys. Spotlight scanning started approximately 1.5 hours after sun set and was finished before dawn. The abundance of hares and rabbits per 100 hectare surveyed area was calculated as well as the abundance per hectare. Additionally, the frequency of occurrence (FO) was determined based on the total number of study fields (field approach; FO_{field} n=90) and on the total number of surveys (survey approach; FO_{survey} n=180).

Study Sites

Two study sites each containing two sets of individual study fields were selected in Germany (Lower Saxony (Set 1/2) and Schleswig-Holstein (Set 3/4)). The presence of hares and rabbits in these regions was confirmed through recordings in previous years (e.g. information gathered from local game keepers). In total 90 study fields were chosen, in which the BBCH development stage of winter cereals was less than 30, thus providing appropriate conditions for spotting lagomorphs in the crops. Every set of study fields comprised between 20 and 26 cereal fields, on which hares and rabbits were counted during two consecutive nights.

Data recording and analysis

All data were collated and analysed using standard spread sheet applications. The surveyed area for each study field was calculated by multiplying the transect length with the range of the hand-held spotlight (=150 m) and thus quadratic dimensions were assumed. If the latter was not true marginal subareas were identified by the means of Google Earth Pro and either subtracted or added to the calculated surveyed area. In addition, if the study field itself or parts of the study field were less than 150 m deep, exact measures were taken and used for calculating the illuminated area instead.

II. RESULTS AND DISCUSSION

Hares were found in 61 out of 90 of the cereal fields surveyed (FO_{field} = 67.78%). Hares were present in 86 out of 180 of the field field surveys (FO_{survey} = 47.78%). The mean abundance of hares in the cereal fields was 22.22 hares per 100 ha with 0.22 hares per ha.
Rabbits were counted in 8 out of 90 of the cereal fields surveyed (FO_{field} = 8.89%). Rabbits were observed in 12 out of 180 of the fields surveyed (FO_{survey} = 6.67%). The mean abundance of rabbits in the cereal fields was 2.27 rabbits per 100 ha with less than 0.1 rabbits per hectare.

Frequency of occurrence and abundance of rabbits and hare in cereal fields in Germany

Observation period	**10 April - 21 April 2011**				
BBCH growth stage	**23-29**				
Number of cereal fields	**90**				
Number of surveys	**180**				
	Number of cereal fields with record	**FO_{field} [%]**	**FO_{survey} [%]**	**Abundance [number of animals / 100 ha surveyed area] ± SD**	**Abundance [number of animals / ha surveyed area]**
Rabbit	8	8.89	6.67	2.27 ± 1.51	< 0.1
Hare	61	67.78	47.78	22.22 ± 9.44	0.22

III. CONCLUSION

Both hares and rabbits use cereal fields at BBCH growth stages below 30 as a night time refuge e.g. for foraging and probably reproduction (in hares). Hares were often found in the cereal fields investigated whereas the number of rabbits found was low. Density measures exhibit the same pattern with the hare being frequently abundant in cereal crops.

Please note that the following study CP 10.1/3 was erroneously not included in the Application submitted in January 2014 for the renewal of approval of pyraclostrobin.

Report: CP 10.1/3
Pedall I., Riffel M., 2004b
Population dynamics of common voles in winter cereal fields - Field monitoring in South Western Germany
2004/1025715
Guidelines: EEC 91/414, EEC 96/68
GLP: no

Executive Summary

This generic field study was performed to evaluate the population dynamics of common voles in winter cereal fields. Because wood mice were caught in sufficient numbers as well, this species was also included in the evaluation. The particular objective of the study was to identify the point of time (month and BBCH code) when rodents immigrate into winter cereal fields after emergence of the cereal plants.

Considering the detailed results, the common vole (*Microtus arvalis*) is not seen relevant for the risk assessment for cereals, because cereal fields would be only potential secondary refuges during summer and no stable populations can be established in these crops. The wood mouse (*Apodemus sylvaticus*), however, proved to make more regular use of cereal fields over a longer period, which makes this species relevant for the risk assessment for cereals.

I. MATERIAL AND METHODS

Three winter cereal fields bordering grassland plots in the vicinity of Bruchsal (8°35′23′′; 49°7′26′′, Baden-Württemberg, Germany) were chosen as study sites. This area is typical for the cultivation of cereals in Germany. The size of the study plots ranged between 0.3 and 0.315 ha, each including a grassland subplot as well as a cereal field subplot. The rodents were monitored using live traps on both subplots. To assess the recapture rate the specimens were marked with tattoo dye. In each subplot, six capture periods of about 4 days were carried out from January to July 2003. The endpoints of the study included a quantification of vole population numbers in both subplots for each sample site and the determination of immigration time of voles and wood mice into winter cereal fields. Additionally, a list of species trapped in grassland subplots and adjacent cereal fields was prepared.

II. RESULTS AND DISCUSSION

Findings for the common vole: The common vole was the predominant small mammal species observed in the grassland. The numbers of vole individuals in the cereal fields was lower by a factor of 6 compared to the grassland plots. The results of the study are presented in the three following tables below.

Point of immigration time of common voles into cereal fields

	BBCH crop stage
Tolerance value for immigration	39 / 41
Cereal growth stage for a constant population	65

Average population density of common voles in three study sites from January to July 2003 [Individuals/ha]

	Sampling 1	**Sampling 2**	**Sampling 3**	**Sampling 4**	**Sampling 5**	**Sampling 6**
Month	Jan/Feb	Feb/March	March/April	April/May	May/June	June/July
Grassland	23.8	38.1	42.9	71.4	9.5	2.4
Cereals	0	0	0	9.8	13.7	0

Recapture rate of common voles in the field and grassland (for all study sites)

	Field [%]	**Grassland [%]**
Common vole	25	63

Findings for the wood mouse: In contrast to the common vole the wood mouse was occasionally caught in the grassland subplots, but was more common in the cereal field plots and outnumbered the common vole by a factor of 3 there. Based on the low recapture rates, the wood mouse population was considered unstable and it is assumed that it consisted of wandering individuals mostly. The results of the study are depicted in the two following tables below.

Point of immigration time of wood mice into cereal fields

	BBCH crop stage
Tolerance value for immigration	20
Cereal growth stage for regular occurrence of cereal fields	39 / 41

Recapture rate of wood mice in the field and grassland

	Field [%]	Grassland [%]
Wood mouse	40	25

Discussion

Common voles were observed in the grassland and field subplots of every sample site. The grassland subplots were continuously inhabited with much higher population densities compared to the cereal fields. Furthermore, the grassland population was noticeably more constant. The period of colonization of cereal fields proved to be very short and did not exceed the period of two months. It commenced at cereal growth stage of BBCH 39 / 41 (May) with single catches, whereas a more regular use of cereal fields by common voles could be proven for June (growth stage BBCH 65) based on recapture data. The colonization ceased at time of harvest when vole numbers dropped to almost zero. The populations inhabiting both grassland subplots and cereal subplots were also significantly affected by mowing of grassland plots. The population maximum was observed before the harvest of cereal fields and mowing of grassland subplots. The number of voles in the grassland subplots exceeded the number in the cereal subplots by a factor of six.
Generic literature data corroborates that agricultural operations prove detrimental to populations of common voles as was shown in various studies throughout Europe. During harvest the vegetation cover is removed exposing the voles to natural predators. Mechanical operations such as harrowing or ploughing destroy existing vole burrows (Spitz 1977). Hence the voles are able to colonize arable land for a restricted period of the year only. In a field study in Germany vole population inhabiting pasture and arable land were compared in regard to various parameters within the course of several years. Due to ploughing no voles were found on arable land during winter (Jacob 2000). This underlines again that cereal fields would be only potential secondary refuges for the common vole during summer, and that due to harvesting and ploughing no stable populations could be established in these crops.
Wood mice were only sporadically observed in the grassland subplots, but their numbers were consistently higher in the cereal fields and outnumbered the common vole by a factor of 3. The period of colonization of cereal fields proved to be longer than for common voles. Wood mice were first recorded in cereal fields at growth stage BBCH 20 (February / March) and a regular use of fields was recorded up from BBCH 39 / 41 (May). The wood mice numbers reached a maximum in May and declined towards the harvest. Harvesting had a noticeable impact on the wood mouse population and lead to the end of colonization of cereal fields.

III. Conclusion

Considering the detailed data presented above, the common vole (*Microtus arvalis*) is not seen relevant for the risk assessment for cereals, because cereal fields would be only potential secondary refuges during summer and no stable populations can be established in these crops. The wood mouse (*Apodemus sylvaticus*), however, proved to make more regular use of cereal fields over a longer period, which makes this species relevant for the risk assessment for cereals.

References to study BASF DocID 2004/1025715

Spitz, F. (1977). Le campagnol des champs (Microtus arvalis (Pallas)) en Europe. EPPO Bulletin 7: 156-175.

Jacob, J. (2000). Populationsökologische Untersuchungen an Kleinnagern auf unterschiedlich bewirtschafteten Flächen der Unstrut-Aue. Dissertation, Universität Jena, pp. 102.

CP 10.1.1 Effects on birds

Data of the representative formulation BAS 500 06 F and of the active substance pyraclostrobin are evaluated and the respective risk assessments are presented based on the already registered use of BAS 500 06 F in cereals and maize.

Table 10.1.1-1: Endpoints for birds

Test system	Test species	Study endpoints	Endpoints used in present risk assessment [4]
Acute toxicity	*Colinus virginianus*	LD_{50} > 2000 mg a.s./kg b.w.[1]	LD_{50} (geometric mean) = 1701 mg a.s./kg b.w.[5]
	Serinus canaria	LD_{50} > 1446 mg a.s./kg b.w.[2]	
Reproductive toxicity (long-term)	*Colinus virginianus*	NOEL = 105 mg a.s./kg b.w./d[1)3)]	NOEC = 1000 mg a.s./kg diet NOEL = 105 mg a.s./kg b.w./d[2]

1) Endpoints confirmed in the ecotoxicology section of the Review Report for the active substance pyraclostrobin (SANCO/1420/2001-Final. 8. September 2004).
2) Based on data from a new study and therefore not evaluated during the previous Annex I inclusion process. For the detailed study summary please refer to CA 8.1.1.1/1.
3) Daily Dose [mg/kg b.w./d] calculated based on study data for food consumption and body weight
4) Endpoints according to EFSA/2009/1438
5) For calculation of LD_{50} (geometric mean) please refer to Table 10.1.1-3.

RISK ASSESSMENT

Summary of avian toxicity testing

The avian toxicity studies with pyraclostrobin relevant for the dietary risk assessment for birds are summarized in Table 10.1.1-2. The listed studies have been already evaluated during the previous Annex I listing process except for the acute toxicity study in the canary (BASF DocID 2013/1400375). The two available and already evaluated short term toxicity studies in bobwhite quail and mallard duck are not listed below, because they are no longer part of the core data package according to EFSA/2009/1438.

Table 10.1.1-2: Summary of avian studies on the acute and reproductive toxicity of pyraclostrobin

Test system	Test species	Results	Reference (BASF DocID)
Acute oral toxicity	*Colinus virginianus*	LD_{50} > 2000 mg a.s./kg b.w.[1)]	1997/11136
	Serinus canaria	LD_{50} > 1446 mg a.s./kg b.w.	2013/1400375
Sub-chronic toxicity and reproduction	*Colinus virginianus*	NOEC = 1000 mg a.s./kg diet NOEL = 105 mg a.s./kg b.w./d [1) 2)]	1999/11207
	Anas platyrhynchos	NOEC = 1000 mg a.s./kg diet NOEL = 128 mg a.s./kg b.w./d[2)]	1999/11206

1) Endpoints confirmed in the ecotoxicology section of the Review Report for the active substance pyraclostrobin (SANCO/1420/2001-Final. 8. September 2004).
2) Daily Dose [mg/kg b.w./d] calculated based on study data for food consumption and body weight.

Calculated avian toxicity endpoint for use in the acute tier 1 risk assessment

For pyraclostrobin acute oral studies in the bobwhite quail and the canary are available (Table 10.1.1-2). No mortality occurred in any of the two acute oral toxicity bird studies at the highest dose tested. However, in the study with the bobwhite quail treatment related effects were observed at the two highest dose groups (i.e. dose groups of 1000 and 2000 mg a.s./kg b.w.) and therefore no extrapolation of the LD_{50} >2000 mg a.s./kg b.w.for the bobwhite quail is possible according to EFSA/2009/1438 (2.1.2, p.16). In the study with the canary no clear signs of toxicity were seen up to the highest dose tested of 1446 mg a.s./kg b.w. It would be thus permissible to extrapolate an LD_{50} value upwards. However, for a conservative evaluation an extrapolation of the LD_{50} >1446 mg a.s./kg b.w. for the canary is not conducted.

According to EFSA/2009/1438, a geometric mean of the endpoints can be used in the acute dietary risk assessment as two different species were tested and the endpoint of the most sensitive species (i.e. the canary) is not by a factor of 10 below the overall geometric mean. The most sensitive endpoint with an LD_{50} > 1446 mg a.s./kg b.w./d in the canary is clearly higher than the 'assessment factor LD_{50}' of 170.1 mg a.s./kg b.w./d (Table 10.1.1-3). Hence, the geometric mean of both acute toxicity endpoints will be used.

The overall geometric mean LD_{50} value, i.e. the **LD_{50}** (geometric mean) **= 1701 mg a.s./kg b.w.** is the relevant endpoint to be used for the acute avian risk assessment.

Table 10.1.1-3: Calculation of the relevant avian toxicity endpoint for the acute risk assessment of pyraclostrobin

Characteristic	*Colinus virginianus*	*Serinus canaria*
Experimentally obtained LD_{50} [mg a.s./kg b.w.]	> 2000[1)]	> 1446[2)]
Birds tested/group [no.]	10	10
Mortality at highest dose level [%]	0	0
Extrapolation factor[3)]	--	--
LD_{50} (extrapolated)[4)] [mg a.s./kg b.w.]	--	--
LD_{50} (overall geometric mean)[5)] [mg a.s./kg b.w.]	1701	
Assessment factor LD_{50}[6)]: LD_{50} (overall geometric mean) / 10 [mg a.s./kg b.w.]	170.1	

1) BASF DocID 1997/11136
2) BASF DocID 2013/1400375
3) Extrapolation factor can be applied to an LD_{50} value in case 10 birds were tested and no mortalities occurred at the highest dose tested (EFSA GD 2009, 2.1.2, p.16). For a conservative evaluation an extrapolation of the endpoints derived is not conducted.
4) LD_{50} multiplied by the extrapolation factor
5) Determination of the geometric mean out of the LD_{50} values of >2000 and >1446 mg a.s./kg b.w. of the acute oral toxicity studies (EFSA/2009/1438, 2.4.2)
6) 'Assessment factor LD_{50}' of all the tested species, geometric mean divided by a factor of 10 (EFSA/2009/1438, 2.4.2)

Calculated avian toxicity endpoint for use in the <u>reproductive</u> tier 1 risk assessment

In order to obtain the relevant toxicity endpoint to be used in the screening and tier 1 reproductive risk assessment, the lowest NOEL from available bird reproduction studies and the 'assessment factor LD_{50}' [LD_{50} (overall geometric mean) / 10] from acute toxicity studies (EFSA/2009/1438, p. 33 and 35) have to be considered.

The lowest toxicity value from the avian reproduction study for pyraclostrobin is NOEL = 105 mg a.s./kg b.w./d derived from the study with bobwhite quail (BASF DocID 1999/11207). The acute toxicity endpoint was calculated to be LD_{50} = 1701 mg a.s./kg b.w. (Table 10.1.1-3).

Following EFSA/2009/1438 (p. 33 and 35), either the overall lowest NOEL or the 'assessment factor LD_{50} 'is to be selected as relevant reproductive toxicity endpoint. Since the lowest of both is the NOEL, the value of **NOEL = 105 mg a.s./kg b.w./d** will be used in the screening step and the tier 1 reproductive risk assessment for pyraclostrobin.

Results from available toxicity testing with the formulation

The avian toxicity study with BAS 500 06 F (BASF DocID 2008/1078602) relevant for the risk assessment for birds is summarized in Table 10.1.1-4. Please note that the study was erroneously included in the Application submitted in January 2014 in chapter M-CP 10.1.1.1 and is now given in chapter M-CP 10.1.1.2.

Table 10.1.1-4: Summary of avian study on the acute toxicity of BAS 500 06 F

Test system	Test species	Results	Reference (BASF DocID)
Acute oral toxicity	*Colinus virginianus*	LD_{50} > 2000 mg/kg b.w.	2008/1078602

This study was not evaluated during the previous Annex I listing process. Therefore, the study is submitted within this dossier together with a detailed study summary provided in chapter 10.1.1.2. In addition, an acute risk assessment for BAS 500 06 F considering the **LD_{50} > 2000 mg/kg b.w. for BAS 500 06 F** is provided below.

Metabolites of pyraclostrobin

The metabolism of pyraclostrobin in potential food items of wild living birds or mammals (i.e. green plant matter, fruits, or seeds) was investigated in plant metabolism studies in grapes (BASF DocIDs 1998/10988 and 2000/1000201), potatoes (BASF DocIDs 1999/11419 and 2000/1000048) and wheat (BASF DocID 1999/11137). Most metabolites occurred only at trace amounts far below 10% TRR in the potential food items. The only metabolite that occurred at higher levels in potential food items was the desmethyl metabolite (BF 500-3, synonym: 500M07). It was found at levels of up to 15.3% TRR in grapes, 21.4% TRR in green matter of potatoes, 13.1% TRR in wheat forage, and 10.5% TRR in wheat grain. However, this desmethyl metabolite was also detectable in rats (BASF DocID 1999/11781), goats (BASF DocID 2000/1000004) and hens (BASF DocID 1999/11480). Hence, it can be concluded that the mammalian toxicity studies with pyraclostrobin cover this metabolite, and that the dietary risk assessment for pyraclostrobin provided for birds and mammals covers the potential risk from this metabolite.

Water metabolites are of minor importance for the risk assessment for wild living birds and mammals, considering the predominant route of exposure being via food items like plants, seeds, or arthropods. Water uptake itself or exposure via the aquatic compartment, however, can play a role in the drinking water and the secondary poisoning risk assessment for fish-eaters. Hence in the following the risk from relevant surface water metabolites to birds and mammals is considered. The metabolites BF 500-5, BF 500-11, BF 500-13, BF 500-14 were found at levels slightly above 10% TAR in surface water (for details see M-CA 7.2). However, the risk from these metabolites to birds and mammals is considered to be covered by the presented risk assessments for the parent compound pyraclostrobin due to the following reasons:

i) PEC values of the metabolites are by a factor of ~30-66 below the one of the parent (highest global PECsw, max [µg/L] following FOCUS Step1 are for the metabolites BF 500-5 = 0.256 µg/L, BF 500-11 = 0.407 µg/L, BF 500-13 = 0.533 µg/L, BF 500-14 = 0.573 µg/L, and for the parent BAS 500 F = 17.031 µg/L; for details see M-CP 9.2).

ii) There are no specific toxicity studies with the metabolites BF 500-5, BF 500-11, BF 500-13, BF 500-14 available in birds or mammals. However, the low toxicity of the metabolites was confirmed in standard acute fish toxicity studies with *O. mykiss* (for details see M-CP 10.2).

Summary of relevant avian toxicity endpoints to be used in the screening and tier 1 risk assessments

The avian toxicity endpoints relevant for the screening and tier 1 risk assessments are given in Table 10.1.1-5.

Table 10.1.1-5 Avian toxicity endpoints for screening and tier 1 risk assessments of pyraclostrobin and BAS 500 06 F

Test substance	Test system	Relevant toxicity endpoint
Pyraclostrobin	Acute oral toxicity	LD_{50} (geometric mean) = 1701 mg a.s./kg b.w.
	Sub-chronic toxicity and reproduction	NOEL = 105 mg a.s./kg b.w./d
BAS 500 06 F	Acute oral toxicity	LD_{50} > 2000 mg a.s./kg b.w.

Relevant exposure scenarios

The relevant scenarios for the intended representative use patterns of the formulation BAS 500 06 F (Table 10.1.1-6) are given for cereals in Table 10.1.1-7 and for maize in Table 10.1.1-8.

Table 10.1.1-6: Critical use pattern for BAS 500 06 F

Crop	EFSA/2009/1438 crop group	Application time (BBCH growth stage)	No. of applications	Interval [d]	Maximum application rate per treatment	
					Pyraclostrobin [kg/ha]	BAS 500 06 F [L/ha]
Cereals	Cereals	25 - 69	2	21	0.25	1.25
Maize	Maize	30 - 65	1	--	0.20	1.0

Table 10.1.1-7: Summary of exposure scenarios to be considered for risk assessments for spray applications of BAS 500 06 F in cereals at BBCH 25 – 69

Scenario	Generic focal species
Cereals Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"
Cereals BBCH 10 - 29	Small omnivorous bird “lark”
Cereals BBCH 30 -39	Small omnivorous bird “lark”
Cereals BBCH ≥ 40	Small omnivorous bird “lark”

Table 10.1.1-8: Summary of exposure scenarios to be considered for risk assessments for spray applications of BAS 500 06 F in maize at BBCH 30 – 65

Scenario	Generic focal species
Maize BBCH ≥ 20	Small insectivorous bird “wagtail”
Maize BBCH 30 - 39	Medium granivorous bird "gamebird"
Maize BBCH 30 - 39	Medium herbivorous/granivorous bird "pigeon"
Maize BBCH 30 - 39	Small omnivorous bird “lark”
Maize BBCH ≥ 40	Medium granivorous bird "gamebird"
Maize BBCH ≥ 40	Medium herbivorous/granivorous bird "pigeon"
Maize BBCH ≥ 40	Small omnivorous bird “lark”

CP 10.1.1.1 Acute oral toxicity

In this section, the dietary TER acute values for the screening step and tier 1 assessment obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) are presented for cereals in Table 10.1.1.1-1 and for maize in Table 10.1.1.1-2.

Cereals

Table 10.1.1.1-1: Pyraclostrobin: Acute dietary risk assessment for birds in cereals - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Cereals	0.25	2	21	10.0	1701.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{90}**	**Daily Dietary Dose (multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	158.8	39.70	1.1	43.67	39.0	

First Tier Risk Assessment:					
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**	**Shortcut value**	**TER**	**No refinement required**
	Cereals Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose" Grass + cereals 100% cereal shoots	30.5	202.8	
	Cereals BBCH 10 - 29	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	24.0	257.7	
	Cereals BBCH 30 - 39	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	12.0	515.5	
	Cereals BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	7.2	859.1	

Maize

Table 10.1.1.1-2: Pyraclostrobin: Acute dietary risk assessment for birds in maize - results from EFSA calculator tool

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT_{50}	LD_{50}	
	Maize	0.2	1	365	10.0	1701.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{90}**	**Daily Dietary Dose (multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	158.8	31.76	1.0	31.76	53.6	

First Tier Risk Assessment:					
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**	**Shortcut value**	**TER**	**No refinement required**
	Maize BBCH ≥ 20	Small insectivorous bird “wagtail” ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods	12.6	675.0	
	Maize BBCH 30 - 39	Medium granivorous bird "gamebird" Small seeds 100% seed	3.3	2577.3	
	Maize BBCH 30 - 39	medium herbivorous/granivorous bird "pigeon" Non-grass herbs 100% leaves	27.8	305.9	
	Maize BBCH 30 - 39	Small omnivorous bird “lark” Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	12.0	708.8	
	Maize BBCH ≥ 40	Medium granivorous bird "gamebird" Small seeds 100% seed	1.6	5315.6	
	Maize BBCH ≥ 40	medium herbivorous/granivorous bird "pigeon" Non-grass herbs 100% leaves	13.9	611.9	
	Maize BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods	6.0	1417.5	

In conclusion, even under the conservative assumptions of the screening and tier 1 acute dietary risk assessments all TER_A values for pyraclostrobin exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for birds from the use of BAS 500 06 F in cereals and maize according to the recommended use pattern.

CP 10.1.1.2 Higher tier data on birds

Please note that this section, albeit suggested by its header, does not only include higher tier data on birds. The calculations presented for cereals in Table 10.1.1.2-1 and for maize in Table 10.1.1.2-2 are the dietary TER reproductive values of the screening step and tier 1 assessment obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm).

Cereals

Table 10.1.1.2-1: Pyraclostrobin: Reproductive risk assessment for birds in cereals - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Repro End Point (mg/kg b.w./d)**	**Time-weighted average (twa)**
	Cereals	0.25	2	21	10	105.0	0.53
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{90}**	**Daily Dietary Dose (multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	64.8	16.20	1.2	10.30	10.2	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	**No refinement required**
	Cereals Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose" Grass + cereals 100% cereal shoots			16.2	40.8	
	Cereals BBCH 10 - 29	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			10.9	60.6	
	Cereals BBCH 30 -39	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			5.4	122.3	
	Cereals BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			3.3	200.1	

Maize

Table 10.1.1.2-2: Pyraclostrobin: Reproductive risk assessment for birds in maize - results from EFSA calculator tool

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT_{50}	Repro End Point (mg/kg b.w./d)	Time-weighted average (twa)
	Maize	0.2	1	365	10	105.0	0.53
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{90}**	**Daily Dietary Dose (multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	64.8	12.96	1.0	6.87	15.3	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	**No refinement required**
	Maize BBCH ≥ 20	Small insectivorous bird “wagtail” ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods			4.8	206.4	
	Maize BBCH 30 - 39	Medium granivorous bird "gamebird" Small seeds 100% seed			1.5	660.4	
	Maize BBCH 30 - 39	medium herbivorous/granivorous bird "pigeon" Non-grass herbs 100% leaves			11.4	86.9	
	Maize BBCH 30 - 39	Small omnivorous bird “lark” Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods			5.4	183.4	
	Maize BBCH ≥ 40	Medium granivorous bird "gamebird" Small seeds 100% seed			0.8	1238.2	
	Maize BBCH ≥ 40	medium herbivorous/granivorous bird "pigeon" Non-grass herbs 100% leaves			5.7	173.8	
	Maize BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods			2.7	366.9	

In conclusion, even under the conservative assumptions of the screening and tier 1 reproductive dietary risk assessments all TER_{LT} values for pyraclostrobin exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low reproductive risk for birds from the use of BAS 500 06 F in cereals and maize according to the recommended use pattern.

Acute toxicity of the formulation

An acute oral study with the formulation BAS 500 06 F on the bobwhite quail is available and summarized below. Please note that the study was erroneously included in the Application submitted in January 2014 in chapter M-CP 10.1.1.1.

The results of the study show no increased formulation toxicity as the formulation is practically acutely non-toxic to birds (LD_{50} > 2000 mg BAS 500 06 F/kg b.w.).

A detailed study summary is presented in the following. An acute risk assessment for BAS 500 06 F considering the LD_{50} from formulation toxicity testing is provided further below.

Report:	CP 10.1.1.2/1 [redacted]., 2008b BAS 500 06 F - Acute toxicity in the bobwhite quail (Colinus virginianus) after single oral administration (LD50) 2008/1078602
Guidelines:	EPA 540/9-82-024, EPA 71-1, EPA 540/9-85-007, EPA 850.2100, EPA 712-C-96-139
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

An acute oral avian toxicity test with BAS 500 06 F was conducted. The test item was administered via a single-dose of 500, 1000 or 2000 mg BAS 500 06 F/kg body weight to 6-month old northern bobwhite quails. Ten birds (5 males and 5 females) were used in each group. The doses were emulsified in drinking water and administered by gavage into the crop. Feed was removed for 21-22 hours prior to dosing.
All groups were observed for mortality, signs of clinical toxicity, impact on food consumption and body weight for 14 consecutive days post dosing. All groups received food and water *ad libitum* throughout the test. The test was terminated after 14 days.

In males, no mortality and no toxic signs were observed in the control and any of the test item treatments up to the highest tested concentration of 2000 mg BAS 500 06 F/kg bw. One female of the 2000 mg/kg bw group died on the day after dosing, but it was concluded that the mortality cannot be clearly attributed to the test item. Diarrhea, which was observed in the first time after dosing, is a consequence of the fasting period and is usually observed in all dose groups as well as in the control group and is not considered to be a toxic effect. In the 2000 mg/kg bw group the feed uptake during the 1st week after dosing was reduced to 70% in males and to 65% of the control group in females. In the 2nd week after dosing the feed uptake returned to normal. The body weights were not statistically significantly different from the control after 7 and 14 days in any of the dose groups. However, in the 2000 mg/kg bw group a slight tendency towards a decreased body weight was observed 7 days after dosing. No treatment-related macroscopic abnormalities were detected in the gross post-mortem examination.

In an acute toxicity test with the bobwhite quail the LD_{50} of BAS 500 06 F was > 2000 mg/kg body weight. The NOEL was 1000 mg/kg body weight.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F; batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 202.7 g/L (nominal 200 g/L).

Test species: Bobwhite quail (*Colinus virginianus*); indistinguishable from wild birds; age: approx. 6 months; source: H. & E. Küberich, Geesdorf/Wiesenheid, Germany.

B. STUDY DESIGN

Test design: Birds were administered different doses of the test item BAS 500 06 F in drinking water in a total amount of 10 g per kg body weight by gavage into the crop; 5 males and 5 females per dose group were used; observation period of 14 days; assessment of mortality and signs of clinical toxicity was carried out three times on day of dosing, daily thereafter; assessment of body weight was carried out on day 7 and 14. Gross pathological examinations of all birds at termination of the test.

Endpoints: LD_{50}, mortality, clinical signs, feed consumption, body weight (bw), gross pathological examinations were conducted on all birds sacrificed at the termination of the test.

Test concentrations: 0 (Control), 500, 1000 and 2000 mg BAS 500 06 F/kg body weight.

Test conditions: Birds fasted for about 21 - 22 h before administration of the test item; temperature 20.0°C to 24.4°C, deviation for 2 days, 21 h and 45 min from the temperature range of 19°C - 23°C; relative humidity: 38% - 75%, deviation of 10 h and 15 minutes above the limit of 45% - 70%; deviation of 2 h and 30 minutes below the limit of 45% - 70%; photoperiod: 8 hours light : 16 hours dark.

Statistics: Descriptive statistics, Dunnett-test for body weight data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements:
The results of the analytical verification of the test item concentration in the diet were within a range of 101% to 102% of the nominal concentrations during the test. The biological results are therefore based on the nominal values.

Biological results:
In males, no mortality and no toxic signs were observed in the control and any of the test item treatments up to the highest tested concentration of 2000 mg BAS 500 06 F/kg bw. One female of the 2000 mg/kg bw group died on the day after dosing. According to the test guideline 10% mortality is acceptable for the control group. Thus it was concluded that the mortality cannot be clearly attributed to the test item. Diarrhea in the first time after dosing is a consequence of the fasting period and is usually observed in all dose groups as well as in the control group and is not considered to be a toxic effect. In the 2000 mg/kg b.w. group the feed uptake during the 1st week after dosing was reduced to 70% in males and to 65% of the control group in females. In the 2nd week after dosing the feed uptake returned to normal. The body weights were not statistically significantly different from the control after 7 and 14 days in any of the dose groups. However, in the 2000 mg/kg b.w. group a slight tendency towards a decreased body weight was observed 7 days after dosing. No treatment-related macroscopic abnormalities were detected in the gross post-mortem examination. The relevant endpoints are summarized in the table below.

Acute toxicity of BAS 500 06 F to the bobwhite quail (Colinus virginianus)

Mortality	Dose [mg BAS 500 06 F/kg b.w.]
Highest dose causing no treatment-related mortality	males: 2000 females: 1000[1)]
LD_{50} (14 d)	> 2000
NOEL	1000

b.w . = body weight
1) It was concluded in the study report that the mortality cannot be clearly attributed to the test item.

III. CONCLUSION

In an acute toxicity test with the bobwhite quail, the LD_{50} of BAS 500 06 F was > 2000 mg/kg body weight. The NOEL was 1000 mg/kg body weight.

Acute risk assessment considering the LD_{50} from toxicity testing with BAS 500 06 F

Please note that the use of the endpoint from the acute toxicity study (BASF DocID 2008/1078602) of BAS 500 06 F on bobwhite quails (i.e. setting LD_{50} to 2000 mg/kg b.w. in the tier 1 calculations with EFSA calculator tool) will lead to an over conservative approach as no clear substance-related mortality occurred throughout the study period, and it can thus be expected that the true LD_{50}, which would be actually relevant, is clearly higher.

Nevertheless, a tier 1 acute dietary exposure assessment and TER calculation with the LD_{50} > 2000 mg BAS 500 06 F /kg b.w. from the acute oral bobwhite quail study with the formulation will be carried out (see below).

Exposure

BAS 500 06 F is intended to be used in cereals and maize with a maximum single application rate of 1.25 L BAS 500 06 F/ha in cereals and 1.0 L BAS 500 06 F/ha in maize (see Table 10.1.1-6 for the critical use pattern). Taking into account the density of the formulation of 1.04 g/cm^3, this will result in an application rate of 1.3 kg BAS 500 06 F/ha and 1.04 kg BAS 500 06 F/ha for the critical use patterns in cereals and maize, respectively. The relevant scenarios for the proposed use pattern are given in Table 10.1.1-7 (cereals) and in Table 10.1.1-8 (maize).

Calculation of the screening and tier 1 risk assessment for BAS 500 06 F according to EFSA/2009/1438

The screening and tier 1 TER values for the acute dietary risk assessment for birds as obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) are presented for cereals in Table 10.1.1.2-3 and in Table 10.1.1.2-4 for maize.

Table 10.1.1.2-3: BAS 500 06 F: Acute risk assessment for birds in cereals - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg formulation/ ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Cereals	1.3	2	21	10.0	>2000.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{90}**	**Daily Dietary Dose (multiple)**	**TER**	**Please perform first tier risk assessment (see below)**
	Small omnivorous bird	158.8	206.44	1.1	227.08	>8.8	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	**No refinement required**
	Cereals Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose" Grass + cereals 100% cereal shoots			30.5	>45.9	
	Cereals BBCH 10 - 29	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			24.0	>58.3	
	Cereals BBCH 30 -39	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			12.0	>116.6	
	Cereals BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods			7.2	>194.3	

Table 10.1.1.2-4: BAS 500 06 F: Acute risk assessment for birds in maize - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg formulation/ ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Maize	1.04	1	365	10.0	>2000.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{90}**	**Daily Dietary Dose (multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	158.8	165.15	1.0	165.15	>12.1	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**		**Shortcut value**	**TER**	**No refinement required**	
	Maize BBCH ≥ 20	Small insectivorous bird “wagtail” ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods		12.6	>152.6		
	Maize BBCH 30 - 39	Medium granivorous bird "gamebird" Small seeds 100% seed		3.3	>582.8		
	Maize BBCH 30 - 39	medium herbivorous/granivorous bird "pigeon" Non-grass herbs 100% leaves		27.8	>69.2		
	Maize BBCH 30 - 39	Small omnivorous bird “lark” Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods		12.0	>160.3		
	Maize BBCH ≥ 40	Medium granivorous bird "gamebird" Small seeds 100% seed		1.6	>1201.9		
	Maize BBCH ≥ 40	medium herbivorous/granivorous bird "pigeon" Non-grass herbs 100% leaves		13.9	>138.4		
	Maize BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods		6.0	>320.5		

In conclusion, even under the conservative assumptions of the tier 1 acute dietary risk assessments for the formulation BAS 500 06 F all TER_A values exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for birds from the use of BAS 500 06 F in cereals and maize according to the recommended use pattern.

Effects of secondary poisoning

The log P_{ow} of the active substance pyraclostrobin is 3.99 (SANCO/1420/2001-final), which triggers an assessment for the potential risk through secondary poisoning according to EFSA/2009/1438.

Risk assessment for earthworm-eating birds

The risk assessment for earthworm-eating birds will be based on the worst-case PEC_{soil} (twa, 21 days) derived from M-CP 9.1. The calculations and the resulting TER_{LT} values are summarized in Table 10.1.1.2-5.

Table 10.1.1.2-5: Risk assessment for pyraclostrobin concerning earthworm-eating birds (tier 1) – dry soil approach

Parameter	Pyraclostrobin	Reference
PEC_{soil} (twa, 21 days)[1] [mg/kg soil]	0.201	M-CP 9.1
K_{ow}	9772	SANCO/1420/2001-final
K_{oc} (arithmetic mean)	9304	SANCO/1420/2001-final
f_{oc} (default)	0.02	EFSA/2009/1438
BCF[2]	0.635	--
PEC_{worm} [mg/kg][3]	0.128	--
Daily dose [mg/kg b.w./d][4]	0.134	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	105.0	Table 10.1.1-1
TER_{LT}[5]	**783.86**	**--**

1) The PEC_{soil} (twa, 21 days) value calculated for applications of BAS 500 06 F in cereals was the highest PEC_{soil} (twa, 21 days) for all crop scenarios. For details see chapter 9.1.
2) Bioconcentration factor (BCF) = $(0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
3) $PEC_{worm} = PEC_{soil} \times BCF$
4) Daily dose = $1.05 \times PEC_{worm}$
5) TER_{LT} = NO(A)EL / Daily dose

Risk assessment for fish-eating birds

The risk assessment for fish-eating birds will be based on the worst-case PEC_{sw} (twa, 21 days) derived from M-CP 9.2. The calculations and the resulting TER_{LT} values are summarized in Table 10.1.1.2-6.

Table 10.1.1.2-6: Risk assessment for pyraclostrobin concerning fish-eating birds (tier 1)

Parameter	Pyraclostrobin	Reference
$PEC_{sw,}$ (twa, 21 days) [mg/L][1)]	$1.552 * 10^{-3}$	M-CP 9.2
BCF fish (max. worst case)	736	SANCO/1420/2001-final
PEC_{fish} [mg/kg][2)]	1.142	--
Daily dose [mg/kg b.w./d][3)]	0.182	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	105.0	Table 10.1.1-1
TER_{LT}[4)]	**578.13**	--

1) Highest PEC_{sw} (twa, 21 days) from FOCUS Step 2 (South Europe) calculation for a multiple application scenario of BAS 500 06 F in cereals with parameters from dark water/sediment study as worst-case. For details see chapter M-CP 9.2.
2) $PEC_{fish} = PEC_{sw,}$ (twa, 21 days) x BCF
3) Daily dose = 0.159 * PEC_{fish}
4) TER_{LT} = NO(A)EL / Daily dose

In the above risk assessments for earthworm- and fish-eating birds, the TER values exceed the trigger of 5 set by Commission regulation (EU) 546/2011 for acceptability of effects. It can therefore be concluded that the application of BAS 500 06 F in cereals and maize does not provide reason for concern regarding a potential accumulation of the active substance pyraclostrobin in the food chain or for concern of secondary poisoning.

Biomagnification in terrestrial food chains

No evidence was found for potential of accumulation of pyraclostrobin in animal tissue (Review Report for the active substance pyraclostrobin. Appendix II, endpoints and related information. 1. Toxicology and metabolism. September 2004).

Based on this it can be concluded that the potential for bioaccumulation in animal tissue for pyraclostrobin is low and thus no further assessment on biomagnification is required.

Risk for birds through drinking water

EFSA/2009/1438 proposes an assessment methodology for the risk to birds from active substances in drinking water using small granivorous birds as indicator species in tier 1. Out of the two scenarios, i.e. the leaf and the puddle scenario, the leaf scenario is not relevant for use in cereals and maize, respectively. Consequently, in a drinking water risk assessment for birds only the 'puddle scenario' would need to be considered for the application of BAS 500 06 F in cereals and maize.

According to EFSA/2009/1438 no specific calculations of exposure and TER values are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg b.w./d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg). The ratio calculations for effective application rate to relevant endpoint are detailed in a screening step in Table 10.1.1.2-7. The ratio for acute and reproductive endpoints for pyraclostrobin (0.2 and 3.5, respectively) do not exceed the threshold value of 3000 as given by EFSA/2009/1438 for more sorptive substances (Koc > 500 L/kg), thus no specific calculations of exposure for birds through drinking water for the puddle scenario are necessary (Table 10.1.1.2-7).

In conclusion, the risk through drinking water from the use of BAS 500 06 F in cereals and maize is acceptable.

Table 10.1.1.2-7: Screening step for drinking water risk assessment - ratio of effective application rate to relevant endpoint for birds

Parameter	Pyraclostrobin	Reference
K_{oc} [L/kg]	9304	SANCO/1420/2001-final
DT_{50} (soil) [d]	18	M-CP 9.2
Number of applications	2	Table 10.1.1-6
Interval [d]	21	Table 10.1.1-6
MAF_{mean} [1)]	1.45	--
Max use rate [g/ha]	250 [6)]	Table 10.1.1-6
AR_{eff} [g/ha] [2)]	362.5	--
LD_{50} [mg/kg b.w.]	1701	Table 10.1.1-1
Ratio (acute) [3)]	0.2	--
Reproductive endpoint [mg/kg b.w./d]	105	Table 10.1.1-1
Ratio (repro) [3)]	3.5	--
Trigger [4)]	3000	--
Drinking water assessment required [Yes/No] [5)]	No	--

1) $MAF_{mean} = (1-e^{-nki}) / (1-e^{-ki})$ with $k = ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d]. Worst-case from application scenario in cereals
2) AR_{eff} = Application rate (g/ha) x MAF_{mean}
3) Ratio of AR_{eff} and relevant toxicity endpoint
4) Trigger according to EFSA/2009/1438
5) Drinking water risk assessment is not necessary when trigger value is not exceeded.
6) Highest application rate for pyraclostrobin in cereals

A quantitative drinking water risk assessment is not triggered for the recommended use pattern of BAS 500 06 F in cereals and maize according to EFSA/2009/1438 criteria. Therefore, the risk to birds via drinking water is acceptable.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

M-CP 10.1.2 was updated with a quantitative higher tier risk assessment of the small herbivorous "vole" mammal scenario taking into account literature data on the composition of vole diet and data on foliar residue decline. Further, data from a new field effect study on common voles were included, showing that there are no adverse effects of BAS 500 06 F on common vole populations (BASF DocID 2015/1126803).

In this chapter, data on BAS 500 06 F are evaluated and the risk assessments are presented for the active substance pyraclostrobin and for effects of the formulation based on the already registered use pattern.

Table 10.1.2-1: Endpoints for mammals

Test system	Test species	EU agreed endpoints	Endpoints used in present risk assessment
Acute toxicity	Rat	LD_{50} > 5000 mg a.s./kg b.w.[1)]	LD_{50} > 5000 mg a.s./kg b.w.
Reproductive toxicity (long-term)	Rat	--	Tier1: NOEL = 3 mg a.s./kg b.w./d[2)]
			Higher tier: NOAEL = 8.2 mg a.s./kg b.w./d[2)]

1) Endpoints confirmed in the Ecotoxicology section of the Review report for the active substance pyraclostrobin, September 2004
2) Daily Dose [mg/kg b.w./d] calculated based on study data for food consumption and body weight

RISK ASSESSMENT

Summary of mammalian toxicity testing

The mammalian toxicity studies with pyraclostrobin relevant for a tier 1 dietary risk assessment for wild mammals are summarized in Table 10.1.2-2.

Table 10.1.2-2: Summary of studies relevant for the mammalian risk assessment for pyraclostrobin

Test system	Test species	Results	Reference (BASF DocID)
Acute oral toxicity	Rat	LD_{50} > 5000 mg a.s./kg b.w.[1)]	1998/10965
Reproductive toxicity	Rat	$NOEL_{Reproduction}$ = 32.6 mg a.s./kg b.w./d[2)] $NOEL_{Offspring}$ = 8.2 mg a.s./kg b.w./d[2)] $NOEL_{Parents}$ = 8.2 mg a.s./kg b.w./d[2)]	1999/11869
Developmental toxicity	Rat	$NOEL_{Rel\ Maternal}$ = 10 mg a.s./kg b.w./d[2)] $NOEL_{Rel\ Developmental}$ = 50 mg a.s./kg b.w./d[2)]	1999/11511
	Rabbit	$NOEL_{Rel\ Maternal}$ = 3 mg a.s./kg b.w./d[2)] $NOEL_{Rel\ Developmental}$ = 5 mg a.s./kg b.w./d[2)]	2001/1003803 and 1999/11512

1) Endpoints confirmed in the Ecotoxicology section of the Review report for the active substance pyraclostrobin, September 2004
2) Daily Dose [mg/kg b.w./d] calculated based on study data for food consumption and body weight

Mammalian toxicity endpoint for use in the acute tier 1 risk assessment

There is one acute oral toxicity test with pyraclostrobin available in rats (BASF DocID 1998/10965). This study resulted in an **LD_{50} > 5000 mg a.s./kg b.w.**, which is the relevant endpoint to be used in the acute risk assessment.

Mammalian toxicity endpoint for use in the reproductive tier 1 risk assessment

In order to obtain the relevant toxicity endpoint for the tier 1 reproductive risk assessment, the lowest NOAEL from the two-generation rat study and the lowest relevant endpoint from the developmental studies have to be considered.

For pyraclostrobin, the lowest toxicity endpoint from these studies (Table 10.1.2-2) is the **NOEL = 3 mg a.s./kg b.w./d** derived from the developmental rabbit study. This value is the endpoint used for the screening and tier 1 reproductive risk assessment. For the higher tier risk assessment the relevant endpoint is the NOEL from the 2-generation rat study, i.e. NOEL = 8.2 mg a.s./kg b.w./d. A full rationale supporting the use of this endpoint in the higher tier risk assessment is given in a separate document (BASF DocID 2014/1010736).

Results from available toxicity testing with the formulation

The mammalian toxicity study with BAS 500 06 F relevant for the risk assessment for mammals is summarized in Table 10.1.2-3.

Table 10.1.2-3: Summary of mammalian study on the acute toxicity of BAS 500 06 F

Test system	Test species	Results	Reference (BASF DocID)
Acute oral toxicity	Rat	LD_{50} ~ 500 mg/kg b.w.	2007/1053390[1)]

1) For detailed study summary please refer to M-CP 7.1.1.

A full summary of this study is given in M-CP 7.1.1. An acute risk assessment for BAS 500 06 F considering the **LD_{50} of 500 mg/kg b.w. for BAS 500 06 F** is provided further below.

Metabolites of pyraclostrobin

A full assessment of the relevance of pyraclostrobin metabolites for the birds and mammals risk assessment is given in M-CP 10.1.1. Please refer to M-CP 10.1.1 for details.

Summary of relevant mammalian toxicity endpoints to be used in the screening and tier 1 risk assessments

The mammalian endpoints relevant for the screening step and for the tier 1 risk assessments are given in Table 10.1.2-4.

Table 10.1.2-4: Mammalian toxicity endpoints for screening and tier 1 risk assessments of pyraclostrobin and BAS 500 06 F

Test substance	Test system	Relevant toxicity endpoint
Pyraclostrobin	Acute oral toxicity	LD_{50} > 5 000 mg a.s./kg b.w.
	Sub-chronic toxicity and reproduction	NOEL = 3 mg a.s./kg b.w./d
BAS 500 06 F	Acute oral toxicity	LD_{50} ~ 500 mg/kg b.w.

Relevant exposure scenarios

The relevant scenarios for the already registered use pattern (Table 10.1.2-5) are given for cereals in Table 10.1.2-6 and for maize in Table 10.1.2-7.

Table 10.1.2-5: Critical use pattern for BAS 500 06 F

Crop	EFSA/2009/1438 crop group	Application time (BBCH growth stage)	No. of applications	Interval [d]	Application rate per treatment	
					Pyraclostrobin [kg/ha]	BAS 500 06 F [L/ha]
Cereals	Cereals	25 - 69	2	21	0.25	1.25
Maize	Maize	30 - 65	1	--	0.20	1.0

Table 10.1.2-6: Summary of exposure scenarios to be considered for risk assessments for spray applications of BAS 500 06 F in cereals at BBCH 25 – 69

Scenario	Generic focal species
Cereals Early (shoots)	Large herbivorous mammal “lagomorph”
Cereals BBCH 10-29	Small omnivorous mammal “mouse”
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"
Cereals BBCH 30 - 39	Small omnivorous mammal “mouse”
Cereals BBCH ≥ 40	Small herbivorous mammal "vole"
Cereals BBCH ≥ 40	Small omnivorous mammal “mouse”

Table 10.1.2-7: Summary of exposure scenarios to be considered for risk assessments for spray applications of BAS 500 06 F in maize at BBCH 30 – 65

Scenario	Generic focal species
Maize BBCH ≥ 20	Small insectivorous mammal “shrew”
Maize BBCH 30 - 39	Small herbivorous mammal "vole"
Maize BBCH 30 - 39	Small omnivorous mammal “mouse”
Maize BBCH ≥ 40	Small herbivorous mammal "vole"
Maize BBCH ≥ 40	Small omnivorous mammal “mouse”

CP 10.1.2.1 Acute oral toxicity to mammals

In this section, the dietary TER acute values for the screening step and tier 1 assessment obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) are presented for cereals in Table 10.1.2.1-1 and for maize in Table 10.1.2.1-2.

Cereals

Table 10.1.2.1-1: Pyraclostrobin: Acute dietary risk assessment for mammals in cereals - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Appli-cation Interval**	**DT_{50}**	**LD_{50}**	
	Cereals	0.25	2	21	10.0	>5000.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{90}**	**Daily Dietary Dose (multiple)**	**TER**	**No refinement step required**
	Small herbivorous mammal	118.4	29.60	1.1	32.56	>153.6	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	**No refinement required**
	Cereals Early (shoots)	Large herbivorous mammal “lagomorph” Grass + cereals 100% cereal shoots			42.1	>431.9	
	Cereals BBCH 10-29	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			17.2	>1057.1	
	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods			5.4	>3367.0	
	Cereals BBCH 30 - 39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			8.6	>2114.2	
	Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass			40.9	>444.5	
	Cereals BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			5.2	>3496.5	

Maize

Table 10.1.2.1-2: Pyraclostrobin: Acute dietary risk assessment for mammals in maize - results from EFSA calculator tool

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Appli-cation Interval	DT_{50}	LD_{50}	
	Maize	0.2	1	365	10.0	>5000.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{90}**	**Daily Dietary Dose (multiple)**	**TER**	**No refinement step required**
	Small herbivorous mammal	136.4	27.28	1.0	27.28	>183.3	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**		**Shortcut value**	**TER**	**No refinement required**	
	Maize BBCH ≥ 20	Small insectivorous mammal “shrew” ground dwelling invertebrates with interception 100% ground arthropods		5.4	>4629.6		
	Maize BBCH 30 - 39	Small herbivorous mammal "vole Grass + cereals All maize shoots + later grass		68.2	>366.6		
	Maize BBCH 30 - 39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods		8.6	>2907.0		
	Maize BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals All maize shoots + later grass		34.1	>733.1		
	Maize BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods		4.3	>5814.0		

In conclusion, even under the conservative assumptions of the screening step and the tier 1 acute dietary risk assessment all TER_A values for pyraclostrobin exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects.

CP 10.1.2.2 Higher tier data on mammals

Please note that this section, albeit suggested by its header, does not only include higher tier data on mammals. The calculations presented for cereals in Table 10.1.2.2-1 and for maize in Table 10.1.2.2-2 are the dietary TER reproductive values for the screening step and tier 1 assessment obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm).

Cereals

Table 10.1.2.2-1: Pyraclostrobin: Reproductive risk assessment for mammals in cereals - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Repro End Point (mg/kg bw/d)**	**Time-weighted average (twa)**
	Cereals	0.25	2	21	10	3.0	0.53
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{mean}**	**Daily Dietary Dose (multiple)**	**TER**	**Please perform first tier risk assessment (see below)**
	Small herbivorous mammal	48.3	12.08	1.2	7.68	0.39	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	
	Cereals Early (shoots)	Large herbivorous mammal “lagomorph” Grass + cereals 100% cereal shoots			22.3	0.8	**Higher tier risk assessment required**
	Cereals BBCH 10-29	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			7.8	2.4	**Higher tier risk assessment required**
	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods			1.9	9.9	**No refinement required**
	Cereals BBCH 30 - 39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			3.9	4.8	**Higher tier risk assessment required**
	Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass			21.7	0.9	**Higher tier risk assessment required**
	Cereals BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			2.3	8.2	**No refinement required**

Maize

Table 10.1.2.2-2: Pyraclostrobin: Reproductive risk assessment for mammals in maize - results from EFSA calculator tool

Data from Data_Entry worksheet	Crop	Application rate (kg a.s./ha)	Number of applications	Application Interval	DT_{50}	Repro End Point (mg/kg bw/d)	Time-weighted average (twa)
	Maize	0.2	1	365	10	3.0	0.53
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{mean}**	**Daily Dietary Dose (multiple)**	**TER**	**Please perform first tier risk assessment (see below)**
	Small herbivorous mammal	72.3	14.46	1.0	7.66	0.39	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	
	Maize BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods			1.9	14.9	**No refinement required**
	Maize BBCH 30 - 39	Small herbivorous mammal "vole Grass + cereals All maize shoots + later grass			36.1	0.8	**Higher tier risk assessment required**
	Maize BBCH 30 - 39	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			3.9	7.3	**No refinement required**
	Maize BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals All maize shoots + later grass			18.1	1.6	**Higher tier risk assessment required**
	Maize BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			1.9	14.9	**No refinement required**

In conclusion the tier 1 TER_{LT} values for pyraclostrobin are below the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects for four scenarios in cereals and two scenarios in maize. The scenarios failing the trigger values require refinement of the assessments in a higher tier step. The higher tier risk assessments are presented in the following.

Refined reproductive risk assessments for mammals

The following scenarios failed the trigger of 5 in the tier 1 reproductive risk assessment and will be refined:

Applications to cereals:

Large herbivorous mammal “lagomorph” at early stages
Small omnivorous mammal “mouse” at BBCH 10-29
Small omnivorous mammal “mouse” at BBCH 30-39
Small herbivorous mammal "vole" at BBCH ≥ 40

Applications to maize:

Small herbivorous mammal "vole" at BBCH 30-39
Small herbivorous mammal "vole" at BBCH ≥ 40

A. Small herbivorous mammal "vole" scenarios in cereals and maize

According to EFSA/2009/1438 (Appendix A) the representative (default) species to be used for refinement of the small herbivorous mammal scenario “vole” at BBCH 30-39 (in maize) and BBCH ≥ 40 (in cereals and maize) is the common vole, *Microtus arvalis*.
However, there are also reasons to not consider the common vole a relevant focal species in cereals and maize, because arable crops in general do not constitute the common vole’s primary habitat (for more details, see BASF DocID 2011/1248329 and BASF DocID 2004/1025715; please note that both documents were erroneously not included in the Application submitted in January 2014). Furthermore, common vole populations naturally display cyclical changes and a strong ability to recover from decimation, contributing to its pest status in European countries. This notion that the common vole is not considered a relevant focal species in arable crops is supported by a recent literature review (Jacob et al., 2013), in which population dynamics, habitat and food preferences, pest potential and the use of the common vole in risk assessments are presented and discussed in detail. Therefore, BASF still believes that the small herbivorous mammal scenario is covered by another rodent, the omnivorous wood mouse (*Apodemus sylvaticus* L.). As shown in Table 10.1.2.2-1 for cereals and in Table 10.1.2.2-2 for maize, the related TER_{LT} values of the wood mouse are above the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects indicating an acceptable risk for the intended use of BAS 500 06 F in cereals and maize.

However, the relevance of the “vole” scenario has been controversially discussed by the European Member States and a harmonized approach is currently lacking. Therefore, the quantitative higher tier risk assessments for the vole in cereals (Table 10.1.2.2-11) and maize (Table 10.1.2.2-12) are presented below (in addition to the quantitative higher tier risk assessment for the wood mouse in cereals).

B. Large herbivorous mammal "lagomorph", small omnivorous mammal “mouse”, and small herbivorous mammal “vole” scenarios in cereals and maize

The refined dietary reproductive risk assessment for mammals is based on the following refinement points:

Refinement point 1:	Ecologically relevant reproductive toxicity endpoint for pyraclostrobin
Refinement point 2:	Residue decline of pyraclostrobin in plants for refinement of MAF x twa
Refinement point 3:	Identification of representative mammal species including ecological data (PT and PD values and FIR)
Refinement point 4:	Interception by cereal and maize plants

Refinement point 1: Ecologically relevant reproductive toxicity endpoint for pyraclostrobin

According to the latest regulatory Guidance (EFSA/2009/1438) both the multi-generation study and the prenatal toxicity studies need to be taken into account for defining a relevant toxicity value for the long-term risk assessment to mammals. Hence, BASF evaluates in detail the database of mammalian laboratory toxicology studies with pyraclostrobin relevant for the long-term risk assessment for wild mammals, and proposes an ecologically relevant NOAEL for the refined evaluation of the reproductive risk to wild mammals.

The available database comprises one multi-generation study conducted with rats, one prenatal toxicity study conducted with rats, and two prenatal toxicity studies conducted with rabbits. According to the proposal of EFSA 2009/1438 for the first tier risk assessment the lowest toxicity value of these studies, i.e. the NOAEL = 3 mg/kg b.w./d derived from the prenatal toxicity studies conducted with rabbits, is used for TER calculations. For higher tier risk assessment EFSA/2009/1438 recommends a re-examination of the study data to derive an endpoint of biological and/or ecological relevance. Full details of this evaluation are given along with the present dossier in a separate position paper [Anonymous 2014 a, BASF DocID 2014/1010736]. A short summary of the evaluation is given in the following:

In brief, a three-step approach was followed to derive an overall higher tier toxicity endpoint of ecological and population relevance, including:

i) A detailed analysis of the results from the prenatal toxicity studies with rabbits, as the lowest endpoint from these studies was used for the first tier risk assessment of pyraclostrobin.
ii) A discussion of the general relevance of the available types of toxicity studies (multi-generation, prenatal toxicity) for the route and conditions of exposure to wild mammals in the field.
iii) A comparison of the ecologically relevant NOAELs from the different studies to define and propose an overall ecologically relevant endpoint for higher tier risk assessment.

The **detailed analysis the prenatal toxicity studies with rabbits** provided the following main results and arguments:

- A transient decrease in maternal food consumption during treatment days 1-5 in the rabbit developmental studies was followed by a clear recovery of maternal food consumption till the end of the treatment period. The transient variations in food consumption observed during the first days of treatment did not translate into effects on body weight for animals dosed with up to and including 10 mg/kg bw/d pyraclostrobin.

- In respect of overall reproductive outcome up to and including doses of 10 mg/kg bw/d, the number of live, male and female fetuses was not affected. The % of live fetuses per implant was not altered, and no effects were seen on the sex ratio of fetuses. Furthermore, no dead fetuses were reported at any dose rate.

- In respect of parameters for dams, there was no statistically significant difference in the number of corpora lutea, the number of implantation sites, the rate of pre-implantation loss, and the number of late resorptions between all dose groups, the concurrent controls, and the set of historical control data.

- A slight increase in early resorptions and consequently a higher rate of post-implantation loss was observed at the high doses. However, up to and including doses of 10 mg/kg bw/d slight differences in early resorptions and post-implantation loss were not of any significance and did not translate into effects on the overall number of live fetuses and the % of live fetuses per implant.

- Minor changes on the fetal body weights at doses of 10 mg/kg bw/d and above were not of statistical significance as compared to the concurrent controls at any dose rate.

➢ In conclusion, no effect was seen on the overall reproductive success, population relevant parameters, and potential fitness of the offspring, and hence no impact on size and stability of wild mammal populations is to be expected. Consequently, the ecotoxicologically relevant endpoint from the rabbit prenatal toxicity studies is the NOAEL = 10 mg/kg bw/d.

Further, **the different types of toxicity studies (multi-generation, prenatal toxicity) were compared regarding their general relevance for the route and conditions of exposure** to wild mammals in the field. The multi-generation study was found to reflect best the route of exposure (dietary), duration (several generations continuously exposed to treated food) and endpoints (development, survival and, most important for the reproduction risk, reproductive endpoints) relevant for small mammals in the wild. The lowest endpoint from the multi-generation study with rats is the NOEL = 8.2. mg/kg b.w./d.

Finally, in **order to propose an overall ecologically relevant endpoint for higher tier risk assessment, the specific endpoints from the available studies were compared:** The relevant endpoint from the multi-generation study with rats is the NOEL = 8.2 mg/kg b.w./d, and hence well in range with the endpoint from the prenatal toxicity study with rat (NOEL = 10 mg/kg b.w./d). The presented analysis supports furthermore for the prenatal toxicity study in rabbit a NOAEL = 10 mg/kg bw/d as ecotoxicological relevant endpoint.

Overall conclusion: Based on the detailed analysis of the prenatal toxicity studies with rabbits, considerations on the most relevant study type, and the comparison of the ecotoxicologically relevant endpoints from the different available studies, BASF proposes to use as conservative value for the refined wild mammal risk assessment for pyraclostrobin the NOAEL=8.2 mg/kg b.w./d from the multi-generation study with rats.

Refinement point 2: Residue decline of pyraclostrobin in plants for refinement of MAF x twa

According to EFSA/2009/1438 in the Tier 1 risk assessment a default foliar half-life (DT_{50}) of 10 days is considered for calculation of the multiple application factor (MAF) and the time-weighted average factor (f_{twa}). However, data from substance-specific field residue trials with pyraclostrobin indicate a much faster dissipation of pyraclostrobin in foliar plant tissue. BASF conducted thirty-two GLP field residue trials in wheat and peas to obtain foliar residue decline data that are fully applicable for Northern Europe and Southern Europe (BASF DocID 2013/1045207 and BASF DocID 2013/1044539).

The field trials were specifically designed to obtain a solid refinement data package for higher tier risk assessments on birds and mammals. Herbivorous / omnivorous birds and mammals might feed on the leaves of the crop plants themselves, but preferably consume young nutritious weed plants growing within the treated field. Hence, the test item was applied onto wheat and pea plants at early growth stages, i.e. BBCH 12/13 for both peas and wheat, to obtain results being fully relevant for monocotyledonous and dicotyledonous weeds at early growth stages. Under this premise wheat and peas are considered acceptable as surrogate plants for monocotyledonous and dicotyledonous weeds.

Samples were collected on DAT 0, 1, 2, 3, 4, 5, 7, 10, 12, 14. During the sampling period (DAT 0 to DAT 14) the large majority of the test fields were not irrigated. Only trials L120077 and L120078 were irrigated once at late time points of the sampling period (DAT 6 or DAT 7). Considering the observed rapid decline of pyraclostrobin, a bias of the trial results by wash-off can be excluded.

The measured residue data in the plants were used to derive dissipation times of pyraclostrobin in the plants. Following FOCUS guidance (2006) kinetic evaluations of all 32 residue data sets were carried out. For two trials no acceptable kinetic fit could be calculated and therefore no reliable DT_{50} could be estimated (see Table 10.1.2.2-3). Therefore, the dissipation data of these two trials were excluded for further analysis. The evaluation of the remaining 30 residue data sets showed a high goodness-of-fit of the applied kinetic models to estimate DT_{50} values based on the following three criteria:

1) The visual inspections of the fitted models indicated that the calculated curves matched the observed residue behavior very well supported by very low $\chi 2$ minimum error values (mean = 9.1 %, n = 30).

2) The results of the t-test statistics (each p value < 0.001) proved clearly that the derived constant rates (SFO models) were statistically different from zero.

3) The residuals were generally small and randomly scattered around the zero line.

The calculated DT_{50} values are presented below in Table 10.1.2.2-3. For calculation details to obtain DT_{50} values please refer to the reports (BASF DocID 2013/1078114 and BASF DocID 2013/1291161.) Please note that both reports were erroneously not listed in the Application submitted in January 2014. The summaries of the dissipation calculations and the field residue trials are found at the beginning M-CA 8.1.

In these two residue studies with pea and wheat plants, two data sets were generated at each location. Since the two treated plots (plot 2 & 3) were situated very close to each other, the agricultural practice was virtually identical. Therefore, the two treated plots may be considered as pseudo-replicates and not as fully independent replicates. Consequently, for the statistical analysis carried out (see below), a single DT_{50} value was calculated for each location by calculating the mean of the values for the two plots.

Table 10.1.2.2-3: Foliar residue decline trials with pyraclostrobin in Europe: DT_{50} in wheat and peas (BASF DocIDs 2013/1078114 and 2013/1291161)

Crop	Location	Trial	Plot	DT_{50} [d] per plot	DT_{50} [d] per location[1)]	BASF DocID
Wheat	DE; Limburgerhof, Rheinland-Pfalz	L120103	P2	2.67	2.02	2013/1078114
Wheat	DE; Limburgerhof, Rheinland-Pfalz	L120103	P3	1.37		
Wheat	DE; Kleve, Nordrhein-Westfalen	L120104	P2	1.40	1.69	
Wheat	DE; Kleve, Nordrhein-Westfalen	L120104	P3	1.97		
Wheat	NL; Gennep, Limburg	L120105	P2	2.18	1.87	
Wheat	NL; Gennep, Limburg	L120105	P3	1.56		
Wheat	UK; Oxfordshire	L120106	P2	1.26	1.26	
Wheat	UK; Oxfordshire	L120106	P3	1.25		
Peas	DE; Limburgerhof, Rheinland-Pfalz	L120073	P2	1.28	1.39	
Peas	DE; Limburgerhof, Rheinland-Pfalz	L120073	P3	1.50		
Peas	DE; Kerken, Nordrhein-Westfalen	L120074	P2	1.91	1.73	
Peas	DE; Kerken, Nordrhein-Westfalen	L120074	P3	1.55		
Peas	FR (N); Loir et Cher	L120075	P2	3.76	3.13	
Peas	FR (N); Loir et Cher	L120075	P3	2.50		
Peas	UK; Essex	L120076	P2	2.10	2.08	
Peas	UK; Essex	L120076	P3	2.06		
Geometric mean (n = 8) – Northern Europe DT_{50} [d]					**1.83**	
Wheat	Spain, Seville, Lebrija	L120107	P2	2.50	2.81	2013/1291161
Wheat	Spain, Seville, Lebrija	L120107	P3	3.12		
Wheat	Spain, Seville, Las Cabezas de San Juan	L120108	P2	5.20	5.37	
Wheat	Spain, Seville, Las Cabezas de San Juan	L120108	P3	5.53		
Wheat	Italy, Cuneo, Castagnito d'Alba	L120109	P2	3.14	3.71	
Wheat	Italy, Cuneo, Castagnito d'Alba	L120109	P3	4.27		
Wheat	Italy, Cuneo, Castagnito d'Alba	L120110	P2	4.41	3.86	
Wheat	Italy, Cuneo, Castagnito d'Alba	L120110	P3	3.31		
Peas	Spain, Andalucía, Sevilla	L120077	P2	2.60	2.61	
Peas	Spain, Andalucía, Sevilla	L120077	P3	2.61		
Peas	Spain, Andalucía, Sevilla	L120078	P2	2.57	2.50	
Peas	Spain, Andalucía, Sevilla	L120078	P3	2.43		
Peas	Italy, Emilia Romagna, Ferrara	L120079	P2	- [2)]	2.59	
Peas	Italy, Emilia Romagna, Ferrara	L120079	P3	2.59		
Peas	Italy, Emilia Romagna, Ferrara	L120080	P2	- [2)]	1.93	
Peas	Italy, Emilia Romagna, Ferrara	L120080	P3	1.93		
Geometric mean (n = 8) – Southern Europe DT_{50} [d]					**3.03**	
Geometric mean (n = 16) – Southern and Northern Europe DT_{50} [d]					**2.35**	
~~**Geometric mean (n=30) DT_{50} [d]**~~				~~**2.34**~~	**—**	

1) Arithmetic mean of the two DT_{50} values per each location, with exception of trials L120079 and L120080, for which only a single value per location is available. Arithmetic mean values were used for statistical analyses.

2) Plot discarded, since no acceptable fit could be achieved.

A statistical evaluation of the DT_{50} data set was done to identify which trial data could be merged for the calculation of a geometric DT_{50} value being adequate for the refined risk assessment. This approach follows the peer-review evaluation in the recent EU renewal process of pendimethalin, where the experts agreed to use the geometric mean DT_{50} value based on results of a statistical evaluation of half-life data (EFSA Conclusion for Pendimethalin, EFSA Journal 2016;14(3):4420).

The statisticial analysis of the pyraclostrobin data was based on the following samples sizes: wheat/north (n = 4); wheat/south (n = 4); peas/north (n = 4); peas/south (n = 4). Results are shown below:

Table 10.1.2.2-4: Results of statistical analyses of residue data sets for factors residue zone and plant species

Data sets comparison	p value*
Wheat vs. Peas	**0.59**
North vs. South	0.009
Peas/South vs. Wheat/South	**0.12**
Peas/North vs. Wheat/North	**0.41**
Peas/North vs. Wheat/South	0.025
Peas/South vs. Wheat/North	**0.14**
Wheat/North vs. Wheat/South	0.002
Peas/North vs. Peas/South	**0.50**

* Derived by non-parametric Kruskal-Wallis tests with post-hoc analysis of variance (Dunn test). Note that a t-test is not applicable to the dataset as required assumptions for the t-test could not be fulfilled (i.e. data are not normally distributed), even not with log-transformation of data.

The statistical results showed that there is no significant difference between wheat and pea data (p = 0.59), albeit data sets splitted by region differ significantly (p = 0.009). Taking additionally the results of comparing data subsets into account, it is acceptable to merge the data set "peas/south" with "wheat/south" (p = 0.12) and on the other hand "peas/north" with "wheat/north" (p = 0.41) as the DT_{50} values are consistent across trials in each of the two residue zones irrespective of the plant species. This results finally in two geometric mean DT_{50} values, the first (DT_{50} = 1.83 days, n = 8) covering the Northern zone and a second one (DT_{50} = 3.03 days, n = 8) applicable to the Southern zone.

The lack of a statistical difference between wheat and pea data per zone indicated that extrapolation between plant/crop types is justified, so that the provided residue data of pyraclostrobin are suitable surrogates of food items for small herbivorous mammals such as young grass and weed plant material.

In 2016 BASF conducted three additional foliar residue decline trials in wheat in the Northern residue zone in order to complete the picture on foliar residue decline of pyraclostrobin. In these three trials the application was carried out at a later growth stage (BBCH 25-29) to address uncertainties on the foliar residue dissipation pattern associated with dilution of residues by plant growth. The estimated DT_{50} values (based on a high goodness-of-fit according to FOCUS GD, 2006) are for all three trials < 3 days and therefore well in line with the values based on the already submitted wheat studies. This finding supports that the plant growth stage at the time point of application does not play a major role in the foliar dissipation pattern of pyraclostrobin.

The final study reports are expected to be available in the second quarter of 2017 and can be therefore provided upon request after the commenting period of the dRAR.

Based on the evaluation and statistical analysis of the submitted monocot (wheat) and dicot (pea) data, the calculated geomeans were DT_{50} = 1.83 days for the Northern residue zone and DT_{50} = 3.03 days for the Southern zone. Following a worst-case approach for this EU-wide evaluation, the highest of these two values (DT_{50} = 3.03 days) is used for the refinement of the risk assessments of the mammal scenarios. Therefore, the higher tier calculations and the resulting TER values of the large herbivorous mammal "lagomorph" and small omnivorous mammal “mouse” scenarios in cereals changed slightly and were updated accoordingly.

MAF x twa moving time window approach

The calculation of MAF and twa factor is conducted in accordance with the recommendations from EFSA/2009/1438 (Appendix H). An EXCEL spreadsheet was developed that describes the actual concentration in feed items from the day after first treatment (1 DAFT) up to 200 DAFT. Dissipation between the application events according to single first order kinetics (SFO) was introduced in the EXCEL spreadsheet as well as the build-up of residues through multiple applications. The geometric mean DT_{50} value of 3.03 d for pyraclostrobin is used for the twa calculation.

The calculations follow the basic formula assuming single first order dissipation kinetic:

$$C_{act}(t) = C_0 * e^{-k * t}$$

$C_{act(t)}$	actual concentration at time t
C_0	initial concentration
K	degradation rate constant (= ln(2) / DT_{50})
T	time t

Furthermore, the established spreadsheet calculates - one after the other in a resolution of 0.1 d time steps - the average concentration factors for a 21 d time period, starting from the time of the first treatment (0 DAFT) up to 200 DAFT and scans for the maximum of the resulting twa values (moving time window approach (EFSA 2009/1438, Appendix H)). The high resolution of 0.1 d time steps leads to precise results even under consideration of short DT_{50} values. The calculation of the twa, 21 d is described in the equation below.

Calculation of the twa over 21 d using a "moving time window" approach:

$$twa, 21\ d = max\left[\frac{1}{21 * 10}\sum_{t=t_j\ ,step\ 0.1}^{t_{j+20.9}} C_{act}\ (t)\right]\ for\ j = 0.05,\quad (200 - 21 - 0.05)$$

twa, 21 d	maximum average concentration in feed item for a 21 d interval
$C_{act\ (t)}$	actual concentration at time t
t	time
t_j	start time point for integration
j	time step running variable

For the use of BAS 500 06 F in cereals (2 applications, 21-day interval), the calculation results in a maximum 21-d twa factor of 0.2081 and for the use in maize (1 application), the calculation results in a maximum 21-d twa factor of 0.2064. Please note that the MAF is set to 1 as this factor is already included in the calculation of the maximum 21-d twa factor.

Refinement point 3: Identification of representative mammal species including ecological data (PT and PD values and FIR)

Representative and focal species

Hare

For the large herbivorous mammal scenario at early stages in cereals EFSA/2009/1438 suggests the rabbit (*Oryctolagus cuniculus*) as representative species.

However, data from a recent field study (BASF DocID 2011/1112612, for a detailed study summary please refer to the beginning of chapter M-CP 10.1) suggests that the hare and not the rabbit is the representative focal species at early growth stages of cereals.

In spring 2011, spotlight counts were conducted at two different study sites in central Europe (Germany). In total, 90 cereal fields were monitored and all observed hares and rabbit were recorded. The number of hare per 100 ha surveyed area was ten-fold higher compared to that of rabbits and the frequency of occurrence of the hare (*Lepus europaeus*) was more than seven-fold higher compared to that of rabbits at early growth stages in cereal fields.

The results from this field survey are in agreement with the fact that the habitat requirements of the hare and the rabbit are different (see e.g. Gurney et al. 1998). Whereas the rabbit (*Oryctolagus cuniculus*) prefers areas of short grass like naturally occurring dry heaths or closely grazed agricultural pasture and secure refuge nearby, i.e. diversely structured landscape, the hare (*Lepus europaeus*) prefers arable land predominated by cereals where it feeds (among other items) on cereals at early growth stages (Gurney et al. 1998). Thus, arable land including cereal fields at early growth stages are an important habitat type for the hare (and not so much for the rabbit) in spring.

Wood mouse
According to EFSA/2009/1438 the wood mouse (*Apodemus sylvaticus*) is the representative species for the small omnivorous mammal scenario at BBCH 10-29 and BBCH 30-39.

Common vole
According to EFSA/2009/1438 the common vole (*Microtus arvalis*) is the representative species for the small herbivorous mammal scenario at BBCH 30-39 and BBCH ≥ 40 in maize and BBCH ≥ 40 in cereals.

In conclusion, the refined risk assessment for pyraclostrobin applied as BAS 500 06 F in cereals will be carried out for three species representative of large herbivorous (hare, *Lepus europaeus*), omnivorous (wood mouse, *Apodemus sylvaticus*) and small herbivorous (common vole, *Mircotus arvalis*) mammals using cereal fields as foraging grounds.

Ecological data

Hare (*Lepus europaeus*)

Proportion of diet obtained from the treated area (PT)

It has been shown that hares have large home ranges. Home ranges can vary in size from approximately 20 ha up to >100 ha (for a review, see e.g. Olesen & Asferg, 2006). The occurrence of such large home ranges strongly suggests that it is appropriate to refine the default PT value of 1.

Prosser (2010) presents PT data for the hare, from FERA's (formerly CSL's) previous projects in a single document for easy reference. Prosser (2010) included only animals for which a quantity of data likely to give robust estimates of PT is available. The modelled PT distributions have been fitted by the author to these data, to give a more accurately calculated 90th percentile value of the PT distributions. The 90th percentile value of PT=0.87 from all tracked hares (n=19) for cereals in spring (Prosser, 2010, Table 12) will be used in the refined TER calculations for the hare.

Wood mouse (*Apodemus sylvaticus)*

Fraction of food type in the diet (PD)

The wood mouse is known to be an opportunistic feeder adapted to utilise and exploit various food resources. The default diet (25% weeds, 50% weed seeds, 25% ground arthropods) according to EFSA/2009/1438 used in the tier 1 calculations above, can be refined by data from scientific literature. Most suitable data to conclude on the diet composition of the wood mouse in arable land, especially cereal fields, are found in Green (1979) and Pelz (1989). The data also allow to quantify proportions of the different feed items (PD) for the period when the product BAS 500 06 F is intended to be applied in cereals. For details of this evaluation please refer to Appendix 1 at the end of chapter M-CP 10.1.

The following PD values for the wood mouse in cereals are derived from the evaluation:

- Green plant matter PD = 0.107
- Weed seeds PD = 0.551
- Arthropods PD = 0.187
- Earthworms PD = 0.154

These PD values will be used for the calculation of the Food Intake Rate as summarized in Table 10.1.2.2-6.

Common vole (*Microtus arvalis*)

Fraction of food type in the diet (PD)

For the higher tier risk assessment of the common vole, the fraction of food type in the diet (PD) will be refined.

Rinke (1991) investigated common vole (*Microtus arvalis)* feeding preferences via stomach content analysis in a study area with a high availability of monocotyledonous plants (i.e. meadows) in central Germany. In total, the stomach contents of 363 individuals (186 females and 177 males) caught on five plots of permanent meadow (about 0.5 ha each) during 1984-1987 were analysed. Animals were captured with baited snap traps in spring (April-May), summer (August-September) and autumn (October-December). They were sexed and three age groups based on body weights were established: juveniles (≤ 10g), subadults (10-15g), and adults (> 15g). Stomach contents were analysed microscopically and plant species were determined to the species level. Estimation of volume percentages of mono- and dicotyledonous plants were made based on the area occupied on the slide. The stomach contents of the common voles were classified in five groups as follows: stomach containing (1) 0-20%, (2) 21-40%, (3) 41-60%, (4) 61-80% and (5) 81-100% dicotyledonous plants.

It was shown that the common vole feeds on a broad variety of plant species and exhibits a pronounced selective food intake. Despite the fact that more monocots were available on the study plots (about 70%), the animals showed a clear preference for dicots, even when a larger percentage of monocots were available. Irrespective of season, sex and age, the majority of voles showed >80% dicotyledonous material in their stomach contents. Interestingly, age, sex or season did not strongly impact the common voles' food preference.

The data from Rinke (1991) suggests that the actual diet of the common vole comprises at least >50% dicotyledonous plants. It even consisted of approximately 25% monocots and 75% dicots in spring and summer (see Table 10.1.2.2-5, adapted from Rinke, 1991).

Table 10.1.2.2-5: Diet of common voles (percent volume) in meadows in Germany (adapted from Rinke, 1991) in spring, summer and autumn

	Monocotyledons (percent volume)	Dicotyledons (percent volume)	Number of animals
Spring	24	76	23
Summer	25	75	152
Autum	48	52	188

In conclusion, the 100% monocotyledonous diet as assumed by EFSA (2009) is an unrealistic worst-case estimate, because it has been shown that voles consume both, monocots and dicots. Thus, it is justified to conservatively assume that in spring and summer, the diet of the vole in cereals and maize consists of approximately 25% monocotyledonous plants (grasses) and 75% dicotyledonous plants (non-grass herbs), resulting in PD values of

Grasses	PD = 0.25	
Non-grass herbs	PD = 0.75	PD sum = 1.0

These PD values are used for the refined chronic risk assessment and the calculation of the Food Intake Rate (FIR / b.w.) values (see Table 10.1.2.2-6 below).

Calculation of Food Intake Rate (FIR/b.w.) for the focal species

The FIR/b.w. values for the focal species are calculated following EFSA/2009/1438 (Appendix G - Calculating exposure for the dietary intake approach).

First, based on $PD_{i,fresh}$ (the diet composition in fresh weight) and considering energy content, moisture content and assimilation efficiency, the $FE_{total,\ fresh}$ can be calculated according to the following formula:

$$FE_{total,fresh} = \sum_i \left[PD_{i,fresh} \times FE_i \times \left(1 - \frac{MC_i}{100} \right) \times \frac{AE_i}{100} \right]$$

In which:

$FE_{total,\ fresh}$ = Food energy of total mixed diet [kJ/g fresh weight]
$PD_{i,\ fresh}$ = Fraction of food item [i] in mixed diet [related to fresh weight]
FE_i = Food energy of food item [i] in mixed diet [kJ/g dry weight]
MC_i = Moisture content of food item [i] in mixed diet [%]
AE_i = Assimilation efficiency of food item [i] in mixed diet [%]

Second, using the calculated specific energy content of the mixed fresh diet ($FE_{total,\ fresh}$) the required amount of the mixed diet ($FIR_{total,\ fresh}$) to reach the DEE (daily energy expenditure) of the indicator species can be determined.

$$FIR_{total,fresh} = \frac{DEE}{FE_{total,fresh}}$$

Third, the FIR/b.w. value is calculated as the quotient of $FIR_{total,\ fresh}$ and the species' body weight.

$$FIR/b.w. = FIR_{total,\ fresh} / b.w.$$

The FIR/b.w. value for the hare, the wood mouse, and the common vole are shown below in Table 10.1.2.2-6.

Table 10.1.2.2-6: FIR/b.w. for the hare, the wood mouse and the common vole

Food	$PD_{i,\ fresh}$	FE_i [kJ/g dry weight]	AE_i / 100	MC_i	$FE_{total,\ fresh}$ [kJ/ g fresh weight]	DEE [kJ]	$FIR_{total,\ fresh}$ [g]	Body weight [g]	FIR/ b.w.
Hare									
Grasses / cereal shoots	1.0	17.60	0.47	76.4	1.95	2363.44	1210.66	3800[1)]	**0.319**
Wood mouse									
Grasses / cereal shoots	0.107	17.60	0.47	76.4	0.21	58.83		21.7[1)]	
Weed seeds	0.551	21.70	0.84	9.9	9.05				
Arthropods	0.187	22.70	0.87	68.8	1.15				
Earthworms	0.154	19.40	0.87	84.3	0.41				
Sum	0.999				10.82		5.44		**0.251**
Common vole									
Grasses / cereal shoots	0.25	17.60	0.47	76.4	0.49	65.09		25[1)]	
Non-grass herbs	0.75	17.80	0.76	88.1	1.21				
Sum	1.0				1.70		38.39		**1.536**

[1)] Bodyweight data according to EFSA/2009/1438

Refinement point 4: Interception by cereal and maize plants

Food items like weed seeds, non-grass herbs and grasses on the ground of cereal and maize fields will not receive the total amount of spray solution of a fungicide application. This is due to the fact that significant parts of the spray volume (and hence of the active substance) are intercepted by the crop foliage before reaching the ground.

Regarding interception EFSA/2009/1438 indicates that in a context of a higher tier risk assessment the deposition values given in FOCUS$_{GW}$ (Anonymous 2000/2002) may be used. Indeed, for cereals and maize the interception factors from FOCUS groundwater can more specifically be attributed to the growth stages in cereals and maize at BBCH ≥30, compared to the growth stages as used in the FOCUS surface water document (FOCUS 2001) for Step 2 calculations. However, for the product BAS 500 06 F the earliest application is intended at BBCH 25 in cereals and BBCH 30 in maize (Table 10.1.2.2-7 and Table 10.1.2.2-8) where the FOCUS$_{GW}$ (Anonymous 2000/2002) and the FOCUS surface water document (FOCUS 2001) present the same interception values.
The FOCUS$_{GW}$ document assumes the following successive decrease of soil deposition rates for cereals during the main growing season.

Table 10.1.2.2-7: Deposition rates in cereals according to $FOCUS_{GW}$ (Anonymous 2000/2002)

Growth stage in spring and winter cereals according to $FOCUS_{GW}$ (Anonymous 2000/2002)[1)]		Interception [%]	Deposition factor
Leaf development	10-19[2)]	25[2)]	0.75
Stem elongation (tillering)	20-29[2)]	50[2)]	0.5
Stem elongation (elongation)	30-39	70	0.3
Flowering	40-89	90	0.1

1) From $FOCUS_{GW}$ guidance document (p. 23)
2) Identical values when compared to FOCUS surface water document (FOCUS 2001)

Table 10.1.2.2-8: Deposition rates in maize according to $FOCUS_{GW}$ (Anonymous 2000/2002)

Growth stage in maize according to $FOCUS_{GW}$ (Anonymous 2000/2002)[1)]		Interception [%]	Deposition factor
Leaf development	10-19[2)]	25[2)]	0.75
Stem elongation (elongation)	20-39[2)]	50[2)]	0.5
Flowering	40-89	75	0.25

1) From $FOCUS_{GW}$ guidance document (p. 23)
2) Identical values when compared to FOCUS surface water document (FOCUS 2001)

For the product BAS 500 06 F the earliest application is intended at growth stage BBCH 25 in cereals and BBCH 30 in maize (Table 10.1.2-5). Hence, the deposition factor of DF = 0.5 for the food items weed seeds in cereals will be used in the refined risk assessments for the small omnivorous mammal “mouse” at BBCH 10-29 and BBCH 30-39. The deposition factor of DF = 0.1 for the food items non-grass herbs and grasses in cereals will be used in the refined risk assessments for the small herbivorous mammal "vole" at BBCH ≥ 40 in cereals. The deposition factor of DF = 0.5 for the food items non-grass herbs and grasses in maize will be used in the refined risk assessment for the small herbivorous mammal "vole" at BBCH 30-39, which covers the scenario for the small herbivorous mammal "vole" at BBCH ≥ 40.

Refined reproductive risk assessment for pyraclostrobin: TER calculations

The refined reproductive risk assessments for large herbivorous mammal and small omnivorous mammal scenarios are shown in Table 10.1.2.2-9 for the hare and in Table 10.1.2.2-10 for the wood mouse for cereals. For small herbivorous mammals refined risk assessments are shown in in Table 10.1.2.2-11 for the vole in cereals and in Table 10.1.2.2-12 for the vole in maize.

Table 10.1.2.2-9: Hare: Refined reproductive risk assessment for cereals

Food type[1)]	FIR/ b.w.	$PD_{i,\ fresh}$	PT	RUD [mg a.s./kg]	MAF	Maximum 21-d twa factor	DF	Use rate [kg a.s./ha]	DDD [mg a.s./kg b.w./d]
Grass/cereal shoots	0.319[1)]	1.0[1)]	0.87	54.2[1)]	1[2)]	0.2081[2)]	1	0.25	0.782
Toxicity endpoint [mg a.s./kg b.w./d]									8.2[3)]
TER_{LT}									10.49

1) According to EFSA/2009/1438
2) The maximum 21-d twa factor is calculated based on a pyraclostrobin-specific DT_{50} = 3.03 days and an application interval of 21 days for two applications. Please note that the MAF is set to 1 as this factor is already included in the calculation of the maximum 21-d twa factor.
3) Overall ecologically relevant toxicity endpoint (see refinement point 1)

Table 10.1.2.2-10: Small omnivorous mammal "mouse" scenario (wood mouse): Refined reproductive risk assessment for cereals

Food type[1)]	FIR/ bw[1)]	PD_i, fresh	PT	RUD [mg a.s./kg]	MAF	Maximum 21-d twa factor	DF	Use rate [kg a.s./ha]	DDD [mg a.s./kg b.w./d]
Grass/cereal shoots	0.251	0.107	1	54.2[1)]	1[3)]	0.2081[3)]	1	0.25	0.076
Weed seeds	0.251	0.551	1	40.2[1)]	1.23[1)]	0.53[1)]	0.5[4)]	0.25	0.452
Ground arthropods	0.251	0.187	1	7.5[1)]	1.23[1)]	0.53[1)]	1	0.25	0.057
Earthworms	0.251	0.154	1	0.128[2)]	1[5)]	1[5)]	1	1[5)]	0.005
DDD_{sum} [mg a.s./kg b.w./d]									0.59
Toxicity endpoint [mg a.s./kg b.w./d]									8.2[5)]
TER_{LT}									13.9

1) According to EFSA/2009/1438
2) RUD in earthworms (PEC_{worm}) based on specific PEC_{soil} (twa, 21 days). See Table 10.1.2.2-15 for details.
3) The maximum 21-d twa factor is calculated based on a pyraclostrobin-specific DT_{50} = 3.03 days and an application interval of 21 days for two applications. Please note that the MAF is set to 1 as this factor is already included in the calculation of the maximum 21-d twa factor.
4) See refinement point 4 for details.
5) Overall ecologically relevant toxicity endpoint (see refinement point 1)

Table 10.1.2.2-11: Small herbivorous mammal "vole" scenario (common vole): Refined reproductive risk assessment for cereals

Food type[1)]	FIR/ bw[1)]	$PD_{i,fresh}$	PT	RUD [mg a.s./kg]	MAF	Maximum 21-d twa factor	DF	Use rate [kg a.s. /ha]	DDD [mg a.s./kg b.w./d]
Grass	1.536	0.25	1	54.2[1)]	1[3)]	0.2081[3)]	0.1[4)]	0.25	0.108
Non-grass herbs	1.536	0.75	1	28.7[1)]	1[3)]	0.2081[3)]	0.1[4)]	0.25	0.172
DDD_{sum} [mg a.s./kg b.w./d]									0.280
Toxicity endpoint [mg a.s./kg b.w./d]									8.2[5)]
TER_{LT}									29.26

1) According to EFSA/2009/1438
2) RUD in earthworms (PEC_{worm}) based on specific PEC_{soil} (twa, 21 days). See Table 10.1.2.2-15 for details.
3) The maximum 21-d twa factor is calculated based on a pyraclostrobin-specific DT_{50} = 3.03 days and an application interval of 21 days for two applications. Please note that the MAF is set to 1 as this factor is already included in the calculation of the maximum 21-d twa factor.
4) See refinement 4 point for details.
5) Overall ecologically relevant toxicity endpoint (see refinement point 1)

Table 10.1.2.2-12: Small herbivorous mammal "vole" scenario (common vole): Refined reproductive risk assessment for maize at BBCH 30-39

Food type[1)]	FIR/ bw[1)]	$PD_{i,fresh}$	PT	RUD [mg a.s./kg]	MAF	Maximum 21-d twa factor	DF	Use rate [kg a.s. /ha]	DDD [mg a.s./kg b.w./d]
Grass	1.536	0.25	1	54.2[1)]	1[2)]	0.2064[2)]	0.5[3)]	0.2	0.433
Non-grass herbs	1.536	0.75	1	28.7[1)]	1[2)]	0.2064[2)]	0.5[3)]	0.2	0.688
DDD_{sum} [mg a.s./kg b.w./d]									1.121
Toxicity endpoint [mg a.s./kg b.w./d]									8.2[4)]
TER_{LT}									7.32

1) According to EFSA/2009/1438
2) The maximum 21-d twa factor is calculated based on a pyraclostrobin-specific DT_{50} = 3.03 days for one application. Please note that the MAF is set to 1 as this factor is already included in the calculation of the maximum 21-d twa factor.
3) See refinement point 4 for details.
4) Overall ecologically relevant toxicity endpoint (see refinement point 1)

Additional qualitative evidence that pyraclostrobin (applied as BAS 500 06 F) has no adverse effects on free-living wood mouse and common vole populations

The low risk to wood mice from applications of BAS 500 06 F in cereals as presented in the quantitative reproductive risk assessment for the wood mouse above was also confirmed by results from a field effect study on the acute and long-term effects of BAS 500 06 F in cereals on populations of small mammals (BASF DocID 2014/1000041). A detailed summary of the study can be found at the beginning of M-CA 8.1. In brief, live trapping (capture-mark-recapture) was carried out from April to October 2013 to compare the abundance and population dynamics of small mammal species in six treated (2 applications of BAS 500 06 F according to the intended use pattern) and six untreated winter cereal fields and adjacent off-crop areas in Germany. Eight different parameters (captures/individuals in- and off-crop, trapping efficiency, minimum number alive, population growth rate, percentage of reproductively active individuals, percentage of juveniles, percentage of females, and adult body weight) were monitored for the whole growth period of winter cereals. Further, although trapping efficiency of common voles was relatively low in this study, no signs of any potential impacts of BAS 500 06 F on voles were observed.

To confirm the initial findings for the common vole, a second field effect study on the acute and long-term effects of BAS 500 06 F on populations of common voles (BASF DocID 2015/1126803) was conducted. The study was performed under realistic worst-case field conditions in meadows, which are – in contrast to e.g. cereal fields - preferred common vole habitats with high abundances of common voles. Meadows were used as a surrogate for other crop types to ensure sufficient numbers of common voles being exposed to the test item BAS 500 06 F. A detailed summary of the study can be found at the beginning of M-CA 8.1. In brief, live trapping (capture-mark-recapture) was carried out in ten trapping sessions from May to October 2015. The abundance and population dynamics of common voles was compared in five treated (2 applications of BAS 500 06 F according to the critical use pattern) and five untreated study fields in Germany. Seven different parameters (trapping success, minimum number alive (MNA), recapture rate, sex ratio, proportion of reproductively active animals, age structure and body weight development) were monitored for the whole study period, which covered the majority of the reproductive season of common voles. Overall, trapping success of common voles was high: 9161 captures of common voles were made, including a total of 2495 individually marked animals. No adverse acute and long-term effects of the fungicide BAS 500 06 F on common vole populations in meadows were detected in any of the parameters investigated.

In conclusion, the studies showed no impacts of the fungicide BAS 500 06 F under field conditions on the common vole (*Microtus arvalis*) and wood mouse (*Apodemus sylvaticus*).

In addition, all relevant TER_{LT} values for pyraclostrobin exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low and acceptable reproductive risk for wild mammals from the use of BAS 500 06 F in cereals and maize.

Acute oral toxicity of the preparation

A study on the acute oral toxicity of BAS 500 06 F on rats was conducted (BASF DocID 2007/1053390) and resulted in an **LD_{50} of around 500 mg BAS 500 06 F/kg b.w.**). The full study summary is included in M-CP 7.1.1.

Please note, an acute risk assessment for BAS 500 06 F with LD_{50} from formulation toxicity testing is provided further below.

Acute risk assessment considering the LD_{50} from toxicity testing with BAS 500 06 F

Toxicity endpoint of the formulation for mammals

Acute oral toxicity studies of formulations are not specifically designed for use in wild mammal risk assessments, but are conducted for the purpose of classification and labelling and include testing of rates with single doses of the intact formulation administered by oral gavage. The type and pattern of exposure of wild mammals following a foliar spray in the field is very different than the exposure pattern tested in acute oral gavage tests.

Hence, the results of such acute oral toxicity tests are of limited relevance for the acute wild mammal risk assessment. Nevertheless, for a conservative estimate, the endpoint of the acute oral toxicity study with BAS 500 06 F on rats, namely LD_{50} = 500 mg/kg b.w., will be used for a tier 1 acute risk assessment of the formulation. The results of the tier 1 acute dietary exposure assessment and TER calculation will be presented below.

Exposure

BAS 500 06 F is intended to be used in cereals and maize with a maximum single application rate of 1.25 L BAS 500 06 F/ha in cereals and 1.0 L BAS 500 06 F/ha in maize (see Table 10.1.2-5 for the critical use pattern). Taking into account the density of the formulation of 1.04 g/cm^3, this will result in an application rate of 1.3 kg BAS 500 06 F/ha and 1.04 kg BAS 500 06 F/ha for the critical use patterns in cereals and maize, respectively. The relevant scenarios for the critical use pattern are given in Table 10.1.2-6 (cereals) and in Table 10.1.2-7 (maize).

Calculation of the screening and tier 1 risk assessment for BAS 500 06 F according to EFSA/2009/1438

The screening and tier 1 TER values for the acute dietary risk assessment for mammals as obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) are presented for cereals in Table 10.1.2.2-13 and in Table 10.1.2.2-14 for maize.

Table 10.1.2.2-13: BAS 500 06 F: Acute risk assessment for mammals in cereals - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg formulation/ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Cereals	1.3	2	21	10.0	500.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{90}**	**Daily Dietary Dose (multiple)**	**TER**	**Please perform first tier risk assessment (see below)**
	Small herbivorous mammal	118.4	153.92	1.1	169.31	3.0	
Tier 1 Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	
	Cereals Early (shoots)	Large herbivorous mammal “lagomorph” Grass + cereals 100% cereal shoots			42.1	**8.3**	**Higher tier risk assessment required**
	Cereals BBCH 10-29	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			17.2	20.3	**No refinement required**
	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods			5.4	64.8	**No refinement required**
	Cereals BBCH 30 - 39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			8.6	40.7	**No refinement required**
	Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass			40.9	**8.5**	**Higher tier risk assessment required**
	Cereals BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			5.2	67.2	**No refinement required**

Table 10.1.2.2-14: BAS 500 06 F: Acute risk assessment for mammals in maize - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg formulation/ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Maize	1.04	1	365	10.0	500.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF_{90}**	**Daily Dietary Dose (multiple)**	**TER**	**Please perform first tier risk assessment (see below)**
	Small herbivorous mammal	136.4	141.86	1.0	141.86	3.5	
Tier 1 Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	
	Maize BBCH ≥ 20	Small insectivorous mammal “shrew” ground dwelling invertebrates with interception 100% ground arthropods			5.4	89.0	**No refinement required**
	Maize BBCH 30 - 39	Small herbivorous mammal "vole Grass + cereals All maize shoots + later grass			68.2	**7.0**	**Higher tier risk assessment required**
	Maize BBCH 30 - 39	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			8.6	55.9	**No refinement required**
	Maize BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals All maize shoots + later grass			34.1	14.1	**No refinement required**
	Maize BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods			4.3	111.8	**No refinement required**

In conclusion the tier 1 TER_A values for the formulation BAS 500 06 F are below the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects for two scenarios in cereals and one scenario in maize. The scenarios failing the trigger values require refinement of the assessments in a higher tier step. The refined acute risk assessments are presented in the following.

Refined acute dietary risk assessments for the formulation

The following scenarios failed the trigger of 10 in the tier 1 acute risk assessment and will be refined:

Applications to cereals:
Large herbivorous mammal “lagomorph” at early stages
Small herbivorous mammal "vole" at BBCH ≥ 40

Applications to maize:
Small herbivorous mammal "vole" at BBCH 30-39

Refined acute risk assessment for BAS 500 06 F

The refined dietary reproductive risk assessment for mammals is based on the following refinement points:

Refinement point 1: Residue decline of pyraclostrobin in plants for refinement of MAF_{90}

Refinement point 2: Identification of representative mammal species including ecological data (PD values and FIR)

Refinement point 1: Residue decline of pyraclostrobin in plants for refinement of MAF_{90}

Dissipation times of residues

Based on the evaluation and statistical analysis of the submitted monocot (wheat) and dicot (peas) data (BASF DocIDs 2013/1045207, and 2013/1044539), the calculated geomeans were DT_{50} = 1.83 days for the Northern residue zone and DT_{50} = 3.03 days for the Southern zone. Following a worst-case approach for this EU-wide evaluation, the highest of these two values (DT_{50} = 3.03 days) is used for the refinement of the risk assessments of the mammal scenarios. Therefore, the higher tier calculation and the resulting TER value of the large herbivorous mammal "lagomorph" scenario in cereals changed slightly and was also updated. The study details were already introduced in the refined reproductive risk assessment of the active substance. The summaries of the dissipation calculations as well as of the field residue trials are found at the beginning of M-CA 8.1.

The refined MAF_{90} value together with the respective DT_{50} value is presented in Table 10.1.2.2-15.

Table 10.1.2.2-15: Refined multiple application factor (MAF_{90}) for green plant matter based on residue decline data for pyraclostrobin in wheat (monocot.) and pea (dicot.) plants

Plant matrix from field trials	Relevant food item according to EFSA/2009/1438	Interval	No. of applications	Geomean DT_{50} [days]	MAF_{90} [2)]	f_{twa}
Cereal scenario						
Wheat + peas	Green plant material (grasses, cereal shoots)	21	2	3.03 [1)]	1.00	1 [3)]

1) See refinement point 1 for acute risk assessment

2) Relevant Multiple application factor (MAF) for use in acute exposure calculations according to EFSA/2009/1438 is the MAF_{90}

3) Please note, in the acute scenario the time-weighted average factor (f_{twa}) is set to 1 according to EFSA/2009/1438.

Refinement point 2: Identification of representative mammal species including ecological data (PD values and FIR)

For the large herbivorous mammals "lagomorph" scenario the hare (*Lepus europaeus*) is the relevant species for cereals at early growth stages. Please refer to the detailed discussion for the selection of this focal species and relevant ecological data under refinement point 3 for the refined reproductive risk assessment of the active substance.

For the small herbivorous mammals "vole" scenario the common vole (*Microtus arvalis*) is the relevant species for cereals and maize. Please refer to the detailed discussion of the relevant ecological data for the common vole under refinement point 3 for the refined reproductive risk assessment of the active substance.

Refined acute risk assessment for large herbivorous mammal "lagomorph" scenario in cereals

The refined acute risk assessments for large herbivorous mammal scenarios are shown in Table 10.1.2.2-16 for the hare.

Table 10.1.2.2-16: Hare: Refined acute risk assessment for the formulation for applications in cereals

Food type[1)]	FIR/ b.w.	$PD_{i,\ fresh}$	PT	RUD [mg a.s./kg]	MAF_{90}	f_{twa}	DF	Use rate [kg a.s./ha]	DDD [mg a.s./kg b.w./d]
Grass/cereal shoots	0.319[1)]	1.0[1)]	1	102.3[1)]	1.00[2)]	1[3)]	1	1.3	42.37
Toxicity endpoint[mg a.s./kg b.w./d]									500
TER_A									11.80

1) According to EFSA/2009/1438
2) Multiple application factor (MAF_{90}) recalculated based on pyraclostrobin-specific DT_{50} = 3.03 days and an application interval of 21 days for two applications. See refinement point 1 for acute risk assessment for details.
3) Please note, the time-weighted average factor (f_{twa}) is set to 1 in the acute scenario according to EFSA/2009/1438.

In conclusion, the refined TER_A values for BAS 500 06 F exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low and acceptable acute risk for lagomorph mammals from the use of BAS 500 06 F in cereals.

Refined acute risk assessment for small herbivorous mammal "vole" scenarios in cereals and maize

The refined acute risk assessments for the formulation BAS 500 06 F for the small herbivorous mammal scenario “vole” is shown in Table 10.1.2.2-17 for cereals and in Table 10.1.2.2-18 for maize.

Table 10.1.2.2-17: Cereals: Refined acute risk assessment for the formulation for the small herbivorous mammal scenario at BBCH ≥40

Food type[1)]		FIR/b.w.	PD_i, fresh	PT	RUD [mg a.s. /kg]	MAF 90	f twa	DF	Use rate [kg a.s. /ha]	DDD [mg a.s./kg b.w./d]
Grass	25%	1.536[1)]	0.25[1)]	1[2)]	102.3[2)]	1.0[3)]	1[4)]	0.1[5)]	1.3	5.106
Non-grass herbs	75%	1.536[1)]	0.75[1)]	1[2)]	70.3[2)]	1.0[3)]	1[4)]	0.1[5)]	1.3	10.526
DDD_{sum} [mg a.s./kg b.w./d]										15.632
Toxicity endpoint [mg a.s./kg b.w./d]										500
TER_A										31.99

1) See refinement point 2 for acute risk assessment.
2) According to EFSA/2009/1438
3) Multiple application factor (MAF_{90}) recalculated based on pyraclostrobin-specific DT_{50} = 3.03 days and an application interval of 21 days for two applications. See refinement point 1 for acute risk assessment for details.
4) Please note, the time-weighted average factor (f_{twa}) is set to 1 in the acute scenario according to EFSA/2009/1438.
5) DF = 0.1 for BBCH > 40 in cereals according to Guidance for FOCUS Groundwater Scenarios

Table 10.1.2.2-18: Maize: Refined acute risk assessment for the formulation for the small herbivorous mammal scenario at BBCH 30-39

Food type[1)]		FIR/b.w.	$PD_{i,\ fresh}$	PT	RUD [mg a.s./kg]	MAF_{90}	f_{twa}	DF	Use rate [kg a.s./ha]	DDD [mg a.s./kg b.w./d]
Grass	25%	1.536[1)]	0.25[1)]	1[1)]	102.3[1)]	1.0	1[2)]	0.5[3)]	1.04	20.423
Non-grass herbs	75%	1.536[1)]	0.75[1)]	1[1)]	70.3[1)]	1.0	1[2)]	0.5[3)]	1.04	42.105
DDD_{sum} [mg a.s./kg b.w./d]										62.528
Toxicity endpoint [mg a.s./kg b.w./d]										500
TER_A										7.99

1) According to EFSA/2009/1438
2) Please note, the time-weighted average factor (f_{twa}) is set to 1 in the acute scenario according to EFSA/2009/1438.
3) DF = 0.5 for BBCH 30-39 in maize according to Guidance for FOCUS Groundwater Scenarios

In conclusion, the refined TER_A value for BAS 500 06 F for the small herbivorous "vole" scenario in cereals exceeds the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low and acceptable acute risk for voles from the use of BAS 500 06 F in cereals. The refined TER_A value for BAS 500 06 F for the small herbivorous "vole" scenario in maize do not exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. Further higher tier assessment is necessary and will be presented below.

Further higher tier acute risk assessment for small herbivorous mammals in ~~cereals and~~ maize:

To address the potential acute risk and to further support the risk assessments from exposure of wild mammals to the formulation beyond standard risk assessments, the following additional lines of evidence are presented:

i) A full rationale evaluating the acute risk of the formulation to wild mammals under realistic exposure conditions, taking into account data from acute dietary toxicity testing with BAS 500 06 F in rat and wood mouse

ii) A body burden model assessing the acute dietary risk posed by formulated pyraclostrobin (BAS 500 06 F) to the common vole

iii) A field effect study on the acute and long-term effects of BAS 500 06 F on populations of small mammals

iv) A field effect study on the acute and long-term effects of BAS 500 06 F on populations of common voles

The low acute risk of the formulation BAS 500 06 F to small mammals is supported by a detailed rationale evaluating the acute dietary risk of the formulation to wild mammals under realistic exposure conditions, taking into account additional data from dietary toxicity testing with BAS 500 06 F in rat and wood mouse (see BASF DocID 2014/1010735; for summaries of the studies BASF DocIDs 2011/1146588 and 2012/1129348 please refer to M-CA 8.1). In brief, both an exploratory non-GLP laboratory rat study (BASF DocID 2011/1146588) and a GLP laboratory wood mouse study (BASF DocID 2012/1129348) investigated the acute dietary toxicity of the formulated product BAS 500 06 F to mammals under more realistic exposure conditions. To better simulate realistic worst-case field conditions, the study protocols included a restricted daily diet regime ensuring high feeding rates of the test animals and testing of field relevant concentrations of pyraclostrobin. That is, tested concentrations cover the maximum dietary concentrations of pyraclostrobin potentially found in feed items of wild mammals in cereals and maize fields, including also an additional safety factor of 10 and beyond to account for interspecies variation.

The study set-up consequently well covers a possible situation in the field where wild mammals are starved and under pressure to eat quickly, and the results hence are of direct relevance for an estimation of the acute dietary risk under more realistic field conditions. The studies show no apparent significant adverse effects either clinical or behavioral from dietary exposure to environmentally relevant concentrations of BAS 500 06 F. No treatment-related mortalities, symptoms of poisoning, or abnormal behavior were observed in any test group. Further, the results of the studies clearly indicated avoidance of the treated food by the test animals. The observed reduction in food consumption prevented the test animals to ingest lethal doses, possibly using food avoidance as a mechanism to reduce exposure and hence reducing risk. A direct comparison of the toxicity of formulated pyraclostrobin administered via acute oral gavage and via the dietary route further shows no mortality in the dietary studies at dose levels that caused mortality in the acute gavage tests, clearly indicating a lower toxicity of formulated pyraclostrobin when taken up over the diet in comparison to single oral gavage exposure.

A **body burden model** is a mechanistic model that describes the internal content of a chemical in the body over time based on the knowledge about the toxicokinetic properties of that chemical and the foraging behavior of the species of interest. Body-burden models are proposed in EFSA/2009/1438 as potential refinement step for risk assessments at higher tier. Here, a body burden model is presented for the common vole as a representative species of the guild of small herbivorous mammals and the active substance pyraclostrobin applied as a formulated product BAS 500 06 F in arable crops (e.g. cereals or maize) to support in a weight of evidence the low acute risk posed by the intended use of BAS 500 06 F to small mammals. Detailed results are given in the full model report (BASF DocID 2014/1001603). For an executive summary please refer to M-CA 8.1.

In brief, the results of the modeling show that the selected toxicological threshold was not exceeded by any individual in any of the 2000 model runs. For a conservative estimate, the applied toxicological threshold was derived from the acute oral rat laboratory study with the product BAS 500 06 F (BASF DocID 2007/1053390) by dividing the respective LD50 by a safety factor of 10. The latter safety factor was applied in order to cover the uncertainty associated with the interspecies extrapolation. This result suggests that the common voles feeding on grass and cereal shoots in a cereal field during 48 hours after treatment with 1.25 L/ha of BAS 500 06 F (corresponding to the rate of 250 g pyraclostrobin per ha) are highly unlikely to experience acute effects. Due to the high level of realism implemented in the foraging submodel, as well as the direct incorporation of uncertainties about input model parameters into the calculations, the presented risk estimate can be characterized as robust.

Finally, it should also be noted that the low acute risk of the formulation BAS 500 06 F to small mammals is supported by the **two available field-effect studies** (BASF DocID 2014/1000041 and BASF DocID 2015/1126803), which did not show any adverse acute or chronic effects of BAS 500 06 F on free-living wood mouse and common vole populations (for more details see the part ‘refined reproductive risk assessment’ above, and the full summaries in M-CA 8.1).

In conclusion, the low acute risk from the formulation to wild mammals, as predicted from the body burden modelling approach and also indicated from laboratory studies under realistic exposure conditions, are confirmed by results from two long-term field-effect studies, in which no adverse effects on wood mice and common voles occurred. Therefore, considering the outcome of these three independent lines of evidence, it is reasonably concluded that the intended uses of BAS 500 06 F in cereals and maize do not pose any unacceptable acute risk to small mammals.

Effects of secondary poisoning

The log P_{ow} of the active substance pyraclostrobin was determined to be 3.99 (SANCO/1420/2001-final) which triggers an assessment for the potential risk through secondary poisoning according to EFSA/2009/1438.

Risk assessment for earthworm-eating mammals

The risk assessment for earthworm-eating mammals will be based on the worst-case PEC_{soil} (twa, 21 days) derived from M-CP 9.1. The calculations and the resulting TER_{LT} values are summarized in Table 10.1.2.2-19.

Table 10.1.2.2-19: Risk assessment for pyraclostrobin concerning earthworm-eating mammals – dry soil approach

Parameter	Pyraclostrobin	Reference
PEC_{soil} (twa, 21 days)[1] [mg/kg soil]	0.201	M-CP 9.1
K_{ow}	9772	SANCO/1420/2001-final
K_{oc} (arithmetic mean)	9304	SANCO/1420/2001-final
f_{oc} (default)	0.02	EFSA/2009/1438
BCF[2]	0.635	--
PEC_{worm} [mg/kg][3]	0.128	--
Daily dose [mg/kg b.w./d][4]	0.163	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	3	Table 10.1.2-1
TER_{LT}[5]	18.37	--

1) The PEC_{soil} (twa, 21 days) value calculated for applications of BAS 500 06 F in cereals was the highest PEC_{soil} (twa, 21 days) for all crop scenarios. For details see M-CP 9.1
2) Bioconcentration factor (BCF) = $(0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
3) $PEC_{worm} = PEC_{soil} \times BCF$
4) Daily dose = $1.28 \times PEC_{worm}$
5) TER_{LT} = NO(A)EL / Daily dose.

The risk assessment for fish-eating mammals will be based on the worst-case PEC_{sw} (twa, 21 days) derived from M-CP 9.2. The calculations and the resulting TER_{LT} values are summarized in Table 10.1.2.2-20.

Table 10.1.2.2-20: Risk assessment for pyraclostrobin concerning fish-eating mammals (tier 1)

Parameter	Pyraclostrobin	Reference
$PEC_{sw,}$ (twa, 21 days) [mg/L][1)]	$1.552 * 10^{-3}$	M-CP 9.2
BCF fish (max. worst case)	736	SANCO/1420/2001-final
PEC_{fish} [mg/kg][2)]	1.142	--
Daily dose [mg/kg b.w./d][3)]	0.162	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	3.0	Table 10.1.2-1
TER_{LT}[4)]	18.50	--

1) Highest PEC_{sw} (twa, 21 days) from FOCUS Step 2 (South Europe) calculation for a multiple application scenario of BAS 500 06 F in cereals with parameters from dark water/sediment study as worst-case. For details see M-CP 9.2.
2) $PEC_{fish} = PEC_{sw}$ x BCF
3) Daily dose = 0.142 x PEC_{fish}
4) TER_{LT} = NO(A)EL / Daily dose

In the above risk assessments for earthworm- and fish-eating mammals, the TER values exceed the trigger of 5 set by Commission regulation (EU) 546/2011 for acceptability of effects. It can therefore be concluded that the application of BAS 500 06 F in cereal and maize does not provide reason for concern regarding a potential accumulation of the active substance pyraclostrobin in the food chain or for concern of secondary poisoning.

Biomagnification in terrestrial food chains

No evidence was found for potential of accumulation of pyraclostrobin in animal tissue (Review report for the active substance pyraclostrobin. Appendix II, endpoints and related information. September, 2004).

Based on this it can be concluded that the potential for bioaccumulation in animal tissue for pyraclostrobin is low and thus no further assessment on biomagnification is required.

Risk for mammals through drinking water

EFSA/2009/1438 proposes an approach for assessing the risk to mammals from active substances in drinking water using small granivorous mammals as indicator species in tier 1. Out of the two scenarios, i.e. the leaf and the puddle scenario, the leaf scenario is not relevant for small mammals. Consequently, only the risk from the 'puddle scenario' will be considered.

According to EFSA/2009/1438 no specific calculations of exposure and TER values are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg b.w./d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg). The ratio calculations for effective application rate to relevant endpoint are detailed in a screening step in Table 10.1.2.2-21. The ratio for acute and reproductive endpoints for pyraclostrobin (<0.1 and 120.8, respectively) do not exceed the threshold value of 3000 as given by EFSA/2009/1438 for more sorptive substances (Koc > 500 L/kg), thus no specific calculations of exposure for mammals through drinking water for the puddle scenario are necessary.

In conclusion, the risk through drinking water from the use of BAS 500 06 F in cereals and maize is acceptable.

Table 10.1.2.2-21: Screening step for drinking water risk assessment - ratio of effective application rate to relevant endpoint for mammals

Parameter	Pyraclostrobin	Reference
K_{oc} [L/kg]	9304	SANCO/1420/2001-final
DT_{50} (soil) [d]	18	M-CP 9.2
Number of applications	2	Table 10.1.2-5
Interval [d]	21	Table 10.1.2-5
MAF_{mean} [1)]	1.45	--
Max use rate [g/ha]	250 [6)]	Table 10.1.2-5
AR_{eff} [g/ha] [2)]	362.5	--
LD_{50} [mg/kg b.w.]	> 5 000	Table 10.1.2-1
Ratio (acute) [3)]	<0.1	--
Reproductive endpoint [mg/kg b.w./d]	3	Table 10.1.2-1
Ratio (repro) [3)]	120.8	--
Trigger [4)]	3 000	--
Drinking water assessment required [Yes/No] [5)]	No	--

1) $MAF_{mean} = (1-e^{-nki}) / (1-e^{-ki})$ with $k = ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d]. Worst-case from application scenario in cereals
2) AR_{eff} = Application rate (g/ha) x MAF_{mean}
3) Ratio of AR_{eff} and relevant toxicity endpoint
4) Trigger according to EFSA/2009/1438
5) Drinking water risk assessment is not necessary when trigger value is not exceeded.
6) Highest application rate for use in cereals

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Appendix to chapter M-CP 10.1.2.2

Appendix 1: Diet composition (PD) of the wood mouse (*Apodemus sylvaticus*)

The formulation BAS 500 06 F is intended for use in cereals between growth stages BBCH 25 to 69 corresponding to the calendar months April to June. For the refined risk assessment, the diet composition of the wood mouse typical for this time period is considered.

Data are available from a study on wood mouse ecology on arable farmland in the UK (Green 1979) detailing the diet composition of 15 wood mice (determined by stomach content analysis) caught on winter wheat fields between April and June (Table 10.1.2.2-22 to Table 10.1.2.2-24). It is noteworthy that seeds of *Poa annua* and *Stellaria media* formed the bulk of the diet in the wood mouse stomachs examined for the time period April to June.

Further information can be derived from a comprehensive multi-annual study conducted on farmland in Germany (Pelz 1989) (Table 10.1.2.2-22 to Table 10.1.2.2-24). The diet composition of wood mice in the study area, consisting mainly of sugar beet, winter wheat and winter barley fields, over a time period of several years was evaluated by stomach content analysis (n = 136 for the time period April to June). The results were estimated from Figure 3.3 in Pelz (1989) for the purpose of this refined risk assessment.

Compared to other authors who also examined the diet composition of the wood mouse (Watts 1968, Rogers & Gorman 1995 and Gorman & Zubaid 1993), the data published by Green (1979) and Pelz (1989) are considered to be most appropriate due to the clear relation of the diet composition to arable land or even cereal fields.

Mean values for the proportion of a certain food type in the overall diet were calculated on a monthly basis, thus weighing both relevant studies equally. Individual analysis of the studies with subsequent averaging would have therefore yielded identical results. The percentage of **green plant matter** recorded in the diet of wood mice is shown in Table 10.1.2.2-22.

Table 10.1.2.2-22: Percentage of green plant matter in the diet of the wood mouse

Month	Habitat	Feed item	% of diet	Reference
April	Arable farms Rhineland, Germany	Vegetative plant tissue	24	Pelz 1989
May			16	
June			9	
April - June	Winter wheat Suffolk, UK	Leaf and other plant tissue	1	Green 1979
April - June	Ploughed land, previously cereal Suffolk, UK		No data	
Mean proportion of green plant matter for April – June, corresponding to BBCH growth stages 25-69 in cereals[1)]			**8.7**	Pelz 1989 Green 1979

1) From existing mean values for a period of months (e.g. April - June), values for individual months were derived by employing the mean value of the period for each single month.

The percentage of **weed seeds** recorded in the diet of wood mice is shown in Table 10.1.2.2-23.

Table 10.1.2.2-23: Percentage of weed seeds in the diet of the wood mouse

Month	Habitat	Feed item	% of diet	Reference
April	Arable land	Dicot. seeds	0	Pelz 1989
May			4	
June			25	
April - June	Winter wheat	Seeds other than cereals	80	Green 1979
April - June	Ploughed land, previously cereal	Seeds other than cereals	No data	
April - June	Ploughed land, previously sugar beet	Seeds other than cereals	No data	
Mean proportion of weed seeds for April – June, corresponding to BBCH growth stages 25-69 in cereals[1)]			**44.8**	Pelz 1989 Green 1979

1) From existing mean values for a period of months (e.g. April - June), values for individual months were derived by employing the mean value of the period for each single month.

The percentage of **arthropods and earthworms** recorded in the diet of wood mice is shown in Table 10.1.2.2-24.

Table 10.1.2.2-24: Percentage of arthropods and earthworms in the diet of the wood mouse

Month	Habitat	Feed item	% of diet	Reference
April	Arable farms Rhineland, Germany	Insects	45	Pelz 1989
May			10	
June			25	
April	Arable farms Rhineland, Germany	Earthworms	26	Pelz 1989
May			40	
June			9	
April - June	Winter wheat Suffolk, UK	Arthropods	12	Green 1979
April - June	Ploughed land, previously cereal Suffolk, UK		No data	
April - June	Ploughed land, previously sugar beet UK		No data	
April - June	Winter wheat Suffolk, UK	Earthworms	0	
April - June	Ploughed land, previously cereal Suffolk, UK		No data	
April - June	Ploughed land, previously sugar beet UK		No data	
Arable crops mean (insects) for April - June, corresponding to BBCH growth stages 25-69 in cereals [1)]			**15.3**	Pelz 1989 Green 1979
Arable crops mean (earthworms) for April - June, corresponding to BBCH growth stages 25-69 in cereals[1)]			**12.5**	

1) From existing mean values for a period of months (e.g. April - June), values for individual months were derived by employing the mean value of the period for each single month.

The potential exposure of wood mice to the active substance is estimated by employing the mean consumption values for green plant matter, weed seeds, arthropods and earthworms as given in Table 10.1.2.2-22 to Table 10.1.2.2-24. Thus, for the refined exposure assessment the proportions of the different feed items in the diet (PD) would be as follows:

Green plant matter	PD = 0.087	
Weed seeds	PD = 0.448	
Arthropods	PD = 0.153	
Earthworms	PD = 0.125	Sum of individual PD values = 0.813

Individual PD values for food items should sum up to 1 when all possible food types are taken into account. Since food items obviously not being found in cereal fields at BBCH 25 - 69, e.g. cereal grain, were excluded from the above evaluation, the individual PD values needed to be normalised via multiplication with a factor of 1.23 (1.23 = 1 / 0.813). Thus, the proportions of the different feed items in the diet (PD) to be finally used in the refined exposure assessment are as follows:

Green plant matter	**PD = 0.107**	
Weed seeds	**PD = 0.551**	
Arthropods	**PD = 0.187**	
Earthworms	**PD = 0.154**	Sum of individual PD values = 1.0

References to Appendix 1

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Watts, C. H. S. (1968). The foods eaten by wood mice (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*) in Wytham Woods, Berkshire. The Journal of Animal Ecology 37:25-42.

CP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

In a literature review a few references on amphibian studies with pyraclostrobin, respectively pyraclostrobin-containing formulations were identified. Summaries of these publications are provided in M-CA.8.1.4. Please refer to this part of the dossier for details. There are no specific investigations or findings indicating impacts on reptiles.

The following amphibian studies with the European common frog (*Rana temporaria)* and the Common toad (*Bufo bufo*) had been conducted due to the new data requirements to address potential adverse effects on amphibians as indicated in publications. These reports have not been evaluated previously on EU level.

Report: CP 10.1.3/1
[redacted] 2014a
Effects of BAS 500 06 F to juvenile Rana temporaria under worst case laboratory conditions
2013/1375098
Guidelines: none
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effect of BAS 500 06 F on survival of juvenile European common frog (*Rana temporaria)* was investigated over a period of 7 days under worst-case laboratory conditions. The animals were exposed to BAS 500 06 F on a bare soil surface *via* direct overspray at nominal rates of 197.6, 312.5 and 494.1 mL product/ha corresponding to 39.5, 62.5 and 98.8 g a.s./ha (equivalent to 15.8%, 25% and 39.5% of the maximum label rate of BAS 500 06 F) in 10 replicates per treatment containing one metamorph each. In addition, a water control and a blank formulation control was set up. An “up-and-down” testing approach was applied to minimize the use of animals. Animals were maintained on the soil and observed for mortality and sub-lethal effects approx. 1, 2 and 4 hours after application and twice per day from 1 day after treatment (DAT) to DAT 6 and at test termination on DAT 7. Body weight and body length of test organisms were assessed at the start and the end of experiment.

Several individual metamorphs managed to escape from the test containers. Escape was not related to treatment and did not influence the results. No mortalities, abnormal behavior or other sub-lethal effects occurred in the control groups and in the treatment group of 197.6 mL BAS 500 06 F/ha.
In the treatment group of 312.5 mL BAS 500 06 F/ha some transient sub-lethal effects, but no mortalities occurred. Sub-lethal effects were observed shortly after treatment and subsequent recovery of animals occurred within 24 h. No further impact of the test substance was observed afterwards.

In the treatment group of 494.1 mL BAS 500 06 F/ha 70% mortality occurred within one day after treatment. Two further animals were sub-lethally affected; however, both animals had recovered at the first observation on DAT 1 and DAT 2, respectively. Body weight and body length development was not influenced by the test item in all treatments.

The LR_{50} (7 d) in a worst-case laboratory study on *Rana temporaria* was determined to be 470 mL BAS 500 06 F/ha (94 g pyraclostrobin/ha). The LR_0 (7 d) was determined to be 313 mL BAS 500 06 F/ha (63 g a.s./ha).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 0005018890, EC formulated product, content of a.s.: pyraclostrobin: 203.8 g/L (nominal: 200 g/L); density: 1.037 g/cm³.

B. STUDY DESIGN

Test species: European common frog (*Rana temporaria*) metamorphs approx. 2 - 4 weeks old (age after metamorphosis), obtained from in-house culture (non-GLP); mean body weights: 0.35 - 0.48 g; mean body lengths: 16 - 17 mm; egg clutches were collected from a pond in Kaltenbrunnertal, Germany.

Test design: The test organisms were exposed to the test item on a bare soil surface in the laboratory *via* direct overspray using a laboratory sprayer. The test duration was 7 days with a step-wise testing ("up-and-down") procedure of different rates of BAS 500 06 F, plus a water control and a blank formulation control with 10 individuals per treatment (one animal per container): at the first day of treatment (t_0) a water control, a blank formulation control and test item rate of 312.5 mL/ha were tested; at the second day of treatment (t_1) a water control and test item rates of 197.6 and 494.1 mL/ha were tested. To maintain soil moisture during the study, 40 mL of water were added in all test containers on DAT 4 (via injection into a cotton wool underlayment of the soil layer). Mortalities and sub-lethal effects were assessed 1, 2 and 4 hours after application and twice daily from DAT 1 - DAT 6 and once on DAT 7. Body weight and body length of test organisms were measured at the start and end of the experiment.

Endpoints: LR_0, LR_{50}, NOER, mortality, sub-lethal effects (behavior, body length and body weight).

Test rates:	Water control (tap water), blank formulation control (nominal rate of 1250 mL/ha), 197.6, 312.5 and 494.1 mL BAS 500 06 F/ha (P2-t_1), corresponding to 39.5, 62.5 and 98.8 g a.s./ha (*i.e.* 15.8%, 25% and 39.5% of the maximum label rate of BAS 500 06 F (1250 mL/ha)).
Test conditions:	Test containers: Makrolon Type II (22.5 x 16.7 cm x 14 cm; floor area: 375 cm^2), containing a 5 g cotton wool underlayment soaked with 50 mL water and covered by a bare soil layer (LUFA soil 2.3; soil moisture at test start: approx. 60% of the max. water holding capacity); containers were covered with a meshed metal lid for ventilation and to avoid escape of animals; mean temperature: 18.2°C - 21.9°C ; mean relative humidity: 64.1% - 90.7%; mean light intensity: 1282 - 1568 lux, moisture content of the soil: 42% - 70% of maximum water holding capacity; feeding: flightless *Drosophila*, aphids and crickets from DAT 1 to DAT 7 ad libitum.
Analytics:	Analytical verification of test item concentrations in all spray solutions was conducted using an HPLC-method with MS-detection.
Statistics:	Descriptive statistics; Fisher`s exact binomial test with Bonferroni correction (α = 0.05) for determination of NOER; probit analysis using linear maximum likelihood regression for determination of the LR_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in the spray solutions. Mean measured concentrations of pyraclostrobin were between 83.4% and 103.9% of nominal confirming the correct application of the intended rates. No (or only negligible amount of) test item was detected in the spray liquid of the controls.

Biological results: A few individual metamorphs managed to escape from the containers. Escape was not related to treatment and did not influence the results. All observed effects and also recovery of animals occurred within the first 24 h and no further impact of the test substance was observed afterwards. For evaluation of the data set, it was assumed that escaped animals were vital / unaffected (only vital animals could manage to climb the vessel walls and squeeze underneath the container lid). No mortalities or sub-lethal effects occurred in the water control, blank formulation control group and in the lowest treatment group of 197.6 mL BAS 500 06 F/ha.
In the treatment group of 312.5 mL BAS 500 06 F/ha no mortalities occurred. Transient sub-lethal effects were observed within the first 4 hours after application. Affected animals were lethargic and in two cases lying immobile on the soil surface. All affected animals had completely recovered on DAT 1 showing no visual damage, abnormal behavior or impact on biomass development until the end of the exposure phase.
In the treatment group of 494.1 mL BAS 500 06 F/ha, 70% mortality occurred within one day after treatment. Two further animals were transiently sub-lethally affected (*i.e.* lethargy, animals lying flat / immobile on the soil). Both animals had completely recovered at the first observation on DAT 1 and DAT 2, respectively.

Body weight and body length development was not influenced by the test item in all treatments after seven days. The results are summarized in Table 10.1.3-1.

Table 10.1.3-1: Effects of BAS 500 06 F on European common frog (*Rana temporaria*) in a worst-case laboratory trial

Test item rate [mL BAS 500 06 F/ha] (% of max. label rate)	Water control (t_0)	Water control (t_1)	Blank formulation control	197.6 (15.8%)	312.5 (25.0%)	494.1 (39.5%)
Test item rate [g a.s./ha]	--	--	--	39.5	62.5	98.8
Mortality [%] (7 d) [1)]	0	0	0	0	0	70 [4)] *
Mean weight gain [2)] (over 7 d) [g]	0.06	0.00	0.07	0.00	0.06	0.09
Mean length gain [2)] (over 7 d) [mm]	2	0	1	1	1	1
Sub-lethal effects [3)]	none	none	none	none	L, G, I, SL [#], *	P, L, G, I, SL [##], *

Endpoints		
	[mL BAS 500 06 F/ha]	[g a.s./ha]
LR_{50} (7 d) [5)]	470	94
LR_0 (7 d)	313	63
$NOER_{overall}$ (7 d)	198	40

1) Statistical evaluation was done for results after 7 days, assuming survival of the escaped test organisms.
2) Escaped and dead animals were excluded for calculation of mean values.
3) Sublethal effects: G = animal lying flat on the ground; L = lethargy; I = animal immobile on the ground; P = animal in a slightly ducked position; SL = slight lethargy.
4) All of the seven animals died on DAT 1.
5) 95% confidence limits could not be calculated.
* Statistically significant difference compared to the control (Fisher`s Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$).
\# Sub-lethal effects were only observed on DAT 0.
\## Sub-lethal effects were only observed on DAT 0 and DAT 1.

III. CONCLUSION

The LR_{50} (7 d) in a worst-case laboratory study on *Rana temporaria* was determined to be 470 mL BAS 500 06 F/ha (94 g pyraclostrobin/ha). The LR_0 (7 d) was determined to be 313 mL BAS 500 06 F/ha (63 g a.s./ha).

Report:	CP 10.1.3/2 [redacted] 2015 a Effects of residues of BAS 500 06 F on soil to juvenile *Bufo bufo* under laboratory conditions 2014/1221859
Guidelines:	<none>
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effects of soil residues of BAS 500 06 F on survival of juvenile common toad (*Bufo bufo*) were investigated over 7 days under laboratory conditions in 10 replicates per treatment group containing one toad each. The test units (containers with a bare soil layer) were sprayed at nominal rates of 625, 884 and 1250 mL BAS 500 06 F/ha, equivalent to 50%, 70.7% and 100% of the max. label rate. Measured application rates were 775, 1096 and 1550 mL BAS 500 06 F (mean recovery 124% of nominal) corresponding to 155, 219 and 310 g pyraclostrobin/ha. A water control was tested in parallel.
The toads were introduced into the test units immediately after drying of the spray film (approx. 15 min after spraying) and at 1550 mL/ha in addition 1 and 4 hours after application. Mortalities and sub-lethal effects were assessed 1, 2 and 4 hours after introduction of animals, one day after treatment (DAT 1) and daily from DAT 2 - DAT 7. Body weight was measured at test initiation and termination.

The biological results are based on measured application rates. No mortalities or sub-lethal effects occurred in the control groups and at 775 mL BAS 500 06 F/ha. No mortality occurred at 1096 mL BAS 500 06 F/ha, however, transient sub-lethal effects (slight lethargy and immobility) were observed in two toads on DAT 0 with recovery within one day.
At the treatment rate of 1550 mL BAS 500 06 F/ha and exposure of animals shortly after application, 6 toads showed sub-lethal effects on DAT 0, four of them recovered until DAT 1, two died. Two of the toads introduced 1 h after application died at 1550 mL/ha. No treatment related mortality or sub-lethal effects occurred at 1550 mL/ha with animals introduced 4 hours after application. Body weight development was not influenced by the test item.

The LR_{50} (7 d) was determined to be > 1550 mL BAS 500 06 F/ha (> 310 g pyraclostrobin/ha) based on measured application rates for test organisms introduced shortly after as well as 4 hours after application.

The NOEL (7 d) was 775 mL BAS 500 06 F/ha (155 g pyraclostrobin/ha) for test organisms introduced shortly after application and ≥ 1550 mL BAS 500 06 F/ha (> 310 g pyraclostrobin/ha) for test organisms introduced 4 hours after application.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 500 06 F, batch no. 0005018890, content of a.s.: pyraclostrobin: 203.8 g/L (nominal: 200 g/L); density: 1.037 g/cm³

B. STUDY DESIGN

Test species:	Common toad (*Bufo bufo*), metamorphs approx. 3 - 5 weeks old (age after metamorphosis), obtained from in-house culture (non-GLP), body weights: 0.77 - 0.93 g, body lengths: 21 - 23 mm, strings of eggs were collected from two ponds in Kaltenbrunnertal and Gommersheim, Germany.
Test design:	The test container with a bare soil layer were sprayed with the test item in a step-wise (up-to-down) approach to minimize the use of animals. At all test rates, one group of animals was introduced into the test containers 15 min after application. In the 1250 mL BAS 500 06 F/ha treatment two additional groups of animals were introduced 1 and 4 hours after application to take into account a more thorough desiccation of the test item. The test duration was 7 days with different rates of BAS 500 06 F, plus a water control with 10 individuals per treatment (one animal per container). The test trials started on two different days (t_0 and t_1). To maintain soil moisture during the study, 40 mL of water were added in all test containers on DAT 3 and DAT 5 via injection into a cotton wool underlayment of the soil layer. Mortalities and sub-lethal effects were assessed 1, 2 and 4 hours after introduction of animals. Observations were done twice on DAT 1 and daily from DAT 2 - DAT 7. Body weight and body length (snout to vent) of test organisms were measured at the start and end of the experiment.
Endpoints:	LR_{50}, NOEL, mortality, sub-lethal effects (behavior, body length and body weight)
Test rates:	Water control (tap water) as well as 625, 884 and 1250 mL BAS 500 06 F/ha, equivalent to 50%, 70.7% and 100% of the maximum label rate; based on measured concentrations actual rates of 775, 1096 and 1550 mL BAS 500 06 F were applied (mean recovery 124% of nominal) corresponding to 155, 219 and 310 g pyraclostrobin/ha, respectively.

Test conditions:	Test containers: Makrolon Type II (22.5 cm x 16.7 cm x 14 cm; floor area: 375 cm^2), containing a 5 g cotton wool underlayment soaked with 50 mL water and covered with a standard filter paper before introduction of the bare soil layer (LUFA soil 2.3; soil moisture at test start: approx. 45% of the max. water holding capacity); containers were covered with a meshed metal lid for ventilation and to avoid escape of animals; temperature: 18.8°C - 24.3°C; relative humidity: 57.0% - 79.0%;; light intensity: 3.0 - 5.9 klux; light-dark cycle of 16:8 h; moisture content of soil: 35 - 62% of maximum water holding capacity; feeding: flightless *Drosophila* from DAT 1 to DAT 6 ad libitum.
Analytics:	Analytical verification of test item concentrations was conducted in all spray solutions using an HPLC-method with MS-detection. The actual applied amount of test item was measured taking samples during spraying.
Statistics:	Descriptive statistics; Fisher`s exact binomial test with Bonferroni correction ($\alpha = 0.05$) for determination of NOEL; one-way ANOVA for statistical evaluation of body weight data

II. RESULTS AND DISCUSSION

Analytical measurements: Spray solution samples for analytical verification of test item concentrations were taken on each day of treatment. Mean recovery values were between 101 - 105% of nominal. Mean measured concentrations of the applied amount of pyraclostrobin (samples taken during spraying) were between 121 and 125% of nominal. The biological results are based on measured application rates.

Biological results: No mortalities, abnormal behavior or other sub-lethal effects occurred in the control groups and in the 775 mL BAS 500 06 F/ha treatment. After 7 days of exposure 0% mortality occurred in the 1096 mL BAS 500 06 F/ha treatment. Transient sub-lethal effects (slight lethargy and immobility) were observed in two toads at the treatment rate of 1096 mL BAS 500 06 F/ha on DAT 0. On the next day both animals were appearing normal. At 1550 mL BAS 500 06 F/ha and exposure of animals 15 min after application, 6 toads showed sub-lethal effects on DAT 0, four of them recovered until DAT 1, two died. If toads were exposed to 1550 mL BAS 500 06 F/ha 60 min after application, sub-lethal effects were observed at 4 individuals on DAT 0, whereas 2 animals recovered on the next day, the other two died. No treatment related mortality and no sub-lethal effects occurred in the 1550 mL/ha treatment group with animals introduced 4 hours after application. Two mortalities occurred, which are not considered treatment related. One animal was observed to be in a poor condition, lying on the ground with reduced reaction to stimulus on DAT 4 and the animal was found dead on DAT 5. One further animal died due to accidental desiccation of the soil.
Body weight development was not influenced by the test item. The biological results are summarized in the table below.

Table 10.1.3-2: Effects of residues of BAS 500 06 F on common toad (*Bufo bufo*) introduced on pre-sprayed bare soil in a laboratory study

Measured application rate [mL BAS 500 06 F/ha]	**Water control**	**Water control**	**775**	**1096**	**1550**	**1550**	**1550**
Test item rate [g a.s./ha]	--	--	155	219	310	310	310
Day of treatment[#]	t_0	t_1	t_0	t_1	t_0	t_0	t_1
Time after application when exposure of toads started	15 min	15 min	15 min	15 min	15 min	1 h	4 h
Mortality [%] (7 d)	0	0	0	0	20	20	(20) [1)]
Mean weight gain [2)] (over 7 d) [g]	0.12	0.11	0.02	0.11	0.00	0.11	-0.01
Sub-lethal effects [%] [3)]	none	none	none	20 (L, I) [4)]	60 (L, I) [5)]	40 (L, I) [4)]	none

Endpoints (based on measured application rates)			
	Time after application	[mL BAS 500 06 F/ha]	[g a.s./ha]
LR_{50} (7 d)	15 min	> 1550	> 310
NOEL (7 d)	15 min	775	155
LR_{50} (7 d)	4 h	> 1550	> 310
NOEL (7 d)	4 h	≥ 1550	≥ 310

[#] Time of treatment of the step-wise testing approach (t_0 = first day of treatment; t_1 = second day of treatment)
[1)] Mortality considered not to be test item related.
[2)] Dead animals were excluded for calculation of mean values.
[3)] Sub-lethal effects: I = animal immobile on the ground; L = lethargy
[4)] Sub-lethal effects were observed on DAT 0.
[5)] Sub-lethal effects were observed on DAT 0 and DAT 1.

III. CONCLUSION

The LR_{50} (7 d) was determined to be > 1550 mL BAS 500 06 F/ha (> 310 g pyraclostrobin/ha) based on measured application rates for test organisms introduced shortly after as well as 4 hours after application.

The NOEL (7 d) was 775 mL BAS 500 06 F/ha (155 g pyraclostrobin/ha) for test organisms introduced shortly after application and ≥ 1550 mL BAS 500 06 F/ha (> 310 g pyraclostrobin/ha) for test organisms introduced 4 hours after application.

Report: CP 10.1.3/3
[redacted] 2014b
Effects of BAS 500 06 F to juvenile Rana temporaria in winter wheat (semi-field trial)
2013/1375099
Guidelines: none
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effect of BAS 500 06 F on survival of juvenile European common frog (*Rana temporaria)* was investigated over a period of 7 days under realistic conditions in a semi-field trial. The animals were exposed in fenced enclosures in a winter cereal field at growth stage BBCH 25 for 7 days. BAS 500 06 F was applied *via* spray application at nominal rates of 0 (water control), 937.5, 1250.0 and 1562.5 mL product/ha corresponding to 187.5, 250.0 and 312.5 g a.s./ha (equivalent to 75, 100 and 125% of the maximum label rate of BAS 500 06 F). For each test item rate and the water control, 4 replicates with 4 individuals per replicate were used. Animals were observed for mortality and sublethal effects 2 and 4 hours after application, 1 day after treatment (DAT) and on DAT 2, DAT 5 and DAT 7. Body weight and body length of test organisms were assessed at the start (DAT -1) and the end of the experiment (DAT 7).

No mortalities occurred in the control and in all test item treatment groups. No abnormal behavior or sub-lethal effects were observed in the control replicates and the BAS 500 06 F treatment rates of 937.5 mL/ha (75% field rate) and 1250.0 mL/ha (max. field rate). In the treatment group of 1562.5 mL BAS 500 06 F/ha (125% field rate) a single animal was observed showing uncoordinated movement and lethargy during the observations 2 and 4 hours after application on DAT 0. The animal had recovered on DAT 1 showing no visual damage or abnormal behavior until the end of the exposure phase. No other treatment related impact was observed. Mean body weight and mean length development were not influenced by the test item.

The NOER (7 d) for BAS 500 06 F obtained in the study on *Rana temporaria* under semi-field conditions was determined to be ≥ 1563 mL BAS 500 06 F/ha (≥ 312.5 g a.s./ha; 125% of the max. label rate of BAS 500 06 F).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 0005018890, EC formulated product, content of a.s.: pyraclostrobin: 203.8 g/L (nominal: 200 g/L); density: 1.037 g/cm³.

B. STUDY DESIGN

Test species: European common frog (*Rana temporaria*), juveniles approx. 4 months old (*i.e.* after metamorphosis) at test start, obtained from in-house culture (non-GLP); mean body weights: 2.3 - 2.6 g and mean body lengths: 29 - 30 mm at DAT -1; egg clutches were collected from a pond in Kaltenbrunnertal, Germany.

Test design: The test organisms were exposed in fenced enclosures in a winter cereal field (BBCH 25) at BASF Agrarzentrum Limburgerhof, Germany for 7 days. For each rate of BAS 500 06 F and the water control, 4 replicate enclosures with 4 individuals per replicate were used. The animals were introduced into the enclosures one day prior to spray application. Mortalities and sub-lethal effects were assessed 2 and 4 hours after application and on DAT 1, 2, 5 and DAT 7 (observations were done only visually on DAT 0 and DAT 5, detailed observations by means of short removal of animals from enclosures on DAT 1, DAT 2 and DAT 7). Body weight and body length of test organisms were assessed at the start (DAT -1) and end of the experiment (DAT 7).

Endpoints: NOER, mortality, sub-lethal effects (the behavior, body length and body weight).

Test concentrations: Water control (tap water), 937.5, 1250.0 and 1562.5 mL BAS 500 06 F/ha with a water volume of 200 L/ha; corresponding to 187.5, 250.0 and 312.5 g a.s./ha (*i.e.* 75%, 100% and 125% of the maximum label rate of BAS 500 06 F).

Test conditions: Fenced enclosures: height: 0.5 m above ground, buried into the ground at approx. 0.1 m depth, covered with a fine-meshed lid; study plots: approx. 1.2 m x 0.84 m covering about 6 cereal rows (standard sowing distance between rows of approx. 14 cm); temperature: 5.7 - 23.1°C (mean daily temperature: 11.1 - 16.6°C); precipitation: 8.1 mm on DAT -1, < 1 mm on DAT 0 and DAT 3, 7.9 mm on DAT 4 and 2.9 mm on DAT 7; relative humidity 69.4% and wind speed ≤ 2.5 m/s at time of application; animals were provided with additional feed (*Drosophila* and mealworms) on DAT 2.

Analytics: Analytical verification of test item concentrations in all spray solutions was conducted using an HPLC-method with MS-detection. The applied amount of test item in the field was analytically verified using petri dishes, which were placed at the targeted height of the application and on the ground between the cereal rows.

Statistics: Descriptive statistics; Fisher`s exact binomial test with Bonferroni correction ($\alpha = 0.05$) for determination of the NOER.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in spray solutions was conducted in all spray solutions. Mean measured concentrations of pyraclostrobin in the spray solutions of the test item treatments were between 104.7% and 105.5% of nominal confirming the correct application of the intended rates. No test item was detected in the spray liquid of the control. In the petri dishes placed on the target height in the field, mean recovery values of 89% - 101% were measured and therewith confirming the intended application rates. In the petri dishes placed on the ground, mean recovery ranged from 24% (based on nominal values) respectively 27% (based on target height recovery values) to 32%, indicating approximately 70% interception by the crop.

Biological results: No mortalities occurred in the control and in all test item treatment groups. No abnormal behavior or sub-lethal effects were observed in the control replicates and the BAS 500 06 F treatment rates of 937.5 mL/ha (75% field rate) and 1250.0 mL/ha (max. field rate). In the treatment group of 1562.5 mL BAS 500 06 F/ha (125% field rate) a single animal was observed showing uncoordinated movement and lethargy during the observations 2 and 4 hours after application on DAT 0. The animal had recovered on DAT 1 showing no visual damage or abnormal behavior until the end of the exposure phase. No other treatment-related impact was observed. Mean body weight and mean length development were not influenced by the test item. The results are summarized in Table 10.1.3-2.

Table 10.1.3-3: Effects of BAS 500 06 F on European common frog (*Rana temporaria*) in a semi-field study

Test item rate [mL BAS 500 06 F/ha] (% of max. label rate)	**Water control**	**937.5 (75%)**	**1250.0 (100%)**	**1562.5 (125%)**
Test item rate [g a.s./ha]	**--**	**187.5**	**250.0**	**312.5**
Mortality [%]	0	0	0	0
Mean weight gain (DAT 7 - DAT -1) [g]	0.3	0.4	0.3	0.3
Sub-lethal effects				U, L [#]
	Endpoint			
	[mL BAS 500 06 F/ha]		**[g a.s./ha]**	
NOER (7 days)	≥ 1562.5		≥ 312.5	

[#] Sublethal effects (U = uncoordinated movements; L = lethargy) were only observed for a single animal during the observations 2 and 4 hours after application on DAT 0; the animal had recovered on DAT 1 showing no visual damage or abnormal behavior until the end of the exposure phase.

III. CONCLUSION

The NOER (7 d) for BAS 500 06 F obtained in the study on *Rana temporaria* under semi-field conditions was determined to be ≥ 1563 mL BAS 500 06 F/ha (≥ 312.5 g a.s./ha; 125% of the max. label rate of BAS 500 06 F).

Report:	CP 10.1.3/4 [redacted] 2016 a Effects of BAS 500 06 F to juvenile *Bufo bufo* in winter wheat (semi-field trial) 2015/1240163
Guidelines:	<none>
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The effect of BAS 500 06 F on survival of juvenile common toad (*Bufo bufo*) was investigated over 7 days in a semi-field trial. The animals were exposed in enclosures in a winter wheat field at growth stage BBCH 30. BAS 500 06 F was sprayed at nominal rates of 0 (water control), 1250, 1875 and 12500 mL BAS 500 06 F/ha, corresponding to 250 (max. label rate), 375 and 2500 g pyraclostrobin/ha (10x label rate), respectively. The control and the two lower treatments consisted of 4 replicates with 4 individuals each. For the 12500 mL/ha treatment one replicate with 4 organisms was used to minimize animal sacrifices. The test organisms were observed for mortality and sub-lethal effects 2 hours after application and on DAT (days after treatment) 1, 3 and 7. Body weight and body length were assessed at the test initiation and termination.

The biological results are based on nominal application rates. No mortalities and no sub-lethal effects occurred in the control and in the treatment groups up to and including 1875 mL BAS 500 06 F/ha. At the highest treatment rate of 12500 mL BAS 500 06 F/ha 50% mortality was observed during the exposure period. One additional animal was sub-lethally affected at the end of the experimental phase and therefore euthanized. Mean body weight development was not affected up to 1875 mL BAS 500 06 F/ha.

The NOEL (7 d) for BAS 500 06 F in the semi-field study on juvenile common toads (*Bufo bufo*) was determined to be 1875 mL/ha, corresponding to 375 g pyraclostrobin/ha (150% of the max. label rate of BAS 500 06 F).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 500 06 F, batch no. FD-150202-0013, content of a.s.: pyraclostrobin: 200.3 g/L (nominal: 200 g/L); density: 1.033 g/cm³

B. STUDY DESIGN

Test species:	Common toad (*Bufo bufo*), juveniles approx. 3 - 4 months old (*i.e.* after metamorphosis), obtained from in-house culture (non-GLP), body weights: 2.0 - 2.4 g, body lengths: 28 - 30 mm at DAT -1, egg strings were collected from a pond in Kaltenbrunnertal, Germany.
Test design:	The test organisms were exposed in fenced enclosures in a winter wheat field (BBCH 30) located in Limburgerhof, Germany for 7 days. The control and the treatments of 1250 and 1875 mL BAS 500 06 F/ha consisted of 4 replicates with 4 individuals each. For the 12500 mL BAS 500 06 F/ha treatment only one replicate with 4 organisms was used in order to minimize animal sacrifices. Testing was done "step-wise" with application on two different days (t_0 and t_1). The water control group was maintained in parallel to the second treatment and therewith observed for overall 9 days. Toads were introduced into each enclosure one day prior to application. Due to dry weather conditions, plots were watered by hand with an amount corresponding to about 10 mm of rainfall. The test organisms were observed for mortality and sub-lethal effects 2 hours after application and on day after treatment (DAT) 1, 3 and 7. The toads exposed to a treatment rate of 12500 mL BAS 500 06 F/ha were observed additionally on DAT 5. Body weight and body length were assessed at start (DAT -1) and at test termination (DAT 7).
Endpoints:	NOEL based on mortality and sub-lethal effects
Test concentrations:	0 (water control), 1250, 1875 and 12500 mL BAS 500 06 F/ha, corresponding to 250 (max. label rate), 375 and 2500 g a.s./ha, respectively.
Test conditions:	Fenced enclosures: height: 0.5 m above ground, buried into the ground at approx. 0.1 m depth, covered with a fine-meshed lid; study plots: approx. 1.2 m x 0.84 m covering about 6 cereal rows (standard sowing distance between rows of approx. 14 cm); for application a standard sprayer representing commercial application of the test item according to Good Agricultural Practice was used ("Spritzrad"); mean daily temperature: 11.2 - 16.5°C (min.: 4.1°C, max.: 22.7°C); relative humidity 38.7 - 42.4% at time of application, wind speed 0.2 - 3.5 m/s at time of application; animals were provided with additional feed (*Drosophila* and mealworms) on DAT 3.
Analytics:	Analytical verification of test item concentrations was conducted in all spray solutions using an HPLC-method with MS-detection. The applied amount of test item in the field was analytically verified using 'Speedisks' (Octadecyl, C18; CAS no.: 71889-02-6) for sampling, which were placed at the targeted height of the application (i.e. at about plant height) and on the ground underneath the plant canopy.

Statistics: Descriptive statistics; one-way ANOVA for statistical evaluation of body weight data

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical recovery values of the spray solutions were between 99 - 107% of nominal. No test item was detected in the spray liquid of the water control. Mean recovery values of 92 - 150% of nominal were measured in the field on the targeted height roughly confirming the intended application rates. On the ground, individual recovery values ranged from 13 - 43% (based on nominal application rates), indicating high variation of the amount of test item reaching the ground and corresponding to approximately 60 - 90% interception by the crop. The biological results are based on nominal application rates.

Biological results: No mortalities and sub-lethal effects occurred in the control and in the treatment groups up to and including 1875 mL BAS 500 06 F/ha. At the highest treatment rate 50% mortality was observed during the exposure period. One additional animal was sub-lethally affected at the end of the experimental phase and therefore euthanized. Mean body weight development was not affected. The results are summarized in the table below.

Table 10.1.3-4 Effects of BAS 500 06 F on common toad (*Bufo bufo*) in a semi-field study

Test item rate [mL BAS 500 06 F/ha] (% of max. label rate)	**Water control**	**1250 (100%)**	**1875 (150%)**	**12500 (1000%)**
Test item rate [g a.s./ha]	--	250	375	2500
Mortality [%]	0	0	0	50[1)]
Mean weight gain (over 7 d) [g]	0.34	0.31	0.32	--[2)]
Sub-lethal effects	none	none	none	U[3)]
Endpoints (based on nominal application rates)				
	[mL BAS 500 06 F/ha]		**[g a.s./ha]**	
NOEL (7 days)	1875		375	

1) One additional animal was sub-lethally affected at the end of the exposure phase and therefore euthanized.
2) Mean weight gain cannot be calculated due to mortalities
3) Sub-lethal effects: U = uncoordinated movements, observed on DAT 7

III. CONCLUSION

The NOEL (7 d) for BAS 500 06 F in the semi-field study on juvenile common toads (*Bufo bufo*) was determined to be 1875 mL/ha, corresponding to 375 g pyraclostrobin/ha (150% of the max. label rate of BAS 500 06 F), based on nominal application rates.

Risk assessment - other terrestrial vertebrate wildlife: reptiles and amphibians

The laboratory studies conducted under worst-case conditions, using sensitive life stages (metamorphs) and maximizing exposure via direct overspray over animals and bare soil (Belden et al., 2010; Bruehl et al., 2013; BASF DocID 2013/1375098), indicate inherent toxicity of pyraclostrobin, respectively pyraclostrobin containing formulations to amphibians. The results from the two investigations by Belden et al. (2010) and Bruehl et al. (2013) (non-GLP studies, for more details see M-CA 8.1.4) on *Rana temporaria* and *Bufo cognatus* show no impact of the test substance at 22 g a.s./ha, whereas 220 g a.s./ha (reflecting the field rate) caused high mortality (100 and 70% at 220 g a.s./ha for *Rana temporaria* and Bufo cognatus, respectively). The BASF laboratory study with *Rana temporaria* provides comparable results while narrowing down the toxicity range. Results are shown in Table 10.1.3-5.
The above-mentioned laboratory studies show that young, juvenile frogs and toads can be sensitive if oversprayed directly with EC formulated pyraclostrobin (containing organic solvents). BASF conducted additionally a study (BASF DocID 2014/1221859) investigating the impact of the same product when not overspraying the toads directly, but exposing them to bare soil that has been oversprayed before. The toxicity to animals exposed to the soil residues shortly after spraying was significantly reduced as compared to direct overspray, and following a time interval of about four hours between spraying and animals moving onto the contaminated soil, no effects on the toads were observed even at elevated rates of 310 g a.s./ha.

The studies with the EC-formulation (emulsifiable concentrate) indicate toxicity at the field rate while the study with a CS-formulation (capsule suspension) shows no relevant toxicity even at an elevated rate. The EC-formulation containing significant amounts of organic solvents maximizes full and rapid bioavailability of the active substance while the alternative formulation with significantly less organic solvents leads to a slow release of the active substance.

Table 10.1.3-5: List of endpoints obtained in studies with amphibians

Study	Test species	Test substance	Formulation type	Appl. rate [g a.s./ha]	Effect (% mortality)
Belden et al. (2010)	*Bufo cognatus*	BAS 500 00 F (Headline®)	EC (emulsifiable concentrate)	22	0
				220	70
				2200	100
Bruehl et al. (2013)	*Rana temporaria*	BAS 500 00 F (Headline®)	EC	22	0
				220	100
		BAS 500 18 F	CS (capsule suspension)	2200	no significant effect
BASF DocID 2013/1375098	*Rana temporaria*	BAS 500 06 F	EC	40	0
				63	0 sublethal symptoms
				99	70
BASF DocID 2014/1375098	*Bufo bufo*	BAS 500 06 F	EC	155 / 15 min 219 / 15 min 310 / 15 min 310 / 60 min 310 / 4 h[1)]	0 0 (20% sublethal) 20 (60% sublethal) 20 (40% sublethal) (20)[2)] (0% sublethal)

[1)] Animals were exposed to the respective rates either shortly (within 15 minutes) or 1 h or 4 h after spraying.
[2)] The observed toxicity is in this case not considered treatment-related.

In previous chapters it has been demonstrated that there is a low risk of pyraclostrobin to bird and mammal species. Yet, pyraclostrobin and certain pyraclostrobin containing formulations can be toxic to amphibians. The particular sensitivity of amphibians is most likely due to their specific skin morphological and physiological differences (dermal toxicity). The amphibian skin not only constitutes a much lesser barrier as compared to the skin of mammals and birds (Quaranta et al., 2009), however, it is itself a sensitive organ relevant for respiration and uptake of water and regulation of minerals in amphibians (see Wells, 2007 for a review). It seems likely that pyraclostrobin, in case sufficiently large surfaces of the skin are exposed, impacts mitochondrial respiration in the skin and thus disturbs a vital process for respiration and function of the skin. However, if the vital function is not sufficiently (largely) disturbed, the substance being rapidly metabolized and excreted (compare M-CA 5.1 and M-CA 6.2) will not cause any further sub-lethal or chronic latency effects. This is in agreement with the observed finding: effects are observed rather rapidly and if there are no rapid effects or once recovery from initial effects occurs, then there is no delayed or long-term toxicity.

The laboratory studies indicate the potential intrinsic toxicity of pyraclostrobin. However, conditions as simulated in these worst-case laboratory studies bear little relevance to field situations.

In general, amphibians need both terrestrial and aquatic habitats. In Europe, most amphibian species depend on aquatic breeding sites and use the terrestrial habitat within 300 - 1000 m of the surrounding area (e.g. Semlitsch and Bodie 2003, Schabetsberger et al. 2004, Sinsch et al. 2012, Berger et al. 2011). Agricultural landscapes, too, may constitute habitats used by amphibians, if these are in the range of the aquatic breeding sites (summary in Berger et al. 2011).
However, agricultural landscapes are generally perceived as low quality environments for most amphibian species for several reasons, posing high resistance to movement for aquatic breeding amphibians, which is corroborated by studies showing lower abundance and association of amphibians with arable land (Pagetti et al. 2006, e.g. *Bufo bufo*: Kyek et al. 1997, Piha et al. 2007; *Epidalea calamita*: Miaud and Sanuy 2005; *Triturus carnifex, T. marmoratus*: Jehle and Arntzen 2000). In particular, agricultural fields with no or low plant cover are avoided since amphibians have to face high water losses in anthropogenically disturbed areas without sufficient plant cover (Rothermel and Semlitsch 2002, Mazerolle and Desrochers 2005, Cosentino et al. 2011). The risk of desiccation and the lack of cover against predators are likely the major factors leading to avoidance of amphibians of such areas. Mazerolle and Vos (2006) observed that *Rana esculenta* opted to take migration routes with higher cover rather than taking shortcuts through bare field. Similar behaviour was reported by Berger et al. (2011) during telemetry and mark-and recapture studies with *Bufo bufo, Triturus cristatus* and *Pelobates fuscus* in agricultural fields in Germany: individuals migrated either fast through the field taking the shortest route, and subsequently stayed in off-crop habitats, which offer more plant cover and structure; or they returned into cover offered by the pond's surrounding. Individuals of *B. bufo* continued to stay in the arable field when favourable conditions, i.e. burrows, stubble field with clods and sufficient plant material, were found (see also Kneitz 1998). Thus, habitat use and behaviour of amphibians in agricultural landscapes highly depend on availability of cover in the respective area (Berger et al. 2011).

The dependence on a moist and the preference for a structured environment with high plant cover is especially crucial for juvenile amphibians. Due to their smaller size and volume to surface ratio, juvenile amphibians are more sensitive to environmental influences, especially desiccation, than adult life stages (Rittenhouse et al. 2008). Accordingly, Vos et al. (2007) found an even more pronounced preference for meadows in contrast to arable land in *Rana temporaria* juveniles than in adults (individuals found in meadows: arable land; 9.6:1 (juveniles), 4.3:1 (adults)). Crop fields might therefore be used by these sensitive stages only if they offer sufficient plant cover and therefore a favourable microclimate and protection against predators.

Habitat use, of course, depends on biology and ecology of the respective species. A few amphibian species, which are physiologically and behaviourally adapted to arid conditions, may also use agricultural fields with low plant cover as habitats (e.g. *P. fuscus, Epidalea calamita*). However, also for these species availability of shelter and cover are of crucial importance (Seebacher and Alford 2002). By their fossorial behaviour and staying in moist shelters (e.g. stone embankments, burrows) during the day, postponing search for food to the night and using short rainy events for rehydration (Nöllert and Nöllert 1992, Miaud et al. 2000, Eggert 2002) they avoid dehydration and extreme temperatures (Seebacher and Alford 2002).

This predominantly nocturnal activity (and hiding behaviour during the day) can be found in many European amphibian species (e.g. *Rana temporaria*, *B. bufo, Lissotriton vulgaris*, see Günther 1996). Furthermore, nocturnal activity, the use of shelter and plant cover also limits visual encounter by predators, a strong selective force shaping behaviour in organisms (Lima and Dill 1990). At the same time this behaviour reduces significantly potential exposure to pesticide applications generally.

The life-cycle of pond-breeding amphibians is characterized by yearly migrations between terrestrial habitats and breeding sites. From early to mid-spring, adults leave their overwintering habitat and migrate to the breeding ponds. After oviposition, adults leave the breeding ponds and migrate to their summer habitats. Depending on weather conditions, early migrations of amphibian species in central Europe start in February / March (e.g. *B. bufo*, *R. temporaria*), while migration periods of other species continue till approximately May (for details see Berger et al. 2011). During these migrations of adult amphibians, agricultural fields can be crossed early in the season and - when there is no alternative - using fields with low plant coverage, too.
Activity and migratory behaviour of amphibians is highly dependent on climatic variables such as temperature and precipitation (e.g. Beebee 1995, Baldwin et al. 2006, Timm et al. 2007). This is especially true for juveniles, emigrating at high temperatures in summer entering terrestrial habitats (Mazerolle 2001). However, literature data on development of amphibian larvae indicate, that migration of metamorphs concur with late growth stages or the time of harvest of field crops in Germany, e.g. earliest *R. temporaria* and *B. bufo* metamorphs mainly occur beginning or mid of June to mid of July (Blab 1982, Günther 1996), see also summary in Berger et al. (2011). This was also exemplarily shown by Berger et al. (2013) in a study assessing the co-occurrence of juvenile stages of *Rana arvalis*, *T. cristatus, P. fuscus* and *Bombina bombina* with agricultural measures in crop fields in Germany. At this time, common field crops (e.g. winter cereals BBCH > 50) already comprise late growth stages and therefore high plant cover with high interception (interception 90%, BBCH 40-89 for winter cereals, Anonymous 2002).

Fungicide treatments in cereals are mainly performed from beginning of May until the end of June. The earlier treatments could coincide with the return of adult anurans from their breeding ponds and the latest applications could coincide with dispersal of metamorphs. In both cases there will be a dense plant cover (BBCH > 30 for the early applications and BBCH > 39 for the later applications) providing 70-90% crop interception. The earliest application of BAS 500 06 F according to label would be BBCH 25 (> 50% interception); however, this early stage would be relevant only for adult specimen and not for the more vulnerable metamorphs. Furthermore, the semi-field study (see below) performed in cereals at BBCH 25 demonstrated low risk to young frogs, too.

In summary, a number of aspects will significantly reduce potential exposure and thus the risk to pesticides under realistic field conditions:

- avoidance of agricultural sites, particularly those with low plant cover
- behavioral aspects: burrowing, hiding, nocturnal activity
- more sensitive juveniles will migrate into or through agricultural fields later in the year, when there is either dense plant cover and/or no pesticide use.

The risk assessment has to consider the likely exposure next to the toxicity data. In order to do this appropriately it is essential to consider behavioral aspects in addition to simple interception calculations.
Therefore, a semi-field study was designed and conducted. This study covers realistic (worst-case) exposure and behavior; the semi-field study in addition covers all routes of exposure: direct spraying, contact to treated surfaces and uptake of contaminated feed.

First experiments performed in midsummer during high temperatures in cereal fields at late growth stages confirmed that juvenile frogs are dependent on suitable conditions, i.e. sufficient moist soil and moist hiding places. In these first experiments most juvenile frogs had disappeared (either desiccated or escaped) from the plots despite some watering of the plots before the trials.

The final experiment was conducted after the hot summer period at more suitable weather conditions with medium temperatures and some rain before and during the study. Accordingly, all animals survived and could be recovered healthy from the trials. Early seeded winter cereal plots were used for the study at a BBCH of 25; cereal plants were grown otherwise according to standard agricultural practice, providing a good and sufficient plant cover.

The plot size was approx. 1.2 m long and 0.84 m (covering six rows of cereals). This plot size is quite sufficient to allow for ‘normal’ movement of the frogs and thus potential uptake of the pesticide from contaminated surfaces. The laboratory studies demonstrated anyway that the initial contamination is decisive; all effects were observed only in the first few hours after test substance application. Animals not impacted within the first 24 hours did not show any symptoms later during the trial; in actual fact even animals impacted sublethally recovered shortly after application. This indicates that later movement over contaminated soil (once the pesticide film has dried up) does not contribute further to the test substance toxicity.

The semi-field study demonstrated that applications of BAS 500 06 F under realistic conditions will be of low risk to amphibians. Even an elevated rate corresponding to over 300 g a.s./ha did not cause any mortality. At this rate only a single individual had shown a transient behavioral impact, which is considered to be of marginal biological relevance.

A second semi-field study was performed in order to verify the results of the first one. Furthermore, higher application rates were used and the study was conducted with the Common toad, an amphibian species more likely to be encountered on agricultural sites than the European common frog. This study very well confirmed the results of the first semi-field study: no effects were observed in the field at application rates up to 375 g a.s/ha in winter cereals at BBCH 30. 50% mortality (75% when including an animal that was euthanized after showing sub-lethal effects) was observed under extreme conditions at the tenfold application rate of 12.5 L BAS 500 06 F/ha (corresponding to 2500 g a.s./ha).

In conclusion, while BAS 500 06 F may harm amphibians under worst-case conditions in the laboratory, there is a low risk to amphibians from BAS 500 06 F applications under realistic field conditions.

In the case of reptiles there is even less information available than for amphibians (see the revision by Fryday and Thompson, 2009). The risk from dietary exposure can be assumed to be lower for reptiles than for birds and mammals (Fryday and Thompson 2009). This is because reptiles are poikilotherm (i.e., do not maintain a constant body temperature) and as a result feeding activity will peak at warm days and will be zero during hibernation or at cold days. In contrast, birds and mammals will have to maintain a constant body temperature and, hence, will need to feed every day (Fryday and Thompson 2009). Uncertainties remain on the contribution of dermal exposure to the overall exposure to reptiles. However, in contrast to amphibians the skin of reptiles is much less permeable; it functions in general as protection and barrier and it is not an organ used for respiration or water/mineral exchange with the environment. Thus, reptiles are considered less vulnerable to dermal exposure as compared to amphibians. Nevertheless, some uncertainty with respect to the risk to reptiles, i.e. whether they are sufficiently covered by other (more standard) ecotoxicological data will remain and further research is needed.

However, pyraclostrobin containing products have been used for many years in many countries worldwide. So far, there are no publications indicating a potential risk of this compound to reptiles and despite the long term use worldwide the applicant is not aware of findings or (incidence) reports that amphibians or reptiles were harmfully affected by correct applications of pyraclostrobin containing fungicides.

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CP 10.2 Effects on aquatic organisms

The EU agreed endpoints for the active substance pyraclostrobin (BAS 500 F) and its major metabolites plus endpoints from new studies and revised endpoints from EU agreed studies, respectively, are used for the risk assessment on aquatic organisms (see Table 10.2-1). The new representative formulation BAS 500 06 F was not evaluated within the previous Annex I inclusion process. The respective data are presented within this chapter. A comprehensive risk assessment for the active substance, its metabolites and the formulation based on the already registered use pattern is provided below. The risk assessment is based on the "Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters", EFSA Journal 2013; 11(7):3290.

Different code numbers of different numbering systems are assigned to the metabolites of pyraclostrobin. In this chapter all metabolites are identified by the BF-code and synonym metabolite codes are given in brackets only where deemed to be helpful.

Table 10.2-1: EU agreed and new /revised endpoints for aquatic species

Test substance	Test species	EU agreed endpoints	Endpoints used in standard and refined risk assessment
Acute toxicity to fish			
Pyraclostrobin	*Oncorhynchus mykiss*	96 h LC_{50} = 0.00616 mg/L	96 h LC_{50} = 0.00616 mg/L
Pyraclostrobin	*O. mykiss* [1)]	--	96 h LC_{50} = 0.00620 mg/L
Pyraclostrobin	*Cyprinodon variegatus* [1), 2)]	--	96 h LC_{50} = 0.0769 mg/L
Pyraclostrobin	*Cyprinus carpio*	96 h LC_{50} = 0.0177 mg/L	96 h LC_{50} = 0.0177 mg/L
Pyraclostrobin	*Danio rerio* [4)]	96 h LC_{50} = 0.0619 mg/L	96 h LC_{50} = 0.0619 mg/L
Pyraclostrobin	*Lepomis macrochirus*	96 h LC_{50} = 0.0254 mg/L	96 h LC_{50} = 0.0254 mg/L
Pyraclostrobin	*L. macrochirus* [3)]	--	96 h LC_{50} = 0.0114 mg/L
Pyraclostrobin	*Leuciscus idus melanotus* [3)]	96 h LC_{50} = 0.0191 mg/L	96 h LC_{50} = 0.0191 mg/L
Pyraclostrobin	*Oryzias latipes* [3)]	96 h LC_{50} = 0.0533 mg/L	96 h LC_{50} = 0.0533 mg/L
Pyraclostrobin	*Pimephales promelas* [3)]	96 h LC_{50} = 0.0161 mg/L	96 h LC_{50} = 0.0161 mg/L
BF 500-3	*O. mykiss* [1)]	--	96 h LC_{50} > 0.0948 mg/L
BF 500-5	*O. mykiss* [1)]	--	96 h LC_{50} =11.3 mg/L
BF 500-11	*O. mykiss*	96 h LC_{50} > 100 mg/L	96 h LC_{50} > 100 mg/L
BF 500-13	*O. mykiss*	96 h LC_{50} > 50 < 100 mg/L	96 h LC_{50} > 50 < 100 mg/L
BF 500-14	*O. mykiss*	96 h LC_{50} > 39.4 < 82.6 mg/L	96 h LC_{50} > 39.4 < 82.6 mg/L
BAS 500 06 F	*O. mykiss* [1)]	--	96 h LC_{50} = 0.0360 mg/L

Test substance	Test species	EU agreed endpoints	Endpoints used in standard and refined risk assessment
Chronic toxicity to fish			
Pyraclostrobin	*O. mykiss* (ELS study)	98 d NOEC = 0.002 mg/L	98 d NOEC = 0.00235 mg/L
Pyraclostrobin	*C. variegatus* [1), 2)] (ELS study)	--	36 d NOEC = 0.0108
Pyraclostrobin	*P. promelas* [1)] (ELS study)	--	36 d NOEC = 0.00414
Acute toxicity to aquatic invertebrates			
Pyraclostrobin	*Daphnia magna*	48 h EC_{50} = 0.0157 mg/L	48 h EC_{50} = 0.0157 mg/L
BF 500-3	*D. magna* [1)]	--	48 h EC_{50} > 0.100 mg/L
BF 500-5	*D. magna* [1)]	--	48 h EC_{50} > 10.0 mg/L
BF 500-11	*D. magna*	48 h EC_{50} > 100 mg/L	48 h EC_{50} > 100 mg/L
BF 500-13	*D. magna*	48 h EC_{50} > 100 mg/L	48 h EC_{50} > 100 mg/L
BF 500-14	*D. magna*	48 h EC_{50} > 60.9 mg/L	48 h EC_{50} > 60.9 mg/L
BAS 500 06 F	*D. magna* [1)]	--	48 h EC_{50} = 0.065 mg/L
Chronic toxicity to aquatic invertebrates			
Pyraclostrobin	*D. magna*	21 d NOEC = 0.004 mg/L	21 d NOEC = 0.004 mg/L
Acute toxicity to sediment dwelling aquatic invertebrates			
Pyraclostrobin	*Leptocheirus plumulosus* [1), 2)] (spiked sediment)	--	10 d LC_{50} = 4.41 mg/kg dry sediment
Chronic toxicity to sediment dwelling aquatic invertebrates			
Pyraclostrobin	*Chironomus riparius* (spiked water)	28 d NOEC = 0.040 mg/L	28 d NOEC = 0.040 mg/L
Pyraclostrobin	*C. riparius* [1)] (spiked sediment)	--	28 d NOEC = 1.37 mg/kg dry sediment
BF 500-3	*C. riparius* [1)] (spiked sediment)	--	28 d NOEC ≥ 16.0 mg/kg dry sediment
BF 500-6	*C. riparius* [1)] (spiked sediment)	--	28 d NOEC 1.2 mg/kg dry sediment
BF 500-7	*C. riparius* [1)] (spiked sediment)	--	28 d NOEC ≥ 123.5 mg/kg dry sediment

Test substance	Test species	EU agreed endpoints	Endpoints used in standard and refined risk assessment
Toxicity to algae			
Pyraclostrobin	*Pseudokirchneriella subcapitata*	96 h E_rC_{50} > 0.843 mg/L	72 h E_rC_{50} > 0.843 mg/L [4]
		96 h E_bC_{50} = 0.152 mg/L	--
BF 500-3	*P. subcapitata* [1]	--	72 h E_rC_{50} > 1.17 mg/L [5]
BF 500-5	*P. subcapitata* [1]	--	72 h E_rC_{50} = 5.33 mg/L
BF 500-11	*Scenedesmus subspicatus*	72 h E_rC_{50} > 100 mg/L	72 h E_rC_{50} > 100 mg/L
		72 h E_bC_{50} > 100 mg/L	--
BF 500-13	*S. subspicatus*	72 h E_rC_{50} > 100 mg/L	72 h E_rC_{50} > 100 mg/L
		72 h E_bC_{50} = 66 mg/L	--
BF 500-14	*S. subspicatus*	72 h E_rC_{50} > 100 mg/L	72 h E_rC_{50} > 100 mg/L
		72 h E_bC_{50} = 46.6 mg/L	--
BAS 500 06 F	*P. subcapitata*	--	72 h E_rC_{50} = 14.2 mg/L
Toxicity to aquatic macrophytes			
Pyraclostrobin	*Lemna gibba* [1]	--	14 d E_bC_{50} = 1.72 mg/L
Higher tier studies / calculations			
Pyraclostrobin	*O. mykiss* (TTE study; different exposure durations) [1]	--	96 h LC_{50} > 0.027 mg/L (0.5 h exposure) [6]
		--	96 h LC_{50} = 0.022 mg/L (2 h) [6]
		--	96 h LC_{50} = 0.015 mg/L (8 h) [6]
Pyraclostrobin	SSD (based on 96 h NOECs for 8 fish species)	--	HC_5 (NOEC) = 0.00338 mg/L
Pyraclostrobin	*O. mykiss* (ELS study with multiple exposure)	97 d NOEC = 0.005 mg/L	97 d NOEC = 0.005 mg/L
Pyraclostrobin	Geomean based on chronic ELS with three fish species	--	$NOEC_{geomean}$ = 0.00472 mg/L
Pyraclostrobin	outdoor mesocosm (multiple spray application) [3]	6 mo NOEC = 0.008 mg/L	6 mo NOEC = 0.008 mg/L
		6 mo NOEAEC > 0.008 < 0.024 mg/L	6 mo NOEAEC > 0.008 < 0.024 mg/L

ELS = early life stage; NO(E)AEC = No observed (ecologically) adverse effect concentration; LC = life cycle; TTE = Time-To-Effect/Event; SSD = Species Sensitivity Distribution; HC_5 = 5% hazardous concentration

[1] Study not submitted during the previous Annex I inclusion process of pyraclostrobin.

[2] Marine / saltwater species

[3] Study was performed with the previous representative solo-formulation BAS 500 00 F; however, results are given in mg a.s./L

[4] In accordance to recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints of the EU agreed study on the green alga have been (re-)calculated from original data. The re-calculated values are presented here and are used in the aquatic risk assessment; for details on these calculations please refer to the respective supplement. A summary of this study and the re-calculations is provided in M-CA 8.2.

[5] In accordance to recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints obtained in the 96 h / 120 h alga studies are considered as relevant endpoint and are presented here.

[6] Endpoint obtained in the 96 hour TTE study after exposure over respective duration, i.e. the value in brackets (0.5, 2 or 8 h exposure time).

Risk assessment for aquatic organisms

Toxicity

An overview on all available toxicity data for all aquatic organisms is provided in Table 10.2-2 for the active substance and the formulated product and in Table 10.2-3 for the metabolites of pyraclostrobin.

Studies with the formulated product BAS 500 06 F are summarized in this chapter, while summaries of the new studies with the active substance and its major metabolites are provided in M-CA 8.2. For completeness this includes some older studies not been submitted during the previous Annex I inclusion process (e.g. because there was no respective data requirement in the EU) and also EU agreed studies that have been amended in the meantime (e.g. due to recalculations of endpoints or a new evaluation according to current guidelines). Further information on the EU agreed studies, which have been already evaluated within the previous Annex I inclusion process of pyraclostrobin, can be found in the Monograph (Vol. 3, Annex B.9, August 2001) and the EU Review Report (SANCO/1420/2001-final, September 2004). Furthermore, the reports of these studies will be provided to RMS, co-RMS and EFSA and are available upon request for other member states.

Active substance and formulated product

Table 10.2-2: List of studies and endpoints for aquatic organisms with the active substance pyraclostrobin (BAS 500 F) and the formulated product BAS 500 06 F

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)
Active substance: pyraclostrobin			
Fish			
Oncorhynchus mykiss	96 h LC_{50}	**0.00616** #	1999/11414
O. mykiss 1)	96 h LC_{50}	0.0062	2000/5034
Cyprinodon variegatus 1), 2)	96 h LC_{50}	0.0769	2000/5032
Cyprinus carpio	96 h LC_{50}	0.0177 (> 0.0121 < 0.0252) *	1998/11580
Danio rerio 3)	96 h LC_{50}	0.0619 (0.0558 - 0.0680) +	1999/11834
Lepomis macrochirus	96 h LC_{50}	0.0254 (0.0233 - 0.0275)	1998/10951
L. macrochirus 1)	96 h LC_{50}	0.0114	2000/5033
Leuciscus idus melanotus 3)	96 h LC_{50}	0.0191 (> 0.0135 < 0.0270)	1999/11835
Oryzias latipes 3)	96 h LC_{50}	0.0533 (0.0444 - 0.0622)	1999/11821
Pimephales promelas 3)	96 h LC_{50}	0.0161 (0.0141 - 0.0181)	1999/11833
O. mykiss	28 d NOEC	0.00464	1999/11249
O. mykiss	98 d NOEC (ELS study)	**0.00235** #	1999/11343
C. variegatus 1), 2)	36 d NOEC (ELS study)	0.0108	2000/5247
P. promelas 1)	36 d NOEC (ELS study)	0.00414	2000/5053
Aquatic invertebrates			
Daphnia magna	48 h EC_{50}	**0.0157** #	1999/10444 + amendment: 1999/10739
Americamysis bahia 1), 2)	48 h LC_{50}	> 0.00597 4)	2000/5031
Crassostrea virginica 1), 2)	96 h EC_{50}	0.0125	2000/5042
D. magna	21 d NOEC	**0.004** #	1999/11864
A. bahia 1), 2)	28 d NOEC	0.0005	2004/5000004
A. bahia 1), 2)	31 d NOEC (LC study)	(0.000365) 0.00128 14)	2013/7002075

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)
Sediment dwelling aquatic invertebrates			
Leptocheirus plumulosus [1), 2)]	10 d LC_{50} (spiked sediment)	4.41 mg/kg dry sediment	2013/7000055
Chironomus riparius	28 d NOEC (spiked water study)	0.040	2000/1000010
C. riparius [1)]	28 d NOEC (spiked sediment)	1.37 mg/kg dry sediment	2012/1185699
Algae			
Pseudokirchneriella subcapitata	72 h E_rC_{50}	**> 0.843** [5), #]	1999/11020 + supplement: 2009/1037148
	72 h E_yC_{50}	0.148 [5)]	
Navicula pelliculosa [1)]	72 h E_rC_{50}	0.0158 [6)]	2000/5046
	72 h E_bC_{50}	0.00165 [6)]	
Anabaena flos-aquae [1)]	96 h E_rC_{50}	1.41 [7), 8)]	2000/5036
	96 h E_bC_{50}	0.367 [7), 8)]	
Skeletonema costatum [1), 2)]	72 h E_rC_{50}	0.0962 [6), 8)]	2000/5035
	72 h E_bC_{50}	< 9.73 [6), 8)]	
Aquatic macrophytes			
Lemna gibba [1)]	14 d E_bC_{50}	> 1.72 [9)] / **1.72** [10)]	2000/5037
Higher tier studies / calculations			
O. mykiss	96 h LC_{50} (TTE study)	> 0.0270 (0.5 h) [11)]	2000/1014919
		0.0220 (2 h) [11)]	
		0.0150 (8 h) [11)]	
SSD (based on 96 h NOECs for 8 fish species) [12)]	NOECs, median HC_5	0.00338	--
geometric mean (based on ELS NOECs for 3 fish species) [12)]	ELS chronic NOECs geomean	0.00472	--
O. mykiss	97 d NOEC (ELS study with multiple exposure peaks) [13)]	0.005	1999/11537
outdoor mesocosm (appr 6 month, multiple applications) [3)]	NOEC	0.008	2000/1000011 + supplement: 2012/1357084
	NOEAEC	> 0.008 < 0.024	
Formulated product: BAS 500 06 F			
Fish			
O. mykiss [1)]	96 h LC_{50}	**0.036** (0.007 mg a.s./L)	2008/1018046
C. carpio	96 h LC_{50}	0.098 (0.019 mg a.s./L)	2012/1250190
Aquatic invertebrates			
D. magna [1)]	48 h EC_{50}	0.065 (0.013 mg a.s./L)	2004/1004393

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)
Algae			
P. subcapitata [1)]	72 h E_rC_{50}	**14.2** (2.76 mg a.s./L)	2008/1009325
	72 h E_yC_{50}	2.40	

Bold endpoints: Where several endpoints are available for the same group or where several endpoints are available for one study based on different effect parameters, the respective endpoint printed bold is used in the standard TER calculations.
ELS = early life stage; NO(E)AEC = No observed (ecologically) adverse effect concentration; LC = life cycle; TTE = Time-To-Effect/Event; SSD = Species Sensitivity Distribution; HC_5 = 5% hazardous concentration

[#] At tier I level, only the endpoints for the standard aquatic test species are considered even though studies on additional invertebrate/alga species are available partly resulting in lower endpoints. This is justified considering the information from higher tier studies such as a mesocosm study covering the risk to a great number of different aquatic invertebrate and alga species (for detailed justifications see below).

[+] Spearman-Karber estimate of LC_{50} with confidence limits

[*] Geometric mean with corresponding LC_0 (>) and LC_{100} (<) values.

[1)] Study has not been submitted during the previous Annex I inclusion process of pyraclostrobin (summaries for the studies with pyraclostrobin and its metabolites are provided in M-CA 8.2; summaries for the studies with the formulation are provided below).

[2)] Marine / saltwater species

[3)] Study was performed with the solo-formulation BAS 500 00 F (same formulation type as the new representative formulation)

[4)] The 48-h LC_{50} obtained in the 96 h study is used in the risk assessment according to EU Regulation 283/2013 (European Commission, 2013) on the data requirements for active substances and the EFSA Aquatic Guidance Document (EFSA, 2013).

[5)] In accordance with the recent aquatic guidance document (EFSA, 2013; OECD, 2011), the 72 h endpoints of the EU agreed study on the green alga have been (re-)calculated from original data. The re-calculated values are presented here and are used in the aquatic risk assessment; for details on these calculations please refer to the supplement. A summary of this study and the re-calculations is provided within this chapter together with the summaries of the new studies.

[6)] In accordance with recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints obtained in the 96 h / 120 h alga studies are considered as relevant endpoint and are presented here

[7)] In this study the 24, 48, and 72 hour effective concentrations were not calculated because sufficient growth had not yet occurred in any of the test groups to allow for these calculations; thus the 96 h endpoints obtained in the 120 h alga studies are presented here.

[8)] Due to the reasoning given above, the study on this alga species is considered to be not valid.

[9)] Based on frond numbers

[10)] Based on dry weight

[11)] Endpoint obtained in the TTE study after peak exposure over respective duration, i.e. the value in brackets (0.5, 2 or 8 h exposure time).

[12)] For details on SSD / geometric mean calculations and derivation of respective RAC values please refer to the refined risk assessment presented below.

[13)] During the peer review this study had been considered to be not valid; however, this was done apparently based on a misunderstanding of the data; more explanation in the respective text on the fish RA.

[14)] A statistically significant impact based on a small reduction in male weight was given at 0.676 µg/L; however, a biologically relevant impact (based on female weight reduction and a corresponding reduced reproduction level) was observed at the highest test concentration only (but which had no impact on the F1 generation) Accordingly, the NOEC is 1.28 µg/L.

Metabolites of pyraclostrobin (BAS 500 F)

The results from toxicity tests on representative freshwater species with the major aquatic metabolites are summarized in Table 10.2-3.

In the dark water/sediment study the metabolites BF 500-3, BF 500-6 and BF 500-7 occurred as major metabolites (see M-CP 9.1 - 9.2). Additionally, BF 500-3, BF 500-11, BF 500-13 and BF 500-14 occurred in relevant amounts in the irradiated water/sediment study and BF 500-5 was detected as a major metabolite in the study on aerobic mineralization in surface water.

The pyraclostrobin metabolite BF 500-3 was not observed in standard aerobic soil metabolism studies, but only in soil under anaerobic conditions (see M-CP 9.1 - 9.2), however, aerobic conditions are predominant in soil. The sorption behavior of BF 500-3 was investigated in the context of the EU review of pyraclostrobin showing a mean K_{oc} of 9315 mL/g (see M-CP 9.2). Due to its non-occurrence under more relevant aerobic conditions and its high K_{oc}, this metabolite will not be relevant for drainage or runoff entry into surface water. BF 500-3 was observed in sediment with a maximum occurrence of 16.9% TAR in an irradiated water/sediment study. The highest amount of BF 500-3 in the water phase never exceeded 5.0% TAR and thus, this metabolite is only relevant in the sediment phase. Nevertheless, toxicity studies were performed for BF 500-3 on fish, daphnids and algae (on the request of Canadian authorities) that were not included in the previous pyraclostrobin EU review; summaries for these studies are provided in M-CA 8.2. These studies (see Table 10.2-3) showed no toxicity up to the solubility level of this metabolite. The evaluation above shows that both theoretical considerations and also the evidence from toxicity studies demonstrate a very low risk to aquatic organisms resulting from water exposure to metabolite BF 500-3. Therefore, the results from these studies are not considered further in the risk assessment. However, as a major sediment metabolite it has also been tested in a 28 d spiked sediment study with *Chironomus riparius* and the results are used in the respective TER calculations (see below).

The aerobic soil metabolites BF 500-6 and BF 500-7 show very high sorption indicating that they are non-mobile in soil (BF 500-6: median K_{oc} of 107301 mL/g; BF 500-7: median K_{oc} of 149900 mL/g; see M-CP 9.2). Considering the high sorption, the low mobility in soil and the very low water solubility, it can be concluded that BF 500-6 and BF 500-7 will not enter surface water in any ecotoxicologically relevant amount. Furthermore, studies with these metabolites and soil organisms indicate very low toxicity and overall low ecotoxicological potential. To assess the risk to sediment dwelling organisms, 28 d spiked sediment studies on *C. riparius* have been conducted with both metabolites. Study summaries are provided in M-CA 8.2 and the results are considered for the risk assessment below.

The metabolite BF 500-5 was found in relevant amounts in the OECD 309 study (dark) in water and was not detected in the sediment phase of the suspended solid test. This metabolite was therefore considered not to be relevant for the sediment.

The metabolites BF 500-11, BF 500-13 and BF 500-14 were observed in the water phase of irradiated water/sediment systems. The highest amount of the metabolites BF 500-11, BF 500-13 and BF 500-14 in the sediment phase of water/sediment systems never exceeded 0.6, 2.1 and 0.7% TAR, respectively; these metabolites were therefore considered not to be relevant for the sediment.

Further information on the metabolites of pyraclostrobin can be found in document N3.

Table 10.2-3: Summary of toxicity values for aquatic organisms of the major metabolites of pyraclostrobin

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)
Metabolite: BF 500-3 (Reg. No. 340266; 500M07)			
Fish			
O. mykiss [1)]	96 h LC_{50}	> 0.0948	2007/1010836
Aquatic invertebrates			
D. magna [1)]	48 h EC_{50}	> 0.100	2006/1038907
Sediment dwelling organisms			
C. riparius [1)]	28 d NOEC (spiked sediment)	≥ 16.0 mg/kg dry sediment	2013/1237446
Algae			
P. subcapitata [1)]	72 h E_rC_{50} / E_yC_{50}	> 1.17 [2)]	2006/1038445
Metabolite: BF 500-5 (Reg. No. 298327; 500M04)			
Fish			
O. mykiss [1)]	96 h LC_{50}	11.3	2013/1349200
Aquatic invertebrates			
D. magna [1)]	48 h EC_{50}	> 10.0	2013/1349201
Algae			
P. subcapitata [1)]	72 h E_rC_{50}	5.33	2013/1349202
	72 h E_yC_{50}	2.03	
Metabolite: BF 500-6 (Reg. No. 364380; 500M01) [3)]			
Sediment dwelling organisms			
C. riparius [1)]	28 d NOEC (spiked sediment)	1.2 mg/kg dry sediment	2014/1001481
Metabolite: BF 500-7 (Reg. No. 369315; 500M02) [3)]			
Sediment dwelling organisms			
C. riparius [1)]	28 d NOEC (spiked sediment)	≥ 123.5 mg/kg dry sediment	2014/1001482

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)
Metabolite: BF 500-11 (Reg. No. 411847; 500M60)			
Fish			
O. mykiss	96 h LC_{50}	> 100	1999/11909
Aquatic invertebrates			
D. magna	48 h EC_{50}	> 100	1999/11917
Algae			
Scenedesmus subspicatus	72 h E_rC_{50} / E_bC_{50}	> 100	1999/11918
Metabolite: BF 500-13 (Reg. No. 412785; 500M62)			
Fish			
O. mykiss	96 h LC_{50}	> 50 < 100	1999/11913
Aquatic invertebrates			
D. magna	48 h EC_{50}	> 100	1999/11921
Algae			
S. subspicatus	72 h E_rC_{50}	> 100	1999/11922
	72 h E_bC_{50}	66.0	
Metabolite: BF 500-14 (Reg. No. 413039; 500M76)			
Fish			
O. mykiss	96 h LC_{50}	> 39.4 < 82.6	1999/11837
Aquatic invertebrates			
D. magna	48 h EC_{50}	> 60.9	1999/11910
Algae			
S. subspicatus	72 h E_rC_{50}	> 100	1999/11914
	72 h E_bC_{50}	46.6	

1) Study has not been submitted during the Annex I inclusion process of pyraclostrobin (study summaries are provided in M-CA 8.2).
2) In accordance with recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints obtained in the 96 h alga study are considered as relevant endpoints and are presented here.
3) The pyraclostrobin metabolites BF 500-6 and BF 500-7 do not occur to any significant extent in water; they are thus considered to be only of relevance in sediment.

Exposure

The critical use pattern of BAS 500 06 F considered in the risk assessment below is presented in Table 10.2-4.

Table 10.2-4: Critical use pattern

Crop	Application time (BBCH growth stage)	Number of applications	Minimum interval [days]	Application rate per treatment	
				pyraclostrobin [kg a.s./ha]	BAS 500 06 F [L/ha]
cereals	25 - 69	2	21	0.25	1.25
maize	30 - 65	1	--	0.20	1.00

Aquatic organisms may be exposed to BAS 500 06 F through spray drift and to the active substance pyraclostrobin and its metabolites through spray drift, run-off and drainage from the application site into adjacent water bodies. Exposure of aquatic organisms from these routes was estimated by calculating Predicted Environmental Concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) for the active substance and its major metabolites using the FOCUS surface water models. The PEC values were calculated for single and twofold application of BAS 500 06 F in cereals at a use rate of 1.25 L/ha (equivalent to 250 g pyraclostrobin/ha) and for single application in maize at 1.0 L/ha (equivalent to 200 g a.s./ha). A stepwise approach has been followed starting with simple worst-case assumptions in the first two steps and proceeding to more realistic worst-case conditions in the third step and adding spray drift mitigation (i.e. no-spray buffer zones, drift reducing nozzles) and runoff mitigation measures (i.e. vegetated filter strips) in the fourth step of the exposure assessment.
PEC values were calculated with endpoints derived from a dark water/sediment study (Tier 1) and additionally with parameters derived from an irradiated water/sediment study (Tier 2). However, only tier 1 PEC values were considered for the following aquatic risk assessment, except for the metabolites BF 500-11, BF 500-13 und BF 500-14 that were mainly observed in irradiated water/sediment studies. Accordingly, only PEC values derived from irradiated water/sediment studies are reported for these metabolites.

The resulting worst-case PEC_{sw} and PEC_{sed} values for pyraclostrobin and its major metabolites are presented in Table 10.2.3-1 to Table 10.2.3-6. For cereals, only the worst-case PEC values are presented, either resulting from calculations for single or multiple applications. The pyraclostrobin metabolites BF 500-3, BF 500-6 and BF 500-7 do not occur to any significant extent in water. They are thus considered to be of relevance only in sediment and only PEC_{sed} values have been calculated for these metabolites. The highest amount of the metabolites BF 500-11, BF 500-13 and BF 500-14 in the sediment phase of water/sediment systems never exceeded 0.6, 2.1 and 0.7% TAR, respectively; the metabolite BF 500-5 was not detected in the sediment phase of the suspended solid test. These metabolites were therefore considered to be not relevant for the sediment and no PEC_{sed} values were calculated.

A summary of the FOCUS $PEC_{sw, max}$ values for pyraclostrobin and its major metabolites used for the aquatic risk assessment are provided in the Appendix at the end of this chapter. For full details of the assumptions used in the exposure calculations, please see M-CP 9.2.5.

Toxicity exposure ratios

The following TER calculations were conducted based on application of BAS 500 06 F according to the already registered uses in cereals and maize. The initial risk assessments were carried out by comparing the $PEC_{sw, max}$ and $PEC_{sed, max}$ values with the acute and long-term toxicity endpoints.

The toxicity data obtained in the studies with the formulation (re-calculated to the active substance) are similar to the respective data from the studies with pyraclostrobin and therefore indicate no significantly higher (or unexpected) toxicity of the formulation than predicted based on the results of the active substance. For example, the measured toxicity to trout as the most sensitive species of the product BAS 500 06 F - 0.036 mg/L - provides a slightly higher endpoint (i.e. slightly lower toxicity) than predicted from the data on the active substance - 0.032 mg/L (see Table 10.2-5). Accordingly, no synergisms or additional toxicity occurs due to the co-formulants. The acute and chronic risk resulting from BAS 500 06 F is thus sufficiently addressed by the risk assessment for the active substance and no additional TER calculations were performed for the formulated product. This is also in agreement with the proposals of the EFSA Aquatic Guidance Document (EFSA, 2013), because dividing the re-calculated mixture toxicity ($LC_{50}/EC_{50mix\text{-}CA}$) by the measured mixture toxicity (LC_{50}/EC_{50PPP}) would result in model deviation ratios (MDR) between 0.33 and 1.24 (see Table 10.2-5).

Table 10.2-5: Comparison of the toxicity of the formulated product BAS 500 06 F and the active substance pyraclostrobin

Test species	Endpoint & Test system	Measured toxicity of BAS 500 06 F [mg product/L]	Calculated toxicity of BAS 500 06 F based on a.s. content and toxicity § [mg product/L]	Measured toxicity of the a.s. pyraclostrobin [mg a.s./L]	Ratio (MDR) (calculated / measured)
O. mykiss	LC_{50}, acute, 96 h	0.036	0.032	0.00616	0.89
D. magna	EC_{50}, acute, 48 h	0.065	0.0809	0.0157	1.24
P. subcapitata #	E_rC_{50}, static, 72 h	14.2	--	> 0.843	--
	E_yC_{50}, static, 72 h	2.4	0.783	0.152	0.33

MDR = model deviation ratio

§ The mixture toxicity of the formulation was re-calculated based on the measured toxicity data of pyraclostrobin, the analyzed content of pyraclostrobin (i.e. 202.7 g/L) within the formulation and the formulation density (i.e. 1.044 g/cm^3).

As no clear growth rate endpoint was obtained in the study with pyraclostrobin on green algae (i.e. E_rC_{50} > 0.843 mg/L), the endpoint yield was used for comparison of the toxicity data.

TER_A for fish

Two acute toxicity endpoints for *O. mykiss* are available for the active substance; a study performed under static conditions and an additional study performed for the US under flow-through conditions. Both studies provide nearly identical endpoints based on mean measured concentrations, i.e. of 6.16 and 6.2 µg/L, respectively. In the following risk assessment of pyraclostrobin the rounded value of 6.2 µg/L is used. The TER_A values were calculated using worst-case FOCUS Step 1 and 2 $PEC_{sw, max}$ values. The results are presented in Table 10.2-6. The acute risk to fish from the product BAS 500 06 F is addressed by the risk assessment for the active substance (see reasoning above).

Table 10.2-6: Fish acute TER values for pyraclostrobin and its major metabolites using worst-case FOCUS Step 1 and 2 $PEC_{sw, max}$ values

Test organism	Test substance	96 h LC_{50} [µg/L]	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER_A	Trigger value
cereals						
O. mykiss	Pyraclostrobin	6.2	2	2.299	**2.7**	100
O. mykiss	BF 500-5	11300	1	0.256	44141	100
O. mykiss	BF 500-11	> 100000	1	0.407	> 245700	100
O. mykiss	BF 500-13	> 50000 < 100000	1	0.533	> 93809 < 187617	100
O. mykiss	BF 500-14	> 39400 < 82600	1	0.573	> 68761 < 144154	100
maize						
O. mykiss	Pyraclostrobin	6.2	2	1.839	**3.4**	100
O. mykiss	BF 500-5	11300	1	0.102	110784	100
O. mykiss	BF 500-11	> 100000	1	0.163	> 613497	100
O. mykiss	BF 500-13	> 50000 < 100000	1	0.213	> 234742 < 469484	100
O. mykiss	BF 500-14	> 39400 < 82600	1	0.229	> 172052 < 360699	100

TERs shown in **bold** fall below the relevant trigger.

The calculated TER_A values for pyraclostrobin are below the trigger value of 100 based on FOCUS Step 2 calculations following application of BAS 500 06 F in cereals and maize. Therefore, additional TER calculations considering Step 3 and 4 $PEC_{sw, max}$ values are presented in Table 10.2-7.

The TER_A values for the metabolites BF 500-5, BF 500-11, BF 500-13 and BF 500-14 by far exceed the trigger value of 100 based on worst-case FOCUS Step 1 calculations, indicating low ecotoxicological relevance of the metabolites.

Table 10.2-7: Fish acute TER values for pyraclostrobin using worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values

FOCUS Scenarios		96 h LC_{50} [µg/L]	FOCUS Step 3		FOCUS Step 4		
			$PEC_{sw, max}$ [µg/L]	TER_A	Step 4 Mitigation	$PEC_{sw, max}$ [µg/L]	TER_A
winter cereals							
D1	ditch	6.2	1.584	**3.9**	20 m D + R	0.118	**53**
	stream	6.2	1.346	**4.6**	20 m D + R	0.135	**46**
D2	ditch	6.2	1.589	**3.9**	20 m D + R	0.119	**52**
	stream	6.2	1.414	**4.4**	20 m D + R	0.142	**44**
D3	ditch	6.2	1.567	**4.0**	20 m D + R	0.117	**53**
D4	pond	6.2	0.065	**95**	50% N	0.033	188
					5 m D	0.056	111
	stream	6.2	1.180	**5.3**	20 m D + R	0.119	**52**
D5	pond	6.2	0.066	**94**	50% N	0.033	188
					5 m D	0.057	109
	stream	6.2	1.263	**4.9**	20 m D + R	0.127	**49**
D6	ditch	6.2	1.547	**4.0**	20 m D + R	0.115	**54**
R1	pond	6.2	0.065	**95**	50% N	0.042	148
					5 m D	0.056	111
	stream	6.2	1.037	**6.0**	20 m D + R	0.104	**60**
R3	stream	6.2	1.463	**4.2**	20 m D + R	0.147	**42**
R4	stream	6.2	1.038	**6.0**	20 m D + R	0.130	**48**
spring cereals							
D1	ditch	6.2	1.759	**3.5**	20 m D + R	0.119	**52**
	stream	6.2	1.389	**4.5**	20 m D + R	0.140	**44**
D3	ditch	6.2	1.569	**4.0**	20 m D + R	0.117	**53**
D4	pond	6.2	0.065	**95**	50% N	0.033	188
					5 m D	0.056	111
	stream	6.2	1.300	**4.8**	20 m D + R	0.131	**47**
D5	pond	6.2	0.068	**91**	50% N	0.034	182
					5 m D	0.058	107
	stream	6.2	1.319	**4.7**	20 m D + R	0.133	**47**
R4	stream	6.2	1.038	**6.0**	20 m D + R	0.104	**60**

FOCUS Scenarios		96 h LC_{50} [µg/L]	FOCUS Step 3		FOCUS Step 4		
			$PEC_{sw, max}$ [µg/L]	TER_A	Step 4 Mitigation	$PEC_{sw, max}$ [µg/L]	TER_A
maize							
D3	ditch	6.2	1.037	**6.0**	10 m D +R	0.180	**34**
D4	pond	6.2	0.042	148	--	--	--
	stream	6.2	0.895	**6.9**	10 m D +R	0.200	**31**
D5	pond	6.2	0.042	148	--	--	--
	stream	6.2	0.888	**7.0**	10 m D +R	0.198	**31**
D6	ditch	6.2	1.029	**6.0**	10 m D +R	0.179	**35**
R1	pond	6.2	0.042	148	--	--	--
	stream	6.2	0.720	**8.6**	10 m D +R	0.161	**39**
R2	stream	6.2	0.963	**6.4**	10 m D +R	0.215	**29**
R3	stream	6.2	1.011	**6.1**	10 m D +R	0.225	**28**
R4	stream	6.2	0.703	**8.8**	10 m D +R	0.157	**39**

TERs shown in **bold** fall below the relevant trigger.
D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Based on FOCUS Step 3 and 4 calculations the TER_A values for pyraclostrobin are below the standard trigger of 100 for the majority of scenarios following application of BAS 500 06 F in cereals and maize.

Therefore, additional studies and a refined risk assessment have been conducted. Besides the standard fish species *O. mykiss,* seven further fish species were tested in acute 96 h laboratory studies with pyraclostrobin (see Table 10.2-2). To ease test item application, some of the additional tests were conducted using pyraclostrobin in the form of the previous representative solo-formulation BAS 500 00 F (due to the low water solubility of pyraclostrobin) the addition of solvents is appropriate anyway). A comparison of acute toxicity data with trout tested with the solo-formulation and the active substance provided a slightly lower endpoint for the formulation (4 µg/L compared to 6.2 µg/L for the a.s.). These data were considered in the previous Annex I inclusion process. Therefore, the use of data from tests with this formulation is considered a conservative surrogate for active substance testing.
Pyraclostrobin shows a very steep concentration response relationship. Therefore, in several cases no effect was observed at the first concentration whereas 100% mortality was found at the next higher concentration. In other studies, only one mortality measurement could be made between 0 and 100% mortality. To obtain relevant figures for the following calculations, in the first case the LC_{50} was determined as geometric mean between the two concentrations, while in the latter case a Spearman-Karber estimate of the LC_{50} was calculated using the original mortality and analytical data for the relevant concentrations from the study reports. Thereby the results may deviate slightly from those given in some of the reports, where no statistical analysis of the LC_{50} had been performed. The respective results were corrected for analytically determined concentrations. These studies and the respective endpoint re-calculations have already been evaluated during the previous Annex I inclusion process.

Additional species testing reduces uncertainty in the risk assessment by addressing better capturing of inter-species variations in sensitivity. According to the proposals of the EFSA Panel on Plant Protection Products and their Residues (PPR) given in the Aquatic Guidance Document (EFSA, 2013), it is recommended to preferably apply a species sensitivity distribution (SSD) approach for refined risk assessment if data on a sufficient number of species is available for the respective group of aquatic organisms. For fish, the SSD approach should be applied if toxicity data is available for at least 5 different species (for pyraclostrobin data on 8 species are available). The PPR Panel further recommends to use acute NOEC/LC_{10} values to construct the SSD for fish, since a higher protection level is desired for vertebrates than for invertebrates and plants.

Therefore, an SSD analysis based on the 96 h NOEC values for 8 fish species was conducted and the results of these calculations are presented below. The data used for SSD calculations are summarized in Table 10.2-8. For fish species where more than one study was performed (i.e. *O. mykiss* and *L. macrochirus*), the arithmetic mean of the endpoints of these studies was considered for SSD calculations.

Table 10.2-8: Pyraclostrobin endpoints selected for calculation of the Hazardous Concentrations based on Species Sensitivity Distribution (SSD) Analysis

Test species	LC_{50} [µg a.s./L]	NOEC / LC_{10} [µg a.s./L]	Ratio (LC_{50}/NOEC)	Reference (BASF DocID)
O. mykiss Salmonidae	6.16	4.5	1.4	1999/11414
	6.2	3.6	1.7	2000/5034
	6.2 *	**4.1 ***	1.5	--
C. carpio Cyprinidae	17.7 +	**12.1**	1.5	1998/11580
C. variegatus Cyprinodontidae	76.9	**53.5**	1.4	2000/5032
D. rerio Cyprinidae	61.9 #	**23.4**	2.6	1999/11834
L. idus melanotus Cyprinidae	19.1 +	**13.5**	1.4	1999/11835
L. macrochirus Centrarchidae	25.4 #	10.9	2.3	1998/10951
	11.4	6.1	1.9	2000/5033
	18.4 *	**8.5 ***	2.2	--
O. latipes Adrianichthyidae	53.3 #	**16.5**	3.2	1999/11821
P. promelas Cyprinidae	16.1 #	**7.0**	2.3	1999/11833

Bold values were used for SSD calculations.
* Arithmetic mean value of two studies conducted with the same species.
\# Spearman-Karber estimate of LC_{50} (for confidence limits see endpoint tables above, Table 10.2-2).
\+ Geometric mean (for corresponding LC_0 (>) and LC_{100} (<) values see endpoint tables above).

The SSD calculations have been performed using the software tool ETX 2.0 (RIVM, 2004). The results (graphs and statistical results) are shown below. In the first step, the toxicity data (NOEC) were subjected to three different goodness of fit tests (Anderson-Darling, Kolmogorov-Smirnov and the Cramer von Mises), where the normality was checked at different significance levels. The respective analysis of the NOEC values confirms normal distribution of the data (see below).

Analysis of normality

Parameters of the normal distribution

Name	Value	Description
mean	1.114	mean of the log toxicity values
s.d.	0.341	sample standard deviation
n	8	sample size

Goodness of fit tests

Sign. level	Tests for normality n=8					
	Anderson-Darling		Kolmogorov-Smirnov		Cramer von Mises	
0.1	0.631	Accepted	0.819	Accepted	0.104	Accepted
0.05	0.752	Accepted	0.895	Accepted	0.126	Accepted
0.025	0.873	Accepted	0.995	Accepted	0.148	Accepted
0.01	1.035	Accepted	1.035	Accepted	0.179	Accepted
Statistic	0.1757		0.4090		0.0114	

Figure 10.2-1: SSD Histogram (based on 96 h NOEC values for 8 fish species)

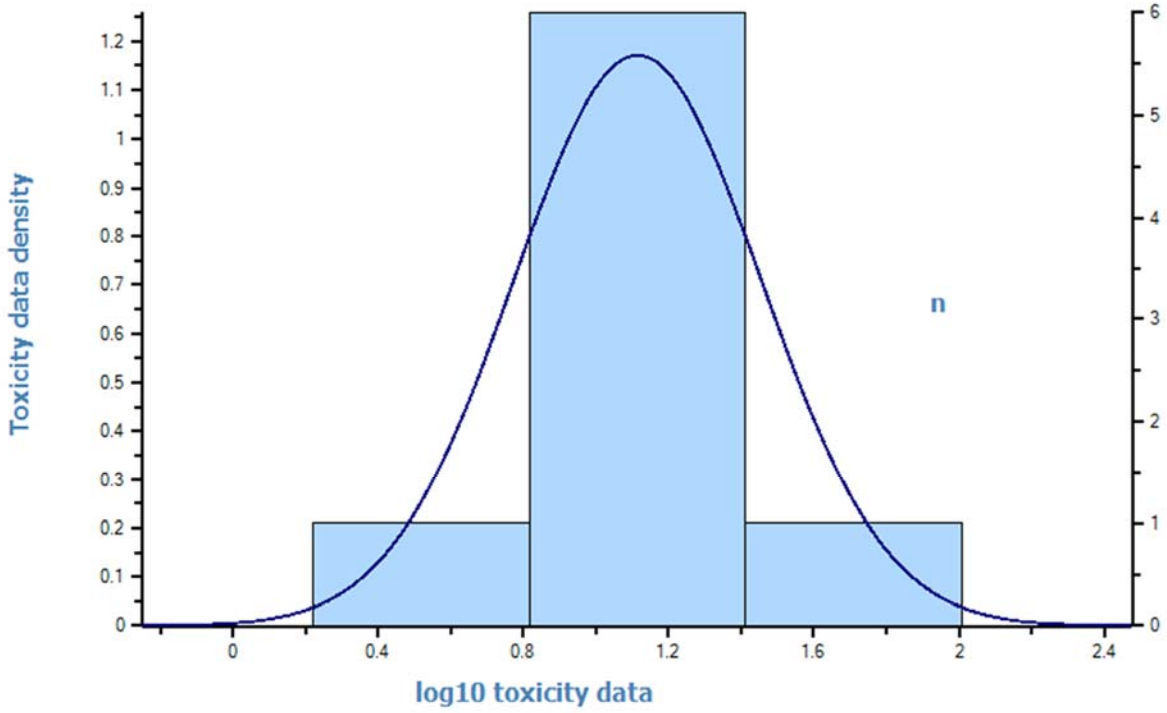
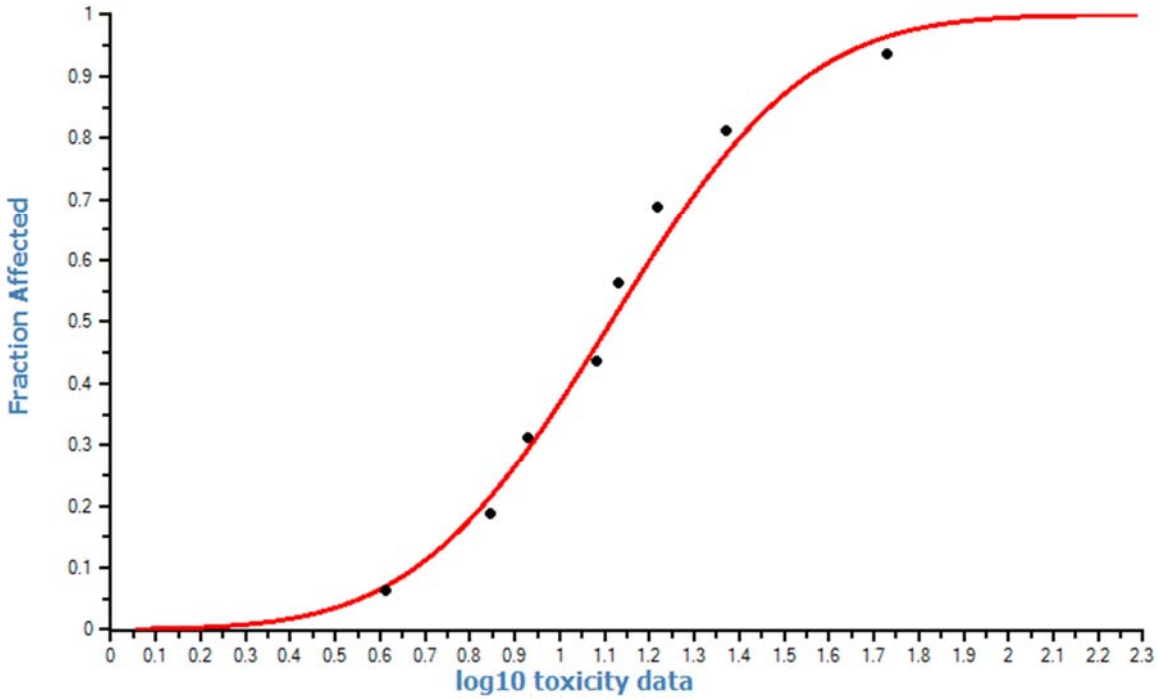


Figure 10.2-2: Species sensitivity distribution (based on 96 h NOEC values for 8 fish species)



The median HC_5 (hazardous concentration to 5% of the tested species that is predicted with 50% certainty) and also the lower limit HC_5 values ($LLHC_5$; hazardous concentration to 5% of the tested species that is predicted with 95% certainty) can be derived from the SSD curves (see table below).

HC_5 results based on acute (96 h) NOEC data for 8 fish species

Name	Value	log10(value)	Description
LL HC_5	1.069	0.029	lower estimate of the HC_5
HC_5	**3.381**	0.529	median estimate of the HC_5
UL HC_5	6.139	0.788	upper estimate of the HC_5
sprHC_5	5.745	0.759	spread of the HC_5 estimate

The **median limit HC_5** for fish can thus be determined to be **3.381 µg a.s./**L.

According to the recommendation given in the EFSA Aquatic GD (2013), an **assessment factor of 3** should be applied on the median HC_5 from an SSD constructed with acute NOEC values for fish for derivation of an SSD-RAC (Regulatory Acceptable Concentration) when latency of effects is not to be expected, which is clearly the case for pyraclostrobin (compare chronic risk assessment for fish below). In this case, the use of an AF of 3 is considered warranted also taking into account the various aspects mentioned in the aquatic GD:

- The quality of the acute toxicity data used to construct the SSD is high. All data are based on endpoints from GLP studies meeting all the listed criteria and including analytical support. A wide taxonomical range has been covered with eight species from five different fish families.
- The lower limit value of the HC_5 is about one third of the median HC_5, the fit of the curve is good and the relevant statistical parameters are met well.
- The SSD-RAC is not higher than the tier 3 RAC derived from effect class 1 of the mesocosm study. In fact, it is about three times lower (might indicate the appropriateness of a lower assessment factor).
- The position of the toxicity data in the lower tail of the SSD are either on the SSD curve or are positioned on the right side of the SSD curve (might indicate the appropriateness of a lower assessment factor).
- The steepness of the SSD curve is less than a factor of 100 between the lowest and the highest NOEC, however, it has to be considered that this is an SSD approach for fish only, where in general the difference between species sensitivities is much less than for the taxonomically more diverse group of invertebrates. In fact, a wide range of sensitivities has been covered in this investigation.
- Since sufficient information is available for the substance, read-across information for compounds with a similar mode of action is not needed.
- The acute to chronic ratio is significantly smaller than 10 (might indicate the appropriateness of a lower assessment factor).

Accordingly, an assessment factor of three is well justified and still conservative.

The endpoint obtained with the marine fish species (*Cyprinodon variegatus)* is twice higher than the next least sensitive species. It might be considered to omit this endpoint from the freshwater fish risk assessment as it may not be representative for freshwater species. However, omitting this higher NOEC value, the resulting median and lower limit HC_5 and the lower limit of this value would be slightly higher (3.89 µg/L, respectively 1.48 µg/L as compared to 3.38 and 1.07 µg/L). As a conservative assumption the lower HC_5 value (i.e. including the higher toxicity endpoint) will be used.

Following this approach and applying the recommended assessment factor an **$SSD\text{-}RAC_{acute}$ of 1.13 µg a.s./L** can be derived. This concentration is considered unlikely to cause unacceptable toxicity to fish, the most sensitive group of aquatic organisms.

TER_A calculations based on the HC_5 value and worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values are shown in Table 10.2-9.

Table 10.2-9: Fish acute TER values for pyraclostrobin based on the median HC_5 and worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values

FOCUS Scenarios		HC_5 (8 species) [µg/L]	FOCUS Step 3		FOCUS Step 4		
			$PEC_{sw, max}$ [µg/L]	TER_A*	Step 4 Mitigation	$PEC_{sw, max}$ [µg/L]	TER_A*
winter cereals							
D1	ditch	3.38	1.584	**2.1**	50% N	0.792	4.3
					5 m D	0.429	7.9
	stream	3.38	1.346	**2.5**	50% N	0.673	5.0
					5 m D	0.491	6.9
D2	ditch	3.38	1.589	**2.1**	50% N	0.794	4.3
					5 m D	0.431	7.8
	stream	3.38	1.414	**2.4**	50% N	0.707	4.8
					5 m D	0.516	6.6
D3	ditch	3.38	1.567	**2.2**	50% N	0.783	4.3
					5 m D	0.424	8.0
D4	pond	3.38	0.065	52	--	--	--
	stream	3.38	1.180	**2.9**	50% N	0.590	5.7
					5 m D	0.431	7.8
D5	pond	3.38	0.066	51	--	--	--
	stream	3.38	1.263	**2.7**	50% N	0.631	5.4
					5 m D	0.461	7.3
D6	ditch	3.38	1.547	**2.2**	50% N	0.773	4.4
					5 m D	0.419	8.1
R1	pond	3.38	0.065	52	--	--	--
	stream	3.38	1.037	3.3	--	--	--
R3	stream	3.38	1.463	**2.3**	50% N	0.731	4.6
					5 m D	0.534	6.3
R4	stream	3.38	1.038	3.3	--	--	--

FOCUS Scenarios		HC_5 (8 species) [µg/L]	FOCUS Step 3		FOCUS Step 4		
			$PEC_{sw, max}$ [µg/L]	TER_A*	Step 4 Mitigation	$PEC_{sw, max}$ [µg/L]	TER_A*
spring cereals							
D1	ditch	3.38	1.759	**1.9**	50% N	0.877	3.9
					5 m D	0.453	7.5
	stream	3.38	1.389	**2.4**	50% N	0.694	4.9
					5 m D	0.507	6.7
D3	ditch	3.38	1.569	**2.2**	50% N	0.784	4.3
					5 m D	0.425	8.0
D4	pond	3.38	0.065	52	--	--	--
	stream	3.38	1.300	**2.6**	50% N	0.650	5.2
					5 m D	0.474	7.1
D5	pond	3.38	0.068	50	--	--	--
	stream	3.38	1.319	**2.6**	50% N	0.659	5.1
					5 m D	0.481	7.0
R4	stream	3.38	1.038	3.3	--	--	--
maize							
D3	ditch	3.38	1.037	3.3	--	--	--
D4	pond	3.38	0.042	80	--	--	--
	stream	3.38	0.895	3.8	--	--	--
D5	pond	3.38	0.042	80	--	--	--
	stream	3.38	0.888	3.8	--	--	--
D6	ditch	3.38	1.029	3.3	--	--	--
R1	pond	3.38	0.042	80	--	--	--
	stream	3.38	0.720	4.7	--	--	--
R2	stream	3.38	0.963	3.5	--	--	--
R3	stream	3.38	1.011	3.3	--	--	--
R4	stream	3.38	0.703	4.8	--	--	--

TERs shown in **bold** fall below the trigger of 3.
* A TER trigger of 3 is considered appropriate for comparison to the median HC_5 (for justification see above).
D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques

Based on the SSD approach the TER_A values for pyraclostrobin indicate that a no-sprayed buffer zone of 5 m or the use of 50% drift reducing nozzles will be sufficient to avoid pyraclostrobin toxicity to fish following application of BAS 500 06 F in winter and spring cereals. For application in maize safe uses could be shown with no need for any additional mitigation measures.

In addition, a time-to-effect study has been performed with pyraclostrobin on rainbow trout as the most sensitive fish species (see M-CA 8.2.1, BASF DocID 2000/1014919). The study was conducted to assess the impact of short exposure pulses typical for moving water bodies such as streams by employing different exposure durations (i.e. 0.5, 2 and 8 hours). The results of this study demonstrate that short term exposure, which might be encountered in plumes of moving water bodies, will be tolerated at much higher levels. For short exposure times (i.e. 0.5 h), simulating average flow velocities in moving water bodies such as creeks and streams, LC_{50} values of > 27 µg/L were found. In a slower moving stream, generating exposure times of about 2 hours, concentrations of up to 18 µg/L are tolerated by the most sensitive species, and even more long-term pulses (8 hours) resulted in 2 - 3 times lower toxicity as compared to the standard 96 hour exposure period. This shows that - different from the values in Table 10.2-9 - there will be low risk to fish for winter and spring cereal application in moving water bodies such as streams without the need of an additional buffer zone.

We propose to base the overall RAC for edge of field surface waters on the hazardous concentrations (HC_5) derived from species sensitivity distributions with fish as recommended in Table 6 of the Aquatic Guidance Document (EFSA, 2013) considering that:

- There is a large dataset from acute and chronic fish studies
- Toxicity is driven by the acute toxicity
- There are no sub-lethal effects at concentrations without mortality even in long-term chronic studies
- Latency of effects is clearly not given
- Pyraclostrobin dissipates quite rapidly from water (short exposure periods only)

Nevertheless, for completeness the chronic risk will be addressed below and TER_{LT} will be provided as well.

TER_{LT} for fish

The long-term risk assessment to fish is based on a number of chronic fish studies. Next to a 28 day juvenile growth test, there is a standard ELS study on trout as well as a higher tier ELS study on trout with variable exposure pattern covering numerous peaks. Furthermore, there are two ELS studies with two additional fish species, fathead minnow and sheepshead minnow.

The higher tier ELS study (BASF DocID 1999/11537) had been considered to be not valid in the ECCO peer review based on “low survival in the control” (ECCO Peer Review Program, Full Report on Pyraclostrobin, 2002). However, this evaluation is assumed to be due to a misunderstanding. Within the report it is stated: “…the survival in the viability control (= fertilization success) after 14 days was only 28%”. This describes the fertilization rate and not the survival of the controls, which in fact was high and well within the standard validation criteria. The fertilization rate was rather low, because eggs and sperm were derived from fish out of the usual breading season. Therefore, a higher number of eggs was fertilized and only fertilized eggs were used further in the test, which then showed a sufficiently high survival (well above the validity criteria). Accordingly, this study is considered valid for the assessment of chronic toxicity to fish from a realistic worst-case, multiple peak exposure scenario covering different life stages and all relevant chronic endpoints.

In addition to the ELS study on *O. mykiss,* ELS studies on two other fish species have been performed (i.e. *C. variegatus* and *P. promelas;* see Table 10.2-2). The results for the three tested fish species can be used to calculate the **geometric mean of 4.72 µg a.s./L.** Considering the standard chronic assessment factor of 10 this results in a **Geomean-RAC$_{chronic}$ of 0.472 µg a.s./L**.

This approach is in line with the EFSA Aquatic GD (2013), which states that the geometric mean-AF approach may be applied for refined risk assessment, when toxicity data for a limited number of additional test species are available. All preconditions for using this approach that were set out in the EFSA Aquatic GD (chapter 8.3.2 and 8.3.3) are fulfilled:

- *similar endpoints:*
  NOEC values are all derived from ELS fish studies and are based on the same effect parameter (i.e. mortality)
- *species of the same taxonomic group:*
  aquatic vertebrates (fish)
- *available data exceed the first tier data requirements:*
  additional studies on *C. variegatus* and *P. promelas*
- *most sensitive species should not be more than a factor of 10 (for chronic tests) below the geometric mean:*
  O. mykiss NOEC= 2.35 µg/L, geomean = 4.72 µg/L, factor = 2
- *< 5 species tested:*
  3 different species
- *if the lowest toxicity value is higher than the Geomean-RAC value, it is acceptable to use the Geometric mean approach:*
  O. mykiss NOEC= 2.35 µg/L > Geomean-RAC value = 0.472 µg/L

Alternatively, the endpoint from the higher tier trout ELS study can be used for the long-term risk assessment for fish. This study employed a slightly more realistic but still worst-case exposure and resulted in a NOEC of 5 µg/L. Applying the standard assessment factor of 10 would derive a RAC of 0.5 µg/L. In the following we use the more conservative lower endpoint based on the Geomean of the three fish ELS studies (4.72 µg/L).

The TER$_{LT}$ values for pyraclostrobin and fish based on worst-case FOCUS Step 2 PEC$_{sw}$ values are shown in Table 10.2-10 to Table 10.2-12.

Table 10.2-10: Fish long-term TER values for pyraclostrobin based on the geomean of three fish ELS studies and worst-case FOCUS Step 3 and 4 $PEC_{sw,\ max,\ ini}$ values for BAS 500 06 F applications in winter cereals

FOCUS Scenarios		NOEC Geomean of 3 ELS studies [µg/L]	FOCUS Step 3		FOCUS Step 4		
			PEC_{sw} [µg/L]	TER_{LT}	Step 4 Mitigation	PEC_{sw} [µg/L]	TER_{LT}
D1	ditch	4.72	1.584	**3.0**	75% N	0.396	12
					5 m D	0.429	11
	stream	4.72	1.346	**3.5**	75% N	0.336	14
					5 m D + 50% N	0.246	19
					10 m D	0.260	18
D2	ditch	4.72	1.589	**3.0**	75% N	0.397	12
					5 m D	0.431	11
	stream	4.72	1.414	**3.3**	75% N	0.353	13
					5 m D + 50% N	0.258	18
					10 m D	0.274	17
D3	ditch	4.72	1.567	**3.0**	75% N	0.392	12
					5 m D	0.424	11
D4	pond	4.72	0.065	73	--	--	--
	stream	4.72	1.180	**4.0**	75% N	0.295	16
					5 m D	0.431	11
D5	pond	4.72	0.066	72	--	--	
	stream	4.72	1.263	**3.7**	75% N	0.316	15
					5 m D	0.461	10
D6	ditch	4.72	1.547	**3.1**	75% N	0.387	12
					5 m D	0.419	11
R1	pond	4.72	0.065	73	--	--	--
	stream	4.72	1.037	**4.6**	75% N	0.259	18
					5 m D	0.379	12
R3	stream	4.72	1.463	**3.2**	75% N	0.365	13
					5 m D + 50% N	0.267	18
					10 m D	0.283	17
R4	stream	4.72	1.038	**4.5**	10 m D + R	0.248	19

TERs shown in **bold** fall below the standard trigger of 10.
D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Table 10.2-11: Fish long-term TER values for pyraclostrobin based on the geomean of three fish ELS studies using worst-case FOCUS Step 3 and 4 $PEC_{sw,\ max}$ values following applications of BAS 500 06 F in spring cereals

FOCUS Scenarios		NOEC Geomean of 3 ELS studies [µg/L]	FOCUS Step 3		FOCUS Step 4		
			PEC_{sw} [µg/L]	TER_{LT}	Step 4 Mitigation	PEC_{sw} [µg/L]	TER_{LT}
D1	ditch	4.72	1.759	**2.7**	75% N	0.437	11
					5 m D	0.453	10
	stream	4.72	1.389	**3.4**	75% N	0.347	14
					5 m D + 50% N	0.254	19
					10 m D	0.269	18
D3	ditch	4.72	1.569	**3.0**	75% N	0.392	12
					5 m D	0.425	11
D4	pond	4.72	0.065	73	--	--	
	stream	4.72	1.300	**3.6**	75% N	0.325	15
					5 m D +50% N	0.237	20
					10 m D	0.252	19
D5	pond	4.72	0.068	69	--	--	
	stream	4.72	1.319	**3.6**	75% N	0.329	14
					5 m D + 50% N	0.241	20
					10 m D	0.255	19
R4	stream	4.72	1.038	**4.5**	75% N	0.267	18
					5 m D	0.379	12

TERs shown in **bold** fall below the standard trigger of 10.
D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Table 10.2-12: Fish long-term TER values for pyraclostrobin based on the geomean of three fish ELS studies using FOCUS Step 3 and 4 $PEC_{sw,\ max}$ values following application of BAS 500 06 F in maize

FOCUS Scenarios		NOEC Geomean of 3 ELS studies [µg/L]	FOCUS Step 3		FOCUS Step 4		
			PEC_{sw} [µg/L]	TER_{LT}	Step 4 Mitigation	PEC_{sw} [µg/L]	TER_{LT}
D3	ditch	4.72	1.037	**4.6**	75% N	0.259	18
					5 m D	0.340	14
D4	pond	4.72	0.042	112	--	--	--
	stream	4.72	0.895	**5.3**	50% N	0.448	11
					5 m D	0.377	13
D5	pond	4.72	0.042	112	--	--	--
	stream	4.72	0.888	**5.3**	50% N	0.444	11
					5 m D	0.374	13
D6	ditch	4.72	1.029	**4.6**	75% N	0.257	18
					5 m D	0.337	14
R1	pond	4.72	0.042	112	--	--	--
	stream	4.72	0.720	6.6	50% N	0.360	13
					5 m D	0.303	16
R2	stream	4.72	0.963	**4.9**	75% N	0.241	20
					5 m D	0.405	12
R3	stream	4.72	1.011	**4.7**	75% N	0.253	19
					5 m D	0.425	11
R4	stream	4.72	0.703	6.7	50% N	0.351	13
					5 m D	0.301	16

TERs shown in **bold** fall below the standard trigger of 10.
D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Based on FOCUS Step 3 and 4 calculations using initial $PEC_{sw, max\ ini}$ values and considering the standard assessment factor of 10, low chronic risk to fish can be demonstrated if certain mitigation measures are considered for the proposed uses of BAS 500 06 F, the level of which depends on the GAP and FOCUS scenario.
However, considering the very narrow acute to chronic ratio and the clear lack of any latency of effects and the relatively short half-life of pyraclostrobin in water it is proposed to derive the overall RAC for edge-of-field surface waters based on hazardous concentrations derived from species sensitivity distribution with fish (= 1.13 µg a.s./L).

In conclusion, taking into account all relevant data and the results of different refinement approaches there is overall weight-of-evidence that no-spray buffer zones of 5 m or the use of drift reducing nozzles (50% drift reduction) for winter and spring cereals and no additional mitigation measure for maize will be sufficient to avoid unacceptable long-term risk to fish following the proposed uses of BAS 500 06 F.

Amphibian tadpoles

There is some limited information from literature on amphibian tadpoles, another aquatic vertebrate group. Hooser et al. (2012) (see CA 8.2.8/6) observed tadpole toxicity to the active substance pyraclostrobin (LC_{50} = 10 µg/L), which is in the range of fish data. The spacing of concentrations was rather rough (factor of three). Therefore, a more precise derivation of the LC_{50} is difficult. They observed higher toxicity for a pyraclostrobin formulated product (LC_{50} = 3.7 µg/L) with apparently slight mortality at 1.7 µg/L already. In another study of the same research group (Hartman et al. 2014, see CA 8.1.4/3) tadpoles of the same species were used again and exposed over a long term period at 1.7 µg/L. In this study they did not find any mortality at all; in contrast they found that tadpoles within this treatment group performed actually better than the control showing a shorter development time, without negative impact at all on biomass development. The slightly positive impact of the fungicide treatment is difficult to evaluate as the overall and particularly the control performance of the tadpoles (i.e. the development time) was rather poor.

Several literature evaluations compare the aquatic toxicity of amphibians to other aquatic organisms and fish in particular (Aldrich, 2009; Fryday and Thompson, 2012). The most comprehensive one was published recently (Weltje et al., 2013). A common conclusion of these data evaluations is that other aquatic endpoints (generally available for pesticides) cover the potential toxicity to amphibians in water.
The literature data shown above is not fully conclusive, however, seems to confirm in general the same trend. Accordingly, we consider that the risk assessment performed for fish as the most sensitive group of aquatic organisms should also cover the risk to amphibians.
It has to be borne in mind though that amphibian tadpoles may inhabit shallower water bodies than fish. If such sensitive water bodies are close to treated fields and if tadpoles inhabit such systems during time of application, there might be a higher risk to this group of organisms. The exact level would need to be determined by a proper and robust test on tadpoles. If for example we assume a twice lower water level, but if toxicity to tadpoles would also be twice lower as compared to the most sensitive fish species (which is roughly the case here considering the a.s. data for tadpoles (Hooser E.A. et al., 2012, see CA 8.2.8/6) and the lowest fish endpoint), then the resulting risk would be the same.

TER_A for *Daphnia magna*

The TER_A values for *Daphnia magna* were calculated for pyraclostrobin and its major metabolites using worst-case FOCUS Step 1 and 2 $PEC_{sw, max}$ values and are given in Table 10.2-13.

Table 10.2-13: Acute TER values for *D. magna* exposed to pyraclostrobin and its major metabolites using worst-case FOCUS Step 1 and 2 $PEC_{sw, max}$ values

Test organism	Test substance	48 h EC_{50} [µg/L]	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER_A	Trigger value
cereals						
D. magna	pyraclostrobin	15.7	2	2.299	**6.8**	100
D. magna	BF 500-5	> 10000	1	0.256	> 39063	100
D. magna	BF 500-11	> 100000	1	0.407	> 245700	100
D. magna	BF 500-13	> 100000	1	0.533	> 187617	100
D. magna	BF 500-14	> 60900	1	0.573	> 106283	100
maize						
D. magna	pyraclostrobin	15.7	2	1.839	**8.5**	100
D. magna	BF 500-5	> 10000	1	0.102	> 98039	100
D. magna	BF 500-11	> 100000	1	0.163	> 613497	100
D. magna	BF 500-13	> 100000	1	0.213	> 469484	100
D. magna	BF 500-14	> 60900	1	0.229	> 265939	100

TERs shown in **bold** fall below the relevant trigger.

The TER_A values for pyraclostrobin are below the trigger of 100 based on FOCUS Step 2 calculations for application in cereals and maize. Therefore, additional TER calculations based on FOCUS Step 3 and 4 PEC values are presented in Table 10.2-14 to Table 10.2-16.

The TER_A values for the metabolites BF 500-5, BF 500-11, BF 500-13 and BF 500-14 exceed the trigger value of 100 based on worst-case FOCUS Step 1 calculations, indicating low ecotoxicological relevance of the metabolites.

Table 10.2-14: Acute TER values for *D. magna* exposed to pyraclostrobin using worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values following applications of BAS 500 06 F in winter cereals

FOCUS Scenarios		48 h EC_{50} [µg/L]	FOCUS Step 3		FOCUS Step 4		
			$PEC_{sw, max}$ [µg/L]	TER_A	Step 4 Mitigation	$PEC_{sw, max}$ [µg/L]	TER_A
D1	ditch	15.7	1.584	**9.9**	5 m D + 75% N	0.107	147
					10 m D + 50% N	0.114	138
					15 m D	0.155	101
	stream	15.7	1.346	**12**	90% N	0.134	117
					5 m D + 75% N	0.123	128
					10 m D + 50% N	0.130	121
					20 m D	0.135	116
D2	ditch	15.7	1.589	**9.9**	5 m D + 75% N	0.108	145
					10 m D + 50% N	0.114	138
					15 m D	0.156	101
	stream	15.7	1.414	**11**	90% N	0.141	111
					5 m D + 75% N	0.129	122
					10 m D + 50% N	0.137	115
					20 m D	0.142	111
D3	ditch	15.7	1.567	**10**	90% N	0.157	100
					5 m D + 75% N	0.106	148
					10 m D + 50% N	0.112	140
					15 m D	0.154	102
D4	pond	15.7	0.065	242	--	--	--
	stream	15.7	1.180	**13**	90% N	0.118	133
					5 m D + 75% N	0.108	145
					10 m D + 50% N	0.114	138
					15 m D	0.156	101
D5	pond	15.7	0.066	238	--	--	--
	stream	15.7	1.263	**12**	90% N	0.126	125
					5 m D + 75% N	0.115	137
					10 m D + 50% N	0.122	129
					20 m D	0.127	124
D6	ditch	15.7	1.547	**10**	90% N	0.155	101
					5 m D + 75% N	0.105	150
					10 m D + 50% N	0.111	141
					15 m D	0.152	103

FOCUS Scenarios		48 h EC_{50} [µg/L]	FOCUS Step 3		FOCUS Step 4		
			$PEC_{sw, max}$ [µg/L]	TER_A	Step 4 Mitigation	$PEC_{sw, max}$ [µg/L]	TER_A
R1	pond	15.7	0.065	242	--	--	--
	stream	15.7	1.037	**15**	20 m D + R	0.104	151
R3	stream	15.7	1.463	**11**	20 m D + R	0.147	107
R4	stream	15.7	1.038	**15**	20 m D + R	0.130	121

TERs shown in **bold** fall below the relevant trigger.
D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Table 10.2-15: Acute TER values for *D. magna* exposed to pyraclostrobin using worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values following applications of BAS 500 06 F in spring cereals

FOCUS Scenarios		48 h EC_{50} [µg/L]	FOCUS Step 3		FOCUS Step 4		
			$PEC_{sw, max}$ [µg/L]	TER_A	Step 4 Mitigation	$PEC_{sw, max}$ [µg/L]	TER_A
D1	ditch	15.7	1.759	**8.9**	5 m D + 75% N	0.113	139
					10 m D + 50% N	0.117	134
					20 m D	0.119	132
	stream	15.7	1.389	**11**	90% N	0.139	113
					5 m D + 75% N	0.127	124
					10 m D + 50% N	0.134	117
					20 m D	0.140	112
D3	ditch	15.7	1.569	**10**	90% N	0.157	100
					5 m D + 75% N	0.106	148
					10 m D + 50% N	0.113	139
					15 m D	0.154	102
D4	pond	15.7	0.065	242	--	--	--
	stream	15.7	1.300	**12**	90% N	0.130	121
					5 m D + 75% N	0.119	132
					10 m D + 50% N	0.126	125
					20 m D	0.131	120
D5	pond	15.7	0.068	231	--	--	--
	stream	15.7	1.319	**12**	90% N	0.132	119
					5 m D + 75% N	0.120	131
					10 m D + 50% N	0.128	123
					20 m D	0.133	180
R4	stream	15.7	1.038	**15**	20 m D + R	0.104	151

TERs shown in **bold** fall below the relevant trigger.
D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Table 10.2-16: Acute TER values for *D. magna* exposed to pyraclostrobin using FOCUS Step 3 and 4 $PEC_{sw, max}$ values following application of BAS 500 06 F in maize

FOCUS Scenarios		48 h EC_{50} [µg/L]	FOCUS Step 3		FOCUS Step 4		
			$PEC_{sw, max}$ [µg/L]	TER_A	Step 4 Mitigation	$PEC_{sw, max}$ [µg/L]	TER_A
D3	ditch	15.7	1.037	**15**	90% N	0.104	151
					10 m D + R	0.180	**87**
D4	pond	15.7	0.042	374	--	--	--
	stream	15.7	0.895	**18**	90% N	0.089	176
					10 m D + R	0.200	**79**
D5	pond	15.7	0.042	374	--	--	--
	stream	15.7	0.888	**18**	90% N	0.089	176
					10 m D + R	0.198	**79**
D6	ditch	15.7	1.029	**15**	90% N	0.103	152
					10 m D + R	0.179	**88**
R1	pond	15.7	0.042	374	--	--	--
	stream	15.7	0.720	**22**	90% N	0.153	103
					5 m D + 50% N	0.153	103
					10 m D + R	0.161	**98**
R2	stream	15.7	0.963	**16**	90% N	0.096	164
					10 m D + R	0.215	**73**
R3	stream	15.7	1.011	**16**	10 m D + R	0.225	**70**
R4	stream	15.7	0.703	**22**	10 m D + R	0.157	100

TERs shown in **bold** fall below the relevant trigger.
D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Based on FOCUS Step 3 and 4 standard calculations using maximum initial PEC_{sw} values, the TER_A values for pyraclostrobin exceed the trigger value of 100, considering vegetated buffer zones of up to 20 m and/or the use of drift reducing nozzles following application of BAS 500 06 F in cereals and maize.

However, a mesocosm study is available indicating low risk to aquatic invertebrates at concentrations of 8 µg a.s./L or less (for more details see also the following chapter on *Daphnia* long term risk assessment).

Comparison of the respective worst-case FOCUS Step 2 $PEC_{sw, max}$ value for the active substance pyraclostrobin (= 2.299 µg a.s./L for cereals and 1.839 µg a.s./L for maize) to the proposed $RAC_{mesocosm}$ demonstrates low risk to aquatic invertebrates following the proposed uses of BAS 500 06 F with no need for additional mitigation measures.

Additional studies have been conducted with the marine invertebrate species *A. bahia* and *C. virginica*, partly resulting in lower endpoints compared to EC_{50} for *D. magna* (see M-CA 8.2). However, the mesocosm study includes a great number of different and more relevant freshwater invertebrate species (for more details see below and chapter M-CA 8.2). Thus, the higher tier assessment based on the mesocosm study is most appropriate to address the risk to aquatic invertebrates.

TER_{LT} for *Daphnia magna*

The TER_{LT} values for *Daphnia magna* were calculated for pyraclostrobin using the FOCUS Step 2 $PEC_{sw, max}$ values and are given in Table 10.2-17.

Table 10.2-17: Long-term TER values for *D. magna* exposed to pyraclostrobin using worst-case FOCUS Step 2 $PEC_{sw, max}$ values

Crop	Test organism	21 d NOEC [µg/L]	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER_{LT}	Trigger value
cereals	*D. magna*	4.0	2	2.299	**1.7**	10
maize	*D. magna*	4.0	2	1.839	**2.2**	10

TERs shown in **bold** fall below the relevant trigger.

The TER_{LT} values for pyraclostrobin are below the long-term trigger value of 10 based on FOCUS Step 2 calculations for application in cereals and maize. Therefore, additional TER calculations based on FOCUS Step 3 and 4 $PEC_{sw, max}$ values are presented in Table 10.2-18 to Table 10.2-20.

Table 10.2-18: Long-term TER values for *D. magna* exposed to pyraclostrobin using worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values following applications of BAS 500 06 F in winter cereals

FOCUS Scenarios		21 d NOEC [µg/L]	FOCUS Step 3		FOCUS Step 4		
			$PEC_{sw, max}$ [µg/L]	TER_{LT}	Step 4 Mitigation	$PEC_{sw, max}$ [µg/L]	TER_{LT}
D1	ditch	4.0	1.584	**2.5**	75% N	0.396	10
					5 m D + 50% N	0.214	19
					10 m D	0.228	18
	stream	4.0	1.346	**3.0**	75% N	0.336	12
					5 m D + 50% N	0.246	16
					10 m D	0.260	15
D2	ditch	4.0	1.589	**2.5**	75% N	0.397	10
					5 m D + 50% N	0.215	19
					10 m D	0.228	18
	stream	4.0	1.414	**2.8**	75% N	0.353	11
					5 m D + 50% N	0.258	16
					10 m D	0.274	15
D3	ditch	4.0	1.567	**2.6**	75% N	0.392	10
					5 m D + 50% N	0.212	19
					10 m D	0.225	18
D4	pond	4.0	0.065	62	--	--	--
	stream	4.0	1.180	**3.4**	75% N	0.295	14
					5 m D + 50% N	0.215	19
					10 m D	0.228	18
D5	pond	4.0	0.066	61	--	--	--
	stream	4.0	1.263	**3.2**	75% N	0.316	13
					5 m D + 50% N	0.231	17
					10 m D	0.244	16
D6	ditch	4.0	1.547	**2.6**	75% N	0.387	10
					5 m D + 50% N	0.210	19
					10 m D	0.222	18
R1	pond	4.0	0.065	62	--	--	--
	stream	4.0	1.037	**3.9**	75% N	0.259	15
					5 m D	0.379	11
R3	stream	4.0	1.463	**2.7**	75% N	0.365	11
					5 m D + 50% N	0.267	15
					10 m D	0.283	14
R4	stream	4.0	1.038	**3.9**	10 m D + R	0.248	16

TERs shown in **bold** fall below the relevant trigger.
D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Table 10.2-19: Long-term TER values for *D. magna* exposed to pyraclostrobin using worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values following applications of BAS 500 06 F in spring cereals

FOCUS Scenarios		21 d NOEC [µg/L]	FOCUS Step 3		FOCUS Step 4		
			$PEC_{sw, max}$ [µg/L]	TER_{LT}	Step 4 Mitigation	$PEC_{sw, max}$ [µg/L]	TER_{LT}
D1	ditch	4.0	1.759	**2.3**	90% N	0.174	23
					5 m D + 50% N	0.226	18
					10 m D	0.235	17
	stream	4.0	1.389	**2.9**	75% N	0.347	12
					5 m D + 50% N	0.254	16
					10 m D	0.269	15
D3	ditch	4.0	1.569	**2.5**	75% N	0.392	10
					5 m D + 50% N	0.212	19
					10 m D	0.225	18
D4	pond	4.0	0.065	62	--	--	--
	stream	4.0	1.300	**3.1**	75% N	0.325	12
					5 m D + 50% N	0.237	17
					10 m D	0.252	16
D5	pond	4.0	0.068	59	--	--	--
	stream	4.0	1.319	**3.0**	75% N	0.329	12
					5 m D + 50% N	0.241	17
					10 m D	0.255	16
R4	stream	4.0	1.038	**3.9**	75% N	0.267	15
					5 m D	0.379	11

TERs shown in **bold** fall below the relevant trigger.
D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Table 10.2-20: Long-term TER values for *D. magna* exposed to pyraclostrobin using FOCUS Step 3 and 4 $PEC_{sw, max}$ values following application of BAS 500 06 F in maize

FOCUS Scenarios		21 d NOEC [µg/L]	FOCUS Step 3		FOCUS Step 4		
			$PEC_{sw, max}$ [µg/L]	TER_{LT}	Step 4 Mitigation	$PEC_{sw, max}$ [µg/L]	TER_{LT}
D3	ditch	4.0	1.037	**3.9**	75% N	0.259	15
					5 m D	0.340	12
D4	pond	4.0	0.042	95	--	--	--
	stream	4.0	0.895	**4.5**	75% N	0.224	18
					5 m D	0.377	11
D5	pond	4.0	0.042	95	--	--	--
	stream	4.0	0.888	**4.5**	75% N	0.222	18
					5 m D	0.374	11
D6	ditch	4.0	1.029	**3.9**	75% N	0.257	16
					5 m D	0.337	12
R1	pond	4.0	0.042	95	--	--	--
	stream	4.0	0.720	**5.6**	50% N	0.360	11
					5 m D	0.303	13
R2	stream	4.0	0.963	**4.2**	75% N	0.241	17
					5 m D + 50% N	0.203	20
					10 m D	0.215	19
R3	stream	4.0	1.011	**4.0**	75% N	0.253	16
					5 m D + 50% N	0.213	19
					10 m D	0.225	18
R4	stream	4.0	0.703	**5.7**	50% N	0.351	11
					5 m D	0.301	13

TERs shown in **bold** fall below the relevant trigger.
D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Based on FOCUS Step 3 and 4 standard calculations using maximum initial PEC_{sw} values, the TER_{LT} values for pyraclostrobin exceed the trigger value of 10, considering vegetated filter strips up to 10 m following application of BAS 500 06 F in winter cereals. Regarding application of BAS 500 06 F in spring cereals and maize safe uses can be demonstrated for all FOCUS scenarios if no spray-buffer zones of up to 10 m (and/or equivalent mitigation measures) are considered.

However, a mesocosm study is available (BASF DocID 2000/1000011 + supplement 2012/1357084) and a refined risk assessment has been performed providing more realistic information about the potential risk to aquatic invertebrates.

A mesocosm study provides a complex and compelling impression of the potential effects of a test substance under realistic conditions. Thus, mesocosm data allows for a general conclusion on aquatic ecosystems since major direct effects will be quite comparable within different systems (Leeuwangh, 1994).

The Dutch Platform for Assessment of Higher-Tier Studies has produced a Guidance Document on how micro-/mesocosm data should be presented and evaluated in a uniform and transparent manner (RIVM Guidance Document; de Jong et al., 2008;). The EFSA Aquatic GD (EFSA, 2013) proposes to largely use this document to present and evaluate micro-/mesocosm studies for regulatory purposes when placing plant protection products on the European market. Consequently, the mesocosm study has been evaluated following the RIVM Guidance document; for details please refer to the supplement to this study (BASF DocID 2012/1357084). Executive summaries of the originally submitted study and the evaluation according to RIVM are provided in M-CA 8.2. The mesocosm evaluation according to this new procedure results basically in the same conclusion as previously given during the Annex I listing process, i.e. a high quality study covering a wide range of sensitive species and measurements providing robust endpoints.

The results of the extensive and complex mesocosm study using a worst-case exposure scenario (i.e. 8 applications of the test substance applied as the solo-formulation BAS 500 00 F) showed that pyraclostrobin can have effects on a few species at concentrations of 24 µg a.s./L. For all plankton species the effects were found to be reversible. These transient effects are thus not considered to pose a significant (ecologically unacceptable) risk to planktonic communities in aquatic ecosystems. However, fish and molluscs may also be affected at this concentration. No effects on any species or endpoint were observed at the second highest test concentration of 8 µg a.s./L, constituting the ecosystem NOEC. The multitude of endpoints and species and environmental conditions in this mesocosm study show clearly that at this (and lower) concentration no adverse effects on aquatic communities can be expected even after multiple applications. The ecologically acceptable concentration (NOEAEC) of pyraclostrobin is thus between 8 µg a.s./L and 24 µg a.s./L.

In addition, this study still encompassed unrealistic worst case conditions (8 applications of the test substance). The number of applications will be much less under practical conditions (1-4). It is highly unlikely, that eight times a 95% worst case situation with respect to drift will be encountered.

Considering the multitude of endpoints covered, the inclusion of sensitive and relevant species, the worst-case character of the mesocosm study related to exposure and the realistic worst-case test system applied, it can be concluded that no assessment factor to the **NOEC of 8 µg a.s./L** is warranted (which is also in line with previous recommendations and guidances). However, the new Aquatic Guidance Document (EFSA 2013) recommends using an assessment factor of two in this case for the ETO-RAC derivation. Following this approach, this results in an **$RAC_{mesocosm}$ of 4 µg a.s./L.**

A comparison of the respective worst-case FOCUS Step 2 $PEC_{sw, max}$ value for the active substance pyraclostrobin (= 2.299 µg a.s./L for cereals and 1.839 µg a.s./L for maize) to the proposed $RAC_{mesocosm}$ demonstrates low risk to aquatic invertebrates following the proposed uses of BAS 500 06 F with no need for any additional mitigation measures.

TER_A for aquatic insect

Acute tests were not conducted with an aquatic insect species. Instead, chronic studies were performed with the active substance pyraclostrobin and its soil metabolites BF 500-3, BF 500-6 and BF 500-7 on *Chironomus riparius*, which address the potential long-term risk of the active substance to aquatic insects (see below). Furthermore, a mesocosm study is available for pyraclostrobin covering various aquatic invertebrate species including aquatic insects.

TER_{LT} for aquatic insect

TER_{LT} values for *Chironomus riparius* were calculated for water and sediment exposure.

The resulting TER_{LT} values for pyraclostrobin following water exposure using FOCUS Step 2 PEC_{sw} values are given in Table 10.2-21.

Table 10.2-21: Long-term TER values for *C. riparius* exposed to pyraclostrobin using worst-case FOCUS Step 2 $PEC_{sw, max}$ values (water exposure)

Test organism	28 d NOEC [µg/L]	Crop	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER_{LT}	Trigger value
C. riparius (spiked water)	40	cereals	2	2.299	17	10
		maize	2	1.839	22	10

The TER_{LT} values for pyraclostrobin exceed the long-term trigger of 10 based on FOCUS Step 2 calculations, indicating low chronic risk to aquatic insects resulting from water exposure following application of BAS 500 06 F in cereals and maize.

In order to assess similarly the risk arising from sediment exposure, TER values were calculated using the 28 d NOECs from chronic spiked-sediment studies with *C. riparius.* The resulting TER_{LT} values for pyraclostrobin and its soil (sediment) metabolites BF 500-3, BF 500-6 and BF 500-7 using FOCUS Step 1 and 2 PEC_{sed} values are given in Table 10.2-22.

Table 10.2-22: Long-term TER values for *C. riparius* exposed to pyraclostrobin and the metabolites BF 500-3, BF 500-6 and BF 500-7 using worst-case FOCUS Step 1 and 2 $PEC_{sed, max}$ values (sediment exposure)

Test substance	Test organism	28 d NOEC [µg/kg dry sediment]	FOCUS Step	$PEC_{sed, max}$ [µg/kg dry sediment]	TER_{LT}	Trigger value
cereals						
pyraclostrobin	*C. riparius* (spiked sediment)	1370	2	187.230	**7.3**	10
BF 500-3	*C. riparius* (spiked sediment)	≥ 16000	1	20.030	≥ 799	10
BF 500-6	*C. riparius* (spiked sediment)	1200	2	60.344	20	10
BF 500-7	*C. riparius* (spiked sediment)	≥ 123500	1	182.976	≥ 675	10
maize						
pyraclostrobin	*C. riparius (spiked sediment)*	1370	2	95.014	14	10
BF 500-3	*C. riparius* (spiked sediment)	≥ 16000	1	8.012	≥ 1997	10
BF 500-6	*C. riparius (spiked sediment)*	1200	2	24.752	48	10
BF 500-7	*C. riparius (spiked sediment)*	≥ 123500	1	73.190	≥ 1687	10

TERs shown in **bold** fall below the relevant trigger.

The TER_{LT} value for pyraclostrobin is just below the trigger of 10 based on FOCUS Step 2 calculation for application in cereals. Therefore, additional TER calculations based on FOCUS Step 3 PEC values for cereals are presented in Table 10.2-23. For application of BAS 500 06 F in maize, the TER_{LT} value for pyraclostrobin exceeds the trigger based on FOCUS Step 2 calculation, indicating low chronic risk to aquatic insects resulting from sediment exposure.

The TER_{Lt} values for the metabolites BF 500-3, BF 500-6 and BF 500-7 exceed the trigger value of 10 based on Step 1 and 2 calculations, indicating low ecotoxicological relevance of the metabolites.

Table 10.2-23: Long-term TER values for *C. riparius* exposed to pyraclostrobin using worst-case FOCUS Step 3 $PEC_{sed,\ max}$ values for application in cereals (sediment exposure)

FOCUS Scenarios		FOCUS Step 3			
		28 d NOEC [µg/kg dry sediment]	$PEC_{sed,\ max}$ [µg/kg dry sediment]	TER_{LT}	Trigger value
winter cereals					
D1	ditch	1370	8.901	154	10
	stream	1370	0.836	1639	10
D2	ditch	1370	5.032	272	10
	stream	1370	4.212	325	10
D3	ditch	1370	1.289	1063	10
D4	pond	1370	0.698	1963	10
	stream	1370	0.065	21077	10
D5	pond	1370	0.649	2111	10
	stream	1370	0.242	5661	10
D6	ditch	1370	3.807	360	10
R1	pond	1370	0.897	1527	10
	stream	1370	4.063	337	10
R3	stream	1370	2.011	681	10
R4	stream	1370	10.583	129	10
spring cereals					
D1	ditch	1370	9.836	139	10
	stream	1370	1.015	1350	10
D3	ditch	1370	1.496	916	10
D4	pond	1370	0.586	2338	10
	stream	1370	0.257	5331	10
D5	pond	1370	0.646	2121	10
	stream	1370	0.093	14731	10
R4	stream	1370	5.553	247	10

Based on FOCUS Step 3 calculations, the TER_{LT} values for pyraclostrobin by far exceed the standard trigger of 10, indicating low risk to aquatic (sediment dwelling) insects resulting from sediment exposure following application of BAS 500 06 F in cereals.

Furthermore, a mesocosm study is available for pyraclostrobin covering various aquatic invertebrate species including aquatic insects. Based on the mesocosm results a $RAC_{mesocosm}$ of 4 µg a.s./L could be received (see text above), demonstrating low risk to aquatic organisms (at FOCUS Step 2 level) following the proposed uses of BAS 500 06 F with no need for any additional mitigation measures.

TER_A for aquatic crustacean

An acute spiked sediment study has been conducted with the estuarine amphipod *Leptocheirus plumulosus.* The sensitivity of this marine crustacean species seems to be within the range of freshwater species (NOEC = 2.74 mg/kg as compared to 1.37 mg/kg for *Chironomus* in the chronic test). Although this study is not required for registration in the EU, TER calculations based on the resulting 10-day LC_{50} value and worst-case FOCUS Step 2 $PEC_{sed, max}$ are presented in Table 10.2-24.

Table 10.2-24: Acute TER values for *L. plumulosus* exposed to pyraclostrobin using worst-case FOCUS Step 2 $PEC_{sed, max}$ values (sediment exposure)

Test organism	10 d LC_{50} [µg/kg dry sediment]	Crop	FOCUS Step	$PEC_{sed, max}$ [µg/kg dry sediment]	TER_A	Trigger value
Leptocheirus plumulosus	4410	cereals	2	187.230	**24**	100
		maize	2	95.014	**46**	100

TERs shown in **bold** fall below the relevant trigger.

The TER_A values for sediment exposure to pyraclostrobin are below the required trigger of 100 based on FOCUS Step 2 calculations for application of BAS 500 06 F in cereals and maize. Therefore, additional TER calculations based on FOCUS Step 3 PEC values are presented in Table 10.2-25.

Table 10.2-25: Acute TER values for *L. plumulosus* exposed to pyraclostrobin using worst-case FOCUS Step 2 $PEC_{sw, max}$ values (sediment exposure)

FOCUS Scenarios		FOCUS Step 3			
		10 d LC_{50} [µg/kg dry sediment]	$PEC_{sed, max}$ [µg/kg dry sediment]	TER_A	Trigger value
winter cereals					
D1	ditch	4410	8.901	495	100
	stream	4410	0.836	5275	100
D2	ditch	4410	5.032	876	100
	stream	4410	4.212	1047	100
D3	ditch	4410	1.289	3421	100
D4	pond	4410	0.698	6318	100
	stream	4410	0.065	67846	100
D5	pond	4410	0.649	6795	100
	stream	4410	0.242	18223	100
D6	ditch	4410	3.807	1158	100
R1	pond	4410	0.897	4916	100
	stream	4410	4.063	1085	100
R3	stream	4410	2.011	2193	100
R4	stream	4410	10.583	417	100
spring cereals					
D1	ditch	4410	9.836	448	100
	stream	4410	1.015	4345	100
D3	ditch	4410	1.496	2948	100
D4	pond	4410	0.586	7526	100
	stream	4410	0.257	17159	100
D5	pond	4410	0.646	6827	100
	stream	4410	0.093	47419	100
R4	stream	4410	5.553	794	100
maize					
D3	ditch	4410	0.719	6134	100
D4	pond	4410	0.312	13738	100
	stream	4410	0.067	65821	100
D5	pond	4410	0.304	14507	100
	stream	4410	0.030	147000	100
D6	ditch	4410	0.388	11366	100
R1	pond	4410	0.387	11395	100
	stream	4410	2.006	2198	100
R2	stream	4410	3.593	1227	100
R3	stream	4410	1.859	2372	100
R4	stream	4410	6.572	671	100

Based on FOCUS Step 3 calculations, the TER_A values for pyraclostrobin exceed the standard trigger of 100, indicating low acute risk.

Furthermore, a mesocosm study is available for pyraclostrobin covering various aquatic invertebrate species including aquatic crustacean species (see above).

TER_{LT} for aquatic crustacean

TER_{LT} calculations for the freshwater crustacean *Daphnia magna* are presented above.

An additional chronic study has been conducted with the marine species *A. bahia* showing lower endpoints compared to the NOEC *for D. magna*. However, the mesocosm study (see above) includes a great number of different and relevant freshwater invertebrate species providing strong evidence that the mysid data are not representative for freshwater organisms. Thus, the higher tier assessment based on the mesocosm study provides the more relevant and more comprehensive data base for the assessment of the risk to freshwater invertebrates.

TER_A for aquatic gastropod mollusc

TER_A values for aquatic gastropod molluscs are not required since BAS 500 06 F is not foreseen for direct application to surface water.
An additional chronic study has been conducted for the US with the marine species *C. virginica* showing endpoints in the range of *D. magna* endpoints. However, the mesocosm study (see above) includes a great number of different and relevant freshwater invertebrate species including aquatic gastropod molluscs. Thus, the higher tier assessment based on the mesocosm study provides the more relevant and more comprehensive data base for the assessment of the risk to freshwater invertebrates.

TER_{LT} for aquatic gastropod mollusc

TER_{LT} values for aquatic gastropod molluscs are not required since BAS 500 06 F is not foreseen for direct application to surface water. Therefore, no investigations have been performed with aquatic gastropod molluscs. Furthermore, mesocosm data are available for the active substance pyraclostrobin and thus information on gastropod mollusc species.

TER for algae

Besides the study on the standard green alga *P. subcapitata*, studies on three further alga species have been conducted, partly resulting in lower endpoints compared to the endpoints for the green alga. However, following the recommendations and validity criteria defined in the current OECD guideline 201 for alga testing (OECD, 2011) the 120 h studies with the blue-green alga *Anabaena flos-aquae* and marine diatom *Skeletonema costatum* are considered to be not valid because in both studies, at least one validity criterion is not met (for details please refer to chapter M-CA 8.2).
The study with the freshwater diatom *Navicula pelliculosa* showed some shortcomings, too, i.e. no clear dose-response relationship could be shown, because effects at the four highest tested concentrations were in a similar range (i.e. growth rate after 120 h at the four highest tested concentrations is between 68 and 72% of control). Moreover, a mesocosm study is available including a great number of different algae species (including *Navicula* species). Thus, the higher tier assessment based on the mesocosm study provided above also covers the risk to algae. At tier 1 level, TER calculations are thus only performed based on the re-calculated 72 hour growth rate endpoint from the 96 hour study with the standard green alga *P. subcapitata.*
The resulting TER values for pyraclostrobin and its major metabolites using FOCUS Step 1 $PEC_{sw, max}$ values are given in Table 10.2-26.

Table 10.2-26: TER values for algae exposed to pyraclostrobin and its major metabolites using worst-case FOCUS Step 1 $PEC_{sw, max}$ values

Test organism	Test substance	72 h E_rC_{50} [µg/L]	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER	Trigger value
cereals						
P. subcapitata	pyraclostrobin	> 843	1	17.031	> 49	10
P. subcapitata	BF 500-5	5330	1	0.256	20820	10
S. subspicatus	BF 500-11	> 100000	1	0.407	> 245700	10
S. subspicatus	BF 500-13	> 100000	1	0.533	> 187617	10
S. subspicatus	BF 500-14	> 100000	1	0.573	> 174520	10
maize						
P. subcapitata	pyraclostrobin	> 843	1	6.813	> 124	10
P. subcapitata	BF 500-5	5330	1	0.102	52255	10
S. subspicatus	BF 500-11	> 100000	1	0.163	> 613497	10
S. subspicatus	BF 500-13	> 100000	1	0.213	> 469484	10
S. subspicatus	BF 500-14	> 100000	1	0.229	> 436681	10

The TER values for pyraclostrobin and its metabolites BF 500-5, BF 500-11, BF 500-13 and BF 500-14 exceed the trigger of 10 based on worst-case FOCUS Step 1 calculations, indicating low risk to algae following application of BAS 500 06 F in cereals and maize with no need for additional mitigation measures.

TER_{LT} for aquatic plants

Additional aquatic plant testing would not be required for this fungicide, however, for registration purposes in the USA a Lemna study is available. The respective TER_{LT} values for aquatic plants (given in Table 10.2-27) were calculated for pyraclostrobin using the growth rate endpoint of the 14 day study with *Lemna gibba* and FOCUS Step 1 $PEC_{sw, max}$ values.

Table 10.2-27: TER_{LT} values for *Lemna gibba* exposed to pyraclostrobin using FOCUS worst-case Step 1 $PEC_{sw, max}$ values

Test organism	14 d E_rC_{50} [µg/L]	Crops	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER_{LT}	Trigger value
L. gibba	1720	cereals	1	17.031	101	10
		maize	1	6.813	252	10

The TER_{LT} values for pyraclostrobin exceed the trigger of 10 based on worst-case FOCUS Step 1 calculations, indicating low risk to aquatic plants following application of BAS 500 06 F in cereals and maize with no need for additional mitigation measures.

Summary of the Refined Risk Assessment

Pyraclostrobin is toxic to aquatic organisms. Based on standard precautionary assumptions, a risk to aquatic organisms is indicated following applications of a product containing this active substance. In order to refine the risk a number of additional and higher tier studies were performed. These include additional species testing, tests using more realistic exposure scenarios, a time-to-effect study and a mesocosm investigation.

The mesocosm study, which covers a wide range of relevant and sensitive aquatic organisms (but only one fish species), results in a clear NOEC of 8 µg/L, even though it simulates a worst case exposure situation. Accordingly, an ETO-RAC of 4 µg/L can be derived.
In order to further refine the risk assessment for fish besides the standard fish species *O. mykiss,* seven additional fish species were tested in acute 96 h laboratory studies with pyraclostrobin. In line with the recommendations of the Aquatic Guidance document (EFSA, 2013) an SSD analysis was conducted based on the 96 h NOEC values. The median HC_5 for fish was determined to be 3.381 µg a.s./L. An assessment factor of 3 was shown to be warranted to be applied on the median HC_5. Following this approach an overall **RAC** for edge-of-field surface waters **of 1.13 µg a.s./L** can be derived.

Overall Conclusion

The fungicidal product BAS 500 06 F and the active substance pyraclostrobin, respectively, show toxicity to aquatic organisms and contamination of aquatic ecosystems must be avoided. However, the refined risk assessment provided for pyraclostrobin and the standard assessment for its major metabolites demonstrate that application of BAS 500 06 F in cereals and maize according to good agricultural practice is of low risk to aquatic ecosystems without additional mitigation measures for maize and if no-spray buffer zones of 5 m or 50% drift reducing nozzles are considered for winter and spring cereals.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

The following fish acute toxicity study performed with the new representative formulation BAS 500 06 F is provided in support of the assessment and has not been previously evaluated on the EU-level.

Report:	CP 10.2.1/1 [redacted] 2008a BAS 500 06 F - Acute toxicity study with the rainbow trout (Oncorhynchus mykiss) 2008/1018046
Guidelines:	OECD 203, EEC 92/69 A V C 1, EPA 72-1, EPA 850.1075, EPA 540/9-82-024, EPA 712-C-96-118
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, juvenile rainbow trout were exposed to nominal concentrations of 0.005, 0.010, 0.022, 0.050 and 0.10 mg BAS 500 06 F/L (corresponding to mean measured concentrations of 0.0056, 0.0105, 0.0166, 0.0393 and 0.0930 mg/L) and a water control in groups of 10 animals in glass aquaria containing 50 L water. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the active substance. After 96 hours of exposure no mortality was observed in the control and at mean measured concentrations of up to and including 0.0166 mg BAS 500 06 F/L, whereas 80% and 100% mortality occurred at the two highest tested concentrations of 0.0393 mg/L and 0.0930 mg/L, respectively. After 96 hours of exposure no sublethal effects were observed for surviving fish.

In a static acute toxicity study with rainbow trout, the LC_{50} (96 h) of BAS 500 06 F was 0.036 mg/L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.017 mg/L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304428): 202.7 g/L (nominal: 200 g/L); density: 1.044 g/cm^3.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*); age: approx. 4 months; mean body length: 5.8 cm (5.2 - 6.3 cm); mean body weight: 1.81 g (1.14 - 2.29 g); supplied by "Forellenzucht Trostadt GbR", Trostadt, Germany.

Test design: Static system (96 hours); 10 fish per aquarium (loading: 0.36 g fish/L) and per concentration, assessments of mortality and symptoms of toxicity within 1 h after start of exposure and 6, 24, 48, 72 and 96 h after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control, 0.005, 0.010, 0.022, 0.050 and 0.100 mg BAS 500 06 F/L (nominal); corresponding to mean measured concentrations of 0, 0.0056, 0.0105, 0.0166, 0.0393 and 0.0930 mg/L.

Test conditions: Glass aquaria with stainless steel frame (60 x 35 x 40 cm), test volume: 50 L, non-chlorinated, filtered tap water mixed with deionized water; temperature: 13°C; pH 8.0 - 8.2; oxygen content: 8.1 mg/L - 10.3 mg/L; total hardness about 1 mmol/L; conductivity approx. 250 µS/cm; acid capacity about 2.5 mmol/L; photoperiod: 16 h light : 8 h dark; light intensity: approx. 100 lux - 490 lux; no aeration, no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.

Statistics: Descriptive statistics; probit analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The initially measured concentrations of pyraclostrobin in the three highest test concentrations were within 10% of the nominal concentrations (i.e. between 93 and 97%). In the two lowest concentrations the initially measured values ranged from 124 to 135% of nominal. At test termination, the analytically determined values for pyraclostrobin decreased to 54 - 93% of nominal. The biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality was observed in the control and at mean measured concentrations of up to and including 0.0166 mg BAS 500 06 F/L, whereas 80% and 100% mortality occurred at the two highest tested concentrations of 0.0393 mg/L and 0.0930 mg/L, respectively. After 96 hours of exposure no sublethal effects were observed for surviving fish. The results are summarized in Table 10.2.1-1.

Table 10.2.1-1: Acute toxicity of BAS 500 06 F on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**Control**	**0.005**	**0.010**	**0.022**	**0.050**	**0.100**
Concentration [mg/L] (mean measured)	**Control**	**0.0056**	**0.0105**	**0.0166**	**0.0393**	**0.0930**
Mortality (96 h) [%]	0	0	0	0	80	100
Symptoms (96 h)	none	none	none	none	none	n.d.
Endpoints [mg BAS 500 06 F/L] (mean measured)						
LC_{50} (96 h)	0.036					
NOEC (96 h)	0.017					

n.d. = not determined; all animals dead

III. CONCLUSION

In a static acute toxicity study with rainbow trout, the LC_{50} (96 h) of BAS 500 06 F was 0.036 mg/L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.017 mg/L (mean measured).

The following fish acute toxicity study was performed with BAS 500 06 F for the registration in Japan. It has not been previously evaluated on the EU-level. As this study does not provide a lower endpoint (compared to the standard acute toxicity study) and as the correct test item application was confirmed by initial analytical measurements, an additional re-calculation of endpoints considering mean measured concentrations of the a.s. is not needed.

Report:	CP 10.2.1/2 [redacted] 2012a BAS 500 06 F - Acute toxicity study in the common carp (Cyprinus carpio) 2012/1250190
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part C.1, OECD 203 (1992)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, juvenile common carp were exposed to nominal concentrations of 0.032, 0.056, 0.10, 0.18 and 0.32 mg BAS 500 06 F/L and a water control in groups of 10 animals in glass aquaria containing 50 L water. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. After 96 hours of exposure no mortality was observed in the control and at the lowest tested concentration of 0.032 mg BAS 500 06 F/L, whereas 10 and 40% mortality was observed at 0.056 mg/L and 0.10 mg/L, respectively. 100% mortality was observed at the two highest tested concentrations of 0.18 and 0.32 mg/L. After 96 hours of exposure, sublethal effects (i.e. swimming at the bottom) were observed for some of the surviving fish in the 0.10 mg/L test item treatment.

In a static acute toxicity study with common carp, the LC_{50} (96 h) of BAS 500 06 F was 0.098 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 0.032 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 0003223026; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304428): 200.7 g/L (nominal: 200 g/L); density: 1.038 g/cm^3.

B. STUDY DESIGN

Test species: Common carp (*Cyprinus carpio*); age: approx. 3 months; mean body length: 6.2 cm (5.6 - 6.9 cm); mean body weight: 2.62 g (1.78 - 3.83 g); supplied by "Fischzucht Rhönforelle GmbH", Gersfeld, Germany.

Test design: Static system (96 hours); 10 fish per aquarium (loading: 0.52 g fish/L) and per concentration, assessments of mortality and symptoms of toxicity within 1 h after start of exposure and 6, 24, 48, 72 and 96 h after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control, 0.032, 0.056, 0.10, 0.18 and 0.32 mg BAS 500 06 F/L (nominal).

Test conditions: Glass aquaria with stainless steel frame (60 x 35 x 40 cm); test volume: 50 L; non-chlorinated, filtered tap water mixed with deionized water; temperature: 22°C - 23°C; pH 8.1 - 8.5; oxygen content: 6.0 mg/L - 8.7 mg/L; total hardness about 100 mg $CaCO_3$/L; conductivity approx. 250 µS/cm; acid capacity about 2.5 mmol/L; photoperiod: 16 h light : 8 h dark; light intensity: approx. 62 lux - 415 lux; no aeration over the first 48 hours, but slight aeration during the subsequent days of exposure due to decreased oxygen content after 48 hours, no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.

Statistics: Descriptive statistics; probit analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning of the test, after 48 hours and, except for the highest concentration, at the end of the test. The measured concentrations of pyraclostrobin ranged from 86.8 to 108.5% of the nominal concentrations at test initiation and from 47.5 to 79.5% of the nominal concentrations at test termination. As the initially measured concentrations confirmed correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure no mortality was observed in the control and at the lowest tested concentration of 0.032 mg BAS 500 06 F/L, whereas 10 and 40% mortality was observed at 0.056 mg/L and 0.10 mg/L, respectively. 100% mortality was observed at the two highest tested concentrations of 0.18 and 0.32 mg/L. After 96 hours of exposure, sublethal effects (i.e. swimming at the bottom) were observed for some of the surviving fish in the 0.10 mg/L test item treatment. The results are summarized in Table 10.2.1-2.

Table 10.2.1-2: Acute toxicity of BAS 500 06 F on common carp (*Cyprinus carpio*)

Concentration [mg/L] (nominal)	**Control**	**0.032**	**0.056**	**0.10**	**0.18**	**0.32**
Mortality (96 h) [%]	0	0	10	40	100	100
Symptoms (96 h) [#]	none	none	none	D	n.d.	n.d.
Endpoints [mg BAS 500 06 F/L] (nominal)						
LC_{50} (96 h)	0.098 (95% confidence limits: 0.080 - 0.13)					
NOEC (96 h)	0.032					

[#] Symptoms after 96 hours of exposure: D = swimming at the bottom
n.d. = not determined; all animals dead

III. CONCLUSION

In a static acute toxicity study with common carp, the LC_{50} (96 h) of BAS 500 06 F was 0.098 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 0.032 mg/L (nominal).

The following acute aquatic invertebrate toxicity study performed with BAS 500 06 F is provided in support of the assessment and has not been previously evaluated on the EU-level.

Report:	CP 10.2.1/3 Funk M., 2004a Effect of BAS 500 06 F on the immobility of Daphnia magna STRAUS in a 48 hours static, acute toxicity test 2004/1004393
Guidelines:	OECD 202, EEC 79/831 A V C 2, EPA 850.1010
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to BAS 500 06 F at nominal concentrations of 0.0056, 0.010, 0.018, 0.032, 0.056 and 0.10 mg/L in 4 replicates per concentration, containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. After 24 and 48 hours of exposure no immobility of daphnids was observed in the control and at test item concentrations of up to and including 0.032 mg BAS 500 06 F/L. After 48 hours of exposure, 25 and 100% of the daphnids were immobile at the two highest tested concentrations of 0.056 and 0.10 mg/L, respectively.

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of BAS 500 06 F was 0.065 mg/L based on nominal concentrations. The NOEC was determined to be 0.032 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304428): 202.7 g/L (nominal: 200 g/L).

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in-house culture (originally obtained from Institute National de Recherché Chimique Appliquée, France), less than 24 hours old at test initiation.

Test design: Static system (48 hours), 6 test concentrations plus control, 4 replicates with 5 daphnids each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC, EC_{50} based on immobility of daphnids.

Test concentrations: Control, 0.0056, 0.010, 0.018, 0.032, 0.056 and 0.10 mg/L (nominal).

Test conditions: Glass vessels; test volume 50 mL; dilution water "M4" (Elendt medium); pH 7.87 - 8.03; oxygen content: 9.11 mg/L - 9.95 mg/L; total hardness: 2.52 mmol/L at test initiation; conductivity: 615 µS/cm at test initiation; temperature 20.0°C - 21.5°C; light intensity: < 1500 lux; photoperiod: 16 h light :8 h dark; no feeding, no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; log-log and probit analysis for calculation of EC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analytically detected concentrations for pyraclostrobin ranged from 83.1 to 100.6% of the nominal concentration at test initiation and from 89.8 to 94.5% of nominal at test termination. As the measured concentrations confirmed correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: After 24 and 48 hours of exposure no immobility of daphnids was observed in the control and at test item concentrations of up to and including 0.032 mg BAS 500 06 F/L. After 48 hours of exposure, 25 and 100% of the daphnids were immobile at the two highest tested concentrations of 0.056 and 0.10 mg/L, respectively. For results see Table 10.2.1-3.

Table 10.2.1-3: Effect of BAS 500 06 F on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Control**	**0.0056**	**0.010**	**0.018**	**0.032**	**0.056**	**0.10**
Immobility (24 h) [%]	0	0	0	0	0	15	70
Immobility (48 h) [%]	0	0	0	0	0	25	100
Endpoints [mg BAS 500 06 F/L] (nominal)							
EC_{50} (48 h)	0.065 (95% confidence limits: 0.057 - 0.075)						
NOEC (48 h)	0.032						

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} of BAS 500 06 F was 0.065 mg/L based on nominal concentrations. The NOEC was determined to be 0.032 mg/L (nominal).

The following algae toxicity study performed with BAS 500 06 F is provided in support of the assessment and has not been previously evaluated on the EU-level.

Report:	CP 10.2.1/4 Hoffmann F., 2008a Effect of BAS 500 06 F on the growth of the green alga Pseudokirchneriella subcapitata 2008/1009325
Guidelines:	OECD 201
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of BAS 500 06 F on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 0.31, 0.77, 1.92, 4.80, 12.0 and 30.0 mg BAS 500 06 F/L (corresponding to mean measured concentrations of 0, 0.39, 0.84, 2.11, 4.80, 13.0 and 34.0 mg/L). Assessment of growth was conducted 0 h, 24 h, 48 h and 72 h after test initiation.

The biological results are based on mean measured concentrations of the test item. No morphological effects on the algae were observed in the control and at mean measured test item concentrations of up to and including 2.11 mg BAS 500 06 F/L. At 4.8 mg/L about 10%, at 13.0 mg/L about 50% and at 34.0 mg/L nearly all cells got round.

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of BAS 500 06 F was determined to be 14.2 mg/L and the E_yC_{50} was 2.4 mg/L, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304428): 202.7 g/L (nominal: 200 g/L).

B. STUDY DESIGN

Test species:	Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.
Test design:	Static system; test duration 72 hours; 6 test concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.
Test concentrations:	Control, 0.31, 0.77, 1.92, 4.80, 12.0 and 30.0 mg/L (nominal); corresponding to mean measured concentrations of 0.39, 0.84, 2.11, 4.80, 13.0 and 34.0 mg/L.
Test conditions:	Erlenmeyer dimple flasks; test volume 60 mL; test medium according to OECD 201; initial cell densities: 1×10^4 cells/mL; pH 8.1 at test initiation and pH 7.82 - 8.04 at test end; temperature: 22°C ± 1°C; continuous light at about 8000 lux; continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.
Statistics:	Descriptive statistics, probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analytically detected concentrations for pyraclostrobin ranged from 115.9 to 142.0% of the nominal concentration at test initiation and from 82.9 to 108.8% of nominal at test termination. The biological results are based on mean measured concentrations.

Biological results: No morphological effects on the algae were observed in the control and at mean measured test item concentrations of up to and including 2.11 mg BAS 500 06 F/L. At 4.8 mg/L about 10%, at 13.0 mg/L about 50% and at 34.0 mg/L nearly all cells got round. The effects on algal growth are summarized in Table 10.2.1-4.

Table 10.2.1-4: Effect of BAS 500 06 F on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**0.31**	**0.77**	**1.92**	**4.80**	**12.0**	**30.0**
Concentration [mg/L] (mean measured)	**Control**	**0.39**	**0.84**	**2.11**	**4.80**	**13.0**	**34.0**
Inhibition in 72 h (growth rate) [%]	-	0.8	7.3	14.7	23.7	44.1	72.7
Inhibition in 72 h (yield) [%]	-	3.6	28.8	49.7	67.2	87.8	97.4
	Endpoints [mg BAS 500 06 F/L] (mean measured)						
E_rC_{50} (0-72 h)	**14.2** (95% confidential limits: 13.1 - 15.4)						
E_rC_{10} (0-72 h)	1.6 (95% confidential limits: 1.4 - 1.7)						
E_yC_{50} (0-72 h)	2.4 (95% confidential limits: 2.3 - 2.5)						
E_yC_{10} (0-72 h)	0.42 (95% confidential limits: 0.38 - 0.46)						

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata*, the E_rC_{50} of BAS 500 06 F was determined to be 14.2 mg/L based on mean measured concentrations.

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Chronic toxicity to fish and aquatic invertebrates
The results obtained in the acute study on *O. mykiss* and *D. magna* with the formulated product BAS 500 06 F are in good agreement with the results expected from the data with the active substance (see reasoning given above). This demonstrates that the formulation does not cause significant unexpected (additional) toxicity to fish and daphnids. No synergisms or additional toxicity is thus expected to occur due to co-formulants. Therefore, the studies conducted with the active substance can be used to assess the chronic risk resulting from BAS 500 06 F application. No further testing with the product is indicated.

CP 10.2.3 Further testing on aquatic organisms

Marine or estuarine organisms
Studies on marine or estuarine species are not required according to EU requirements and no studies have been conducted with the formulation BAS 500 06 F on marine or estuarine organisms. The contamination of estuarine and marine environments is considered to be minimal compared to freshwater habitats adjacent to agricultural land according to the use pattern, the potential route of contamination and the dissipation of the active substance. Thus, the risk to those habitats is covered by the risk assessment for freshwater ecosystems.

Microcosm or mesocosm study
No microcosm or mesocosm study has been performed with the formulated product BAS 500 06 F, however, a mesocosm study has been performed with pyraclostrobin (using the previous representative solo formulation BAS 500 00 F, which is of the same formulation type). The mesocosm study has been evaluated following the RIVM Guidance document (de Jong et al., 2008). Executive summaries of the originally submitted study and the evaluation according to RIVM are provided in M-CA 8.2.
The mesocosm study provided a clear NOEC of 8 µg a.s./L. The ecologically acceptable concentration (NOEAEC) of pyraclostrobin was determined to be between 8 µg a.s./L and 24 µg a.s./L. Based on the results of this study a **$RAC_{mesocosm}$ of 4 µg a.s./L** could be derived (by applying an assessment factor of 2 on NOEC), which was considered for a refined risk assessment (as described above).

Residue data in fish
A respective evaluation has been performed during the previous Annex I inclusion process. No new data are provided. The following gives a brief summary of the previous evaluation:
The log P_{ow} of the active substance pyraclostrobin was determined to be 3.99 (EU Review Report, SANCO/1420/2001-final, September 2004). Hence, a bioaccumulation study in fish has been performed with pyraclostrobin, which is (together with a new fish metabolism study, CA 6.2.5/2) discussed in detail in M-CA 6.2.5 (please refer to CA 6.2.5/1). An apparent steady state was reached after 2 - 4 days of exposure. The bioconcentration factors for whole fish were 379 - 507 (two labels). The half-life for elimination was 0.9 days. The time for elimination of 90% of the activity varied between 2.8 and 3.0 days. The nature of radioactivity in fish tissues after 28 days of exposure consisted of the parent substance (39 - 74%) and 4 metabolites (2 - 9%). Due to the limited bioaccumulation and the rapid metabolization and excretion of the active substance (and its metabolites), there is no risk of bioaccumulation. In addition, pyraclostrobin dissipates rapidly in water prohibiting continuous exposure.

Residues of pyraclostrobin in fish are of no concern and no accumulation in the food chain is to be expected.

Accumulation in aquatic non-target organisms
A respective evaluation has been performed during the previous Annex I inclusion process. No new data are provided. Bioaccumulation of the active substance pyraclostrobin under natural conditions is not expected to occur (see "Residue data in fish" above). Additional studies are not required or necessary to determine bioaccumulation in aquatic non-target organisms.

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Appendix

The Predicted Environmental Concentrations (PEC) of pyraclostrobin and its major metabolites in surface water and sediment used for the aquatic risk assessment are summarized in the tables below.

Table 10.2.3-1: Worst-case FOCUS Step 1 and 2 $PEC_{sw, max}$ and $PEC_{sed, max}$ values for pyraclostrobin and its major metabolites following application of BAS 500 06 F in cereals and maize

Test substance	$PEC_{sw, max}$ [µg/L]		$PEC_{sed, max}$ [µg/kg dry sediment]		
	Step 1	Step 2 - EU North / South	Step 1	Step 2	
				EU North	EU South
cereals					
pyraclostrobin	17.031	2.299	1160.000	103.437	187.230
BF 500-3	-- #	-- #	20.030	17.430	17.430
BF 500-5	0.256	0.179	-- *	-- *	-- *
BF 500-6	-- #	-- #	304.937	30.933	60.344
BF 500-7	-- #	-- #	182.976	19.014	36.588
BF 500-11 §	0.407	0.266	-- *	-- *	-- *
BF 500-13 §	0.533	0.413	-- *	-- *	-- *
BF 500-14 §	0.573	0.352	-- *	-- *	-- *
maize					
pyraclostrobin	6.813	1.839	462.701	53.062	95.014
BF 500-3	-- #	-- #	8.012	7.947 1)	7.948 1)
BF 500-5	0.102	0.101	-- *	-- *	-- *
BF 500-6	-- #	-- #	121.975	12.723	24.752
BF 500-7	-- #	-- #	73.190	7.850	15.040
BF 500-11 §	0.163	0.158	-- *	-- *	-- *
BF 500-13 §	0.213	0.209	-- *	-- *	-- *
BF 500-14 §	0.229	0.223	-- *	-- *	-- *

§ Metabolites were mainly observed in irradiated water/sediment studies. Accordingly, respective PEC values derived from irradiated water/sediment studies are reported.

The metabolite was not detected in relevant amounts. Accordingly, no PEC_{sw} values were reported.

* The metabolites were not detected in relevant amounts. Accordingly, no PEC_{sed} values were reported.

Table 10.2.3-2: Worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values for pyraclostrobin in different water bodies following applications of BAS 500 06 F in winter cereals (considering drift mitigation by drift reducing nozzles at Step 4 level)

FOCUS Scenarios		Step 3 - edge of field		FOCUS Step 4		
		PEC_{sw} [µg/L]	PEC_{sed} [µg/kg dry sediment]	Step 4 - PEC_{sw} [µg/L]		
				50% N	75% N	90% N
D1	ditch	1.584	8.901	0.792	0.396	0.158
	stream	1.346	0.836	0.673	0.336	0.134
D2	ditch	1.589	5.032	0.794	0.397	0.159
	stream	1.414	4.212	0.707	0.353	0.141
D3	ditch	1.567	1.289	0.783	0.392	0.157
D4	pond	0.065	0.698	0.033	0.016	0.007
	stream	1.180	0.065	0.590	0.295	0.118
D5	pond	0.066	0.649	0.033	0.017	0.007
	stream	1.263	0.242	0.631	0.316	0.126
D6	ditch	1.547	3.807	0.773	0.387	0.155
R1	pond	0.065	0.897	0.042	0.035	0.031
	stream	1.037	4.063	0.518	0.259	0.240
R3	stream	1.463	2.011	0.731	0.365	0.163
R4	stream	1.038	10.583	0.543	0.543	0.543

Table 10.2.3-3: Worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values for pyraclostrobin in different water bodies following applications of BAS 500 06 F in winter cereals (considering drift and runoff mitigation Step 4 level)

FOCUS Scenarios		Step 4 - PEC_{sw} [µg/L]								
		5 m D	5 m D + 50% N	5 m D + 75% N	10 m D	10 m D + 50% N	10 m D + R	15 m D	20 m D	20 m D + R
D1	ditch	0.429	0.214	0.107	0.228	0.114	0.228	0.155	0.118	0.118
	stream	0.491	0.246	0.123	0.260	0.130	0.260	0.178	0.135	0.135
D2	ditch	0.431	0.215	0.108	0.228	0.114	0.228	0.156	0.119	0.119
	stream	0.516	0.258	0.129	0.274	0.137	0.274	0.187	0.142	0.142
D3	ditch	0.424	0.212	0.106	0.225	0.112	0.225	0.154	0.117	0.117
D4	pond	0.056	0.028	0.014	0.040	0.020	0.040	0.031	0.026	0.026
	stream	0.431	0.215	0.108	0.228	0.114	0.228	0.156	0.119	0.119
D5	pond	0.057	0.029	0.014	0.040	0.020	0.040	0.032	0.027	0.027
	stream	0.461	0.231	0.115	0.244	0.122	0.244	0.167	0.127	0.127
D6	ditch	0.419	0.210	0.105	0.222	0.111	0.222	0.152	0.115	0.115
R1	pond	0.056	0.040	0.034	0.046	0.036	0.039	0.042	0.039	0.026
	stream	0.379	0.240	0.240	0.240	0.240	0.201	0.240	0.240	0.104
R3	stream	0.534	0.267	0.163	0.283	0.163	0.283	0.193	0.163	0.147
R4	stream	0.534	0.543	0.543	0.543	0.543	0.248	0.543	0.543	0.130

D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Table 10.2.3-4: Worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values for pyraclostrobin in different water bodies following applications of BAS 500 06 F in spring cereals (considering drift mitigation by drift reducing nozzles at Step 4 level)

FOCUS Scenarios		Step 3 - edge of field		FOCUS Step 4		
		PEC_{sw} [µg/L]	PEC_{sed} [µg/kg dry sediment]	Step 4 - PEC_{sw} [µg/L]		
				50% N	75% N	90% N
D1	ditch	1.759	9.836	0.877	0.437	0.174
	stream	1.389	1.015	0.694	0.347	0.139
D3	ditch	1.569	1.496	0.784	0.392	0.157
D4	pond	0.065	0.586	0.033	0.016	0.006
	stream	1.300	0.257	0.650	0.325	0.130
D5	pond	0.068	0.646	0.034	0.017	0.007
	stream	1.319	0.093	0.659	0.329	0.132
R4	stream	1.038	5.553	0.519	0.267	0.267

Table 10.2.3-5: Worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values for pyraclostrobin in different water bodies following applications of BAS 500 06 F in spring cereals (considering drift and runoff mitigation at Step 4 level)

FOCUS Scenarios		Step 4 - PEC_{sw} [µg/L]								
		5 m D	5 m D + 50% N	5 m D + 75% N	10 m D	10 m D + 50% N	10 m D +R	15 m D	20 m D	20 m D + R
D1	ditch	0.453	0.226	0.113	0.235	0.117	0.235	0.158	0.119	0.119
	stream	0.507	0.254	0.127	0.269	0.134	0.269	0.184	0.140	0.140
D3	ditch	0.425	0.212	0.106	0.225	0.113	0.225	0.154	0.117	0.117
D4	pond	0.056	0.028	0.014	0.040	0.020	0.040	0.031	0.026	0.026
	stream	0.474	0.237	0.119	0.252	0.126	0.252	0.172	0.131	0.131
D5	pond	0.058	0.029	0.015	0.041	0.021	0.041	0.033	0.027	0.027
	stream	0.481	0.241	0.120	0.255	0.128	0.255	0.174	0.133	0.133
R4	stream	0.379	0.267	0.267	0.267	0.267	0.201	0.267	0.267	0.104

D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

Table 10.2.3-6: Worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values for pyraclostrobin in different water bodies following application of BAS 500 06 F in <u>maize</u> (considering drift and runoff mitigation at Step 4 level)

FOCUS Scenarios		Step 3		Step 4 - PEC_{sw} [µg/L]						
		PEC_{sw} [µg/L]	PEC_{sed} [µg/kg dry sediment]	50% N	75% N	90% N	5 m D	5 m D + 50% N	10 m D	10 m D +R
D3	ditch	1.037	0.719	0.519	0.259	0.104	0.340	0.170	0.180	0.180
D4	pond	0.042	0.312	0.021	0.010	0.004	0.037	0.019	0.027	0.027
	stream	0.895	0.067	0.448	0.224	0.089	0.377	0.188	0.200	0.200
D5	pond	0.042	0.304	0.021	0.010	0.004	0.037	0.019	0.027	0.027
	stream	0.888	0.030	0.444	0.222	0.089	0.374	0.187	0.198	0.198
D6	ditch	1.029	0.388	0.514	0.257	0.103	0.337	0.168	0.179	0.179
R1	pond	0.042	0.387	0.026	0.018	0.013	0.038	0.024	0.030	0.027
	stream	0.720	2.006	0.360	0.180	0.153	0.303	0.153	0.161	0.161
R2	stream	0.963	3.593	0.482	0.241	0.096	0.405	0.203	0.215	0.215
R3	stream	1.011	1.859	0.505	0.253	0.193	0.425	0.213	0.225	0.225
R4	stream	0.703	6.572	0.351	0.301	0.301	0.301	0.301	0.301	0.157

D = Drift mitigation using no-spray buffer zones, N = drift mitigation due to drift reducing nozzle techniques,
R = runoff mitigation by vegetated filter strips.

CP 10.3 Effects on arthropods

The new representative formulation BAS 500 06 F was not evaluated within the previous Annex I inclusion process. It is an emulsifiable concentrate (EC) containing 200g/L pyraclostrobin.

CP 10.3.1 Effects on bees

The EU agreed endpoints for the active substance pyraclostrobin as described in the EU Review Report (SANCO/1420/2001-final, September 2004) plus endpoints from new studies with the active substance and the new representative formulation BAS 500 06 F are used for the risk assessment on honeybees (see Table 10.3.1-1).

Table 10.3.1-1: Ecotoxicological endpoints for honeybees and bumblebees

Test substance	EU agreed endpoints	Endpoints used in risk assessment
Studies on adult honeybees		
Pyraclostrobin	oral (48 h) LD_{50} > 73.1 µg a.s./bee contact (48 h) LD_{50} > 100.0 µg a.s./bee	oral (48 h) LD_{50} > 73.1 µg a.s./bee contact (48 h) LD_{50} > 100.0 µg a.s./bee
Pyraclostrobin [1)]	--	oral (48 h) LD_{50} > 110.0 µg a.s./bee contact (48 h) LD_{50} > 100.0 µg a.s./bee
BAS 500 06 F	--	oral (48 h) LD_{50} > 381.84 µg/bee contact (72 h) LD_{50} > 368.20 µg/bee
Studies on adult bumblebees		
Pyraclostrobin	--	Oral (96 h) LD_{50} > 97.2 µg a.s./bumblebee Contact (96 h) LD_{50} > 100 µg a.s./bumblebee
Field studies		
BAS 500 06 F	--	Semi-field tunnel test (*Phacelia tanacetifolia*): no unacceptable lethal or sublethal effects on honeybee colonies exposed to 1.25 L product/ha.
Residue studies		
Pyraclostrobin [2)]	--	Applied rate: 143 g pyraclostrobin/ha Highest residue: 16.6 mg pyraclostrobin/kg (90^{th} percentile, found in sunflower pollen)
Pyraclostrobin [2)]	--	Applied rate: 143 g pyraclostrobin/ha Highest residue: 6.7 mg pyraclostrobin/kg (90^{th} percentile, found in oilseed rape pollen)

[1)] New acute study has been conducted since the results for the reference substance were out of range in the original study; the results of the new study will be used in the risk assessments below.

[2)] Study was carried out with BAS 556 03 F as a surrogate for pyraclostrobin. BAS 556 03 F contains 130 g pyraclostrobin/L and 80 g metconazole/L and is an EC formulation like BAS 500 06 F. The study is presented as additional information.

Overall summary

Data on BAS 500 06 F and its potential effects on honeybees are evaluated and appropriate risk assessments are provided for the active substance and the formulation based on the already registered use pattern in cereals and maize (critical GAP: maximum 2 x 250 g pyraclostrobin per ha).

The endpoints and calculated HQs for acute oral and acute contact exposure of honeybees to pyraclostrobin and BAS 500 06 F (see Table 10.3.1-2) are below the trigger value of 50 demonstrating low acute risk to honeybees.

Table 10.3.1-2: Toxicity data, maximum single application rates and HQ values for honeybees

Test substance	Use pattern	Exposure route	Endpoint [µg/bee]	Maximum single application rate	Hazard quotient (HQ)	HQ assessment trigger
Risk assessment on adult honeybees						
Pyraclostrobin	cereals, maize	acute oral	$LD_{50} > 110$	250 g a.s./ha	< 2.3	50
		acute contact	$LD_{50} > 100$		< 2.5	
BAS 500 06 F		acute oral	$LD_{50} > 381.84$	1305 g/ha *	< 3.4	
		acute contact	$LD_{50} > 368.20$		< 3.5	

* Taking into account the density of BAS 500 06 F of 1.044 g/cm^3.

Two studies were conducted with pyraclostrobin containing products in two different crops, sunflower and oilseed rape (OSR) to determine the residues in the bee relevant matrices pollen and nectar. The highest exposure was found in pollen, i.e. 16.6 mg/kg (sunflower) and 6.7 mg/kg (OSR).

Also for non-*Apis* bees currently no risk assessment scheme exists under Regulation (EC) No 1107/2009. Nevertheless, the potential acute toxicity (oral and contact) of pyraclostrobin to adult bumblebees was addressed in a laboratory study (BASF DocID 2016/1000530), which is described in M-CA 8.3.1.1).

Furthermore, no unacceptable effects were observed in a semi-field tunnel study conducted with BAS 500 06 F on honeybee colonies in flowering *Phacelia tanacetifolia* with focus on bee brood at a rate of 1.25 L/ha. The study was carried out according to EPPO Guideline 170 (4) and the OECD Guidance Document on Honey Bee (*Apis mellifera* L.) "Brood Test under Semi-Field Conditions, Series on Testing and Assessment, No. 75, August 2007".

The test item caused no unacceptable effects on honeybee survival, colony development and colony strength when applied at a rate of 1.25 L BAS 500 06 F per ha under semi-field conditions (tunnel) to *P. tanacetifolia* during active foraging conditions. The brood termination rates, the brood indices as well as the brood compensation indices indicate no test item related effect on the brood development following the labelling of the eggs.

Overall conclusion:

The proposed uses of BAS 500 06 F in cereals and maize present a low risk to honeybees and will not adversely affect honeybees or honeybee colonies.

Toxicity

Table 10.3.1-3 presents the results of honeybee and bumblebee toxicity studies. For pyraclostrobin study summaries please refer to M-CA 8.3. A semi-field tunnel study has been carried out in support of the assessment of pyraclostrobin as contained in BAS 500 06 F. Further details regarding the tests with the formulation are provided in M-CP 10.3.1.1.

Table 10.3.1-3: Summary of endpoints of pyraclostrobin and BAS 500 06 F to honeybees and bumblebees

Substance	Endpoint	LD_{50} [µg/bee]	Reference (BASF DocID)
Studies on adult honeybees			
Pyraclostrobin	48 h oral LD_{50}	> 73.1	1999/11457
	48 h contact LD_{50}	> 100	
Pyraclostrobin [1)]	48 h oral LD_{50}	> 110	2013/1003210
	48 h contact LD_{50}	> 100	
BAS 500 06 F	48 h oral LD_{50}	> 381.84	2004/1015008
	72 h contact LD_{50}	> 368.20	
Studies on adult bumblebees			
Pyraclostrobin	96 h oral LD_{50}	> 97.2 µg a.s./bee	2016/1000530
	96 h contact LD_{50}	> 100 µg a.s./bee	
Field studies			
BAS 500 06 F	Semi-field cage test (*Phacelia tanacetifolia*): no unacceptable lethal or sublethal effects on honeybee colonies exposed to 1.25 mL product/ha.		2011/1112669
Residue studies			
BAS 556 03 F [2)] containing 130 g pyraclostrobin/L	Applied rate: 143 g pyraclostrobin/ha Highest residue: 16.6 mg pyraclostrobin/kg (90th percentile, found in sunflower pollen)		2014/1000204
BAS 556 03 F [2)] containing 130 g pyraclostrobin/L	Applied rate: 143 g pyraclostrobin/ha Highest residue: 6.7 mg pyraclostrobin/kg (90th percentile, found in oilseed rape pollen)		2014/1000182

1) New acute study has been conducted since the results for the reference substance were out of range in the original study; the results of the new study will be used in the risk assessments below.

2) Study was carried out with BAS 556 03 F as a surrogate for pyraclostrobin. BAS 556 03 F contains 130 g pyraclostrobin/L and 80 g metconazole/L and is an EC formulation like BAS 500 06 F. The study is presented as additional information.

Exposure

Applications of pesticides can potentially result in exposure of honeybees either through direct over-spray, or by contact with residues on plants while bees are foraging for food, but cereals and maize are crops of low attractiveness to foraging bees. However, in order to consider a worst-case scenario, the maximum application rate for pyraclostrobin as applied with the maximum recommended rate of 2 x 1.25 L BAS 500 06 F per ha in cereals is used for the risk assessment. The use in cereals is also covering the intended use of 1 x 1.0 L BAS 500 06 F per ha in maize.

Table 10.3.1-4: Critical use pattern of BAS 500 06 F

Crop	Application time (BBCH growth stage)	Number of applications	Interval [d]	Application rate per treatment	
				Pyraclostrobin [g a.s./ha]	BAS 500 06 F [L/ha]
cereals	25 - 69	2	21	250	1.25
maize	30 - 65	1	--	200	1.0

Risk assessment for bees

The acute risk to honeybees from the use of BAS 500 06 F in cereals (and maize) was assessed using the maximum single application rate and the LD_{50} values to calculate hazard quotients [*EPPO/OEPP, 2003: Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(2)). Bulletin OEPP/EPPO Bulletin 33: 141-145*] as follows:

$$\text{Hazard Quotient (HQ)} = \frac{\text{Maximum application rate [g formulation/ha]}}{\text{Acute } LD_{50} \text{ [µg formulation/bee]}}$$

HQs for honeybees were calculated for oral exposure and contact exposure to BAS 500 06 F. An HQ < 50 indicates low risk to honeybees in the field.

Table 10.3.1-5: Risk to honeybees from exposure to pyraclostrobin and BAS 500 06 F considering the worst-case application rate

Test substance	Application rate [g/ha]	Endpoint	LD_{50} [µg/bee]	Hazard quotient HQ	Trigger
Risk assessment on adult honeybees					
Pyraclostrobin[1)]	250	48 h oral	> 110	< 2.3	50
		48 h contact	> 100	< 2.5	
BAS 500 06 F	1305 g/ha *	48 h oral	> 381.84	< 3.4	
		72 h contact	> 368.20	< 3.5	

* Taking into account the density of BAS 500 06 F of 1.044 g/cm^3.

1) New acute study has been conducted since the results for the reference substance were out of range in the original study; the results of the new study will be used in the risk assessments below.

The calculated HQs for acute oral and acute contact exposure of honeybees to pyraclostrobin and BAS 500 06 F are below the trigger value of 50. Therefore, low risk to honeybees is expected from the application of BAS 500 06 F in cereals and maize.

In an acute toxicity study on adult bumblebees with pyraclostrobin, the oral LD_{50} after 96 h was ≥ 97.2 µg a.s./bumblebee, while the contact LD_{50} after 96 h was > 100 µg a.s./bumblebee. As currently no risk assessment scheme exists, these results are presented as additional information.

Risk Assessment Based on Higher Tier Studies

In addition to the laboratory tests, a semi-field study (tunnel test) was conducted with BAS 500 06 F according to EPPO Guideline 170 (4) and the OECD Guidance Document on Honey Bee (*Apis mellifera* L.) "Brood Test under Semi-Field Conditions, Series on Testing and Assessment, No. 75, August 2007". The test was carried out to gain additional information about the potential toxicity of BAS 500 06 F covering effects on bee brood under more realistic conditions. The product was applied at a rate of 1.25 L/ha (equivalent to 1305 g/ha) during active foraging of the honeybees onto flowering *Phacelia tanacetifolia* enclosed within a tunnel. Details of the study are given in chapter M-CP 10.3.1.5.

The test item caused no unacceptable effects on honeybee survival, colony development and colony strength, when applied at a rate of 1.25 L BAS 500 06 F/ha under semi-field conditions (tunnel) to *Phacelia tanacetifolia* during active foraging conditions. The brood termination rates, the brood indices as well as the brood compensation indices indicate no test item related effect on the brood development following the labelling of the eggs.

Thus, the results of the higher tier study confirm the assessment based on the HQs, indicating that the use of BAS 500 06 F according to good agricultural practice presents low risk to honeybees and will not adversely affect honeybee colonies.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

Report:	CP 10.3.1.1.1/1 Bocksch S., 2004a Assessment of side effects of BAS 500 06 F to the honey bee, Apis mellifera L. in the laboratory 2004/1015008
Guidelines:	OECD 213, OECD 214
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

In a dose response test, young adult worker bees (*Apis mellifera carnica*) were exposed orally to BAS 500 06 F. Therefore, nominal concentrations of 12.5, 25.0, 50.0, 100.0 and 150.0 µg a.s./bee were tested, resulting in an actual uptake of 10.61, 22.99, 33.52, 58.98 and 75.25 µg a.s./bee. Additionally, honeybees were treated with Perfekthion as reference item at 0.09 to 0.21 µg/bee (nominal) or with a solution of water and sugar as a control. The test was conducted with 5 replicates per treatment group; each replicate contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

No mortality was observed after 48 hours in the control. In the test item treatments mortality between 0.0 and 50% after 48 hours, but no behavioral abnormalities of the surviving bees were observed.

The oral LD_{50} value (48 h) for BAS 500 06 F was 73.15 µg a.s./bee, equivalent to 381.84 µg BAS 500 06 F per bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 200 g/L (202.7 g/L analyzed); density: 1.044 g/cm^3.

Test species: Honeybees *Apis mellifera carnica*; young adult worker bees; derived from a healthy colony which descended from a breeding line of a beekeeper in Rheinland-Pfalz, Germany, collected one day before test start.

B. STUDY DESIGN

Test design: Dose response test for oral toxicity; duration 48 h; 5 replicates per treatment group, each replicate consisting of 10 bees per cage; assessment of mortality after 4, 24 and 48 hours.

Endpoints: Mortality, resulting in an LD_{50} value; behavioral abnormalities.

Reference item: Perfekthion (dimethoate, nominal 400 g/L).

Test concentrations: Control, 12.5, 25.0, 50.0, 100.0 and 150.0 µg a.s./bee (nominally equivalent to 65.3, 130.5, 261.0, 522.0 and 783.0 µg product/bee); resulting in an actual uptake of 10.61, 22.99, 33.52, 58.98 and 75.25 µg a.s./bee (nominally equivalent to 55.4, 120.0, 175.0, 307.9 and 392.8 µg product/bee).

Test conditions: Temperature: 24 – 27°C; relative humidity: 50% - 73%; photoperiod: 24 h darkness.

Statistics: Descriptive statistics, Probit analysis for LD_{50} value.

II. RESULTS AND DISCUSSION

No mortality was observed after 48 hours in the control. In the test item treatments mortality between 0.0 and 50% after 48 hours was observed. The LD_{50} was determined to be 73.15 µg a.s./bee, equivalent to 381.84 µg product/bee. No behavioral abnormalities of the surviving bees could be observed. The results are summarized in Table 10.3.1.1.1-1.

Table 10.3.1.1.1-1: Toxicity of BAS 500 06 F to honeybees (*Apis mellifera*) in an oral toxicity test

Treatment [µg a.s./bee]	Uptake of test item [µg a.s./bee]	Mortality [%]	
		24 h	48 h
Control	--	0.0	0.0
12.5	10.61	0.0	0.0
25.0	22.99	0.0	0.0
50.0	33.52	14.0	14.0
100.0	58.98	34.0	36.0
150.0	75.25	46.0	50.0
Endpoint			
	[µg a.s./bee]	**[µg formulation/bee]**	
LD_{50} (48 h)	73.15 (63.73 - 90.67 [1)])	381.84	

[1)] Upper and lower confidential limits, $\alpha = 0.05$.

The LD_{50} value (24 h) for the reference item in the oral toxicity test was determined to be 0.15 µg a.s./bee.

III. CONCLUSION

The oral LD_{50} value (48 h) for BAS 500 06 F was 73.15 µg a.s./bee, equivalent to 381.84 µg BAS 500 06 F per bee.

CP 10.3.1.1.2 Acute contact toxicity to bees

Report:	CP 10.3.1.1.2/1 Bocksch S., 2004a Assessment of side effects of BAS 500 06 F to the honey bee, Apis mellifera L. in the laboratory 2004/1015008
Guidelines:	OECD 213, OECD 214
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

In a dose response test, young adult worker bees (*Apis mellifera carnica*) were exposed to BAS 500 06 F. The toxicity of the test product was determined in a contact dose response test at nominal concentrations of 12.5, 25.0, 50.0, 100.0 and 150.0 µg a.s./bee. Additionally, honeybees were treated with Perfekthion as reference item at 0.14 to 0.30 µg/bee or with water as a control. The test was conducted with 5 replicates per treatment group, each replicate contained 10 bees. Assessment of mortality was done after 4, 24, 48 and 72 hours.

No mortality was observed in the control. In the test item treatment mortality between 0.0 and 100.0% was observed after 72 hours. No behavioral abnormalities of the surviving bees could be observed.

The contact LD_{50} value (72 h) for BAS 500 06 F was 70.53 µg a.s./bee, equivalent to 368.2 µg BAS 500 06 F per bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 200 g/L (202.7 g/L analyzed); density: 1.044 g/cm^3.

Test species: Honeybees *Apis mellifera carnica*; young adult worker bees; derived from a healthy colony which descended from a breeding line of a beekeeper in Rheinland-Pfalz, Germany, collected one day before test start.

B. STUDY DESIGN

Test design: Dose response test for contact toxicity; duration 72 h; 5 replicates per treatment group, each replicate consisted of 10 bees per cage; assessment of mortality after 4, 24, 48 and 72 hours.

Endpoints: Mortality, resulting in a LD_{50} value; behavioral abnormalities.

Reference item: Perfekthion (dimethoate, nominal 400 g/L).

Test concentrations: Control, 12.5, 25.0, 50.0, 100.0 and 150.0 µg a.s./bee (nominally equivalent to 65.3, 130.5, 261.0, 522.0 and 783.0 µg product/bee).

Test conditions: Temperature: 24 – 27°C; relative humidity: 50% - 73%; photoperiod: 24 h darkness.

Statistics: Descriptive statistics, Probit analysis for LD_{50} value.

II. RESULTS AND DISCUSSION

No mortality was observed in the control. In the test item treatment mortality between 0.0% and 100.0% was observed after 72 hours. The LD_{50} was determined to be 70.53 µg a.s./bee, equivalent to 368.2 µg product/bee. No behavioral abnormalities of the surviving bees could be observed. The results are summarized in Table 10.3.1.1.2-1.

Table 10.3.1.1.2-1: Toxicity of BAS 500 06 F to honeybees (*Apis mellifera*) in a contact toxicity test

Treatment [µg a.s./bee]	Mortality [%]		
	24 h	48 h	72 h
Control	0.0	0.0	0.0
12.5	0.0	0.0	0.0
25.0	0.0	0.0	0.0
50.0	20.0	20.0	20.0
100.0	60.0	78.0	78.0
150.0	62.0	96.0	100.0
Endpoint			
	[µg a.s./bee]		[µg formulation/bee]
LD_{50} (72 h)	70.53 (63.46 - 77.88 [1])		368.2

[1] Upper and lower confidential limits, p = 0.05.

The LD_{50} value (24 h) for the reference item in the contact toxicity test was determined to be 0.17 µg a.s./bee.

III. CONCLUSION

The contact LD_{50} value (72 h) for BAS 500 06 F was 70.53 µg a.s./bee, equivalent to 368.2 µg BAS 500 06 F per bee.

CP 10.3.1.2 Chronic toxicity to bees

According to SANCO/10606/2014 (May 16th, 2014) a study on chronic toxicity on honeybees is not required for AIR 3 substances submitted before January 1st, 2015. In addition, this data point is covered by M-CA 8.3.1.3

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

As BAS 500 06 F does not pose an unacceptable risk to honeybees, further tests are not necessary.

CP 10.3.1.4 Sub-lethal effects

As BAS 500 06 F does not pose an unacceptable risk to honeybees, further tests are not necessary.

CP 10.3.1.5 Cage and tunnel tests

A tunnel study with flowering *Phacelia* as food source was conducted to address chronic effects and effects on bee brood of the active substance pyraclostrobin. This study was conducted with the representative solo-formulation BAS 500 006 F since the test design requires spray application to flowering plants. The study is considered to also address the data point for the active substance.

Report:	CP 10.3.1.5/1 Barth M., 2012a Effects of BAS 500 06 F on the honeybee Apis mellifera L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development 2011/1112669
Guidelines:	EPPO PP 1/170 (4) (2010), OECD 75 (2007)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

A tunnel test was carried out to determine the effects of BAS 500 06 F on honeybee colonies under semi-field conditions. For this purpose, BAS 500 06 F was applied at a rate of 1250 mL BAS 500 06 F/ha to flowering *Phacelia tanacetifolia* with foraging honeybees being present in the crop. Additionally, a water treated control and a reference item were included in the study. Each of the three treatment groups was replicated four times, with one honeybee colony per tunnel. Mortality of the honeybees was assessed daily from 2 days before to 28 days after application (DAT), respectively. Foraging activity was assessed from DAT -2 to DAT 28. Sub-lethal effects were recorded daily. Colony conditions were assessed 1 day prior to application and on DAT 4, 10, 17, 22 and 28. Brood status assessments (brood indices and brood compensation indices) were carried out on brood fixing day (BFD) 0, 5, 11, 18 and 23.

No increased mortality in the test item treatment compared to the control was observed at any time during the test; neither directly after application between DAT 0 – DAT 7, nor during post-exposure between DAT 7 – 28, nor at overall comparisons. Exposure of honeybees to the reference item did not increase the number of dead adults.
No increased mortality of honeybee larvae and pupae was observed in the control, test item and reference item treatments on the days before application, indicating comparable, healthy and well adapted colonies. Throughout the test period between DAT 0 – 28, no statistically significant numbers of dead larvae or pupae were found in the control or test item treatment, in contrast to the period between DAT 8 and DAT 22 in the reference item treatment.
Exposure of honeybees to the test item did not result in abnormal behavior or intoxication symptoms.
Colony strength was similar in all three test treatment groups and was not significantly affected by the test item or the reference item in comparison to the control, respectively.
The mean brood termination rate on BFD 23 was 26.2 and 27.0% in the control and in the test item treatment, respectively, and no significant effect of the test item on brood development was detected. The brood indices of the control and the test item treatments showed no statistically significant differences at any assessment day, while the reference item treatment significantly decreased brood indices at BFD 18 and 23, respectively.

Under semi-field conditions (tunnel test), BAS 500 06 F was applied in a single application at a rate of 1250 ml/ha (equivalent to 250 g a.s./ha) to flowering *Phacelia tanacetifolia* during active foraging conditions. No unacceptable effects on mortality, foraging activity, colony development, colony strength or bee brood were observed after application. Overall, based on the results of this study, BAS 500 06 F does not adversely affect honeybee colonies.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 0003223026, content of a.s: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 200.7 g/L (nominal 200.0 g/L), density: 1.038 g/cm^3.

Test species: *Apis mellifera carnica* P. (honeybees); healthy small bee colonies, one body containing 11 combs, including 4 – 9 brood combs with all brood stages present and 8 – 10 combs with food; source: local beekeeper in Leipzig/Rehbach, Germany.

B. STUDY DESIGN

Test plots: The test site was located near Cunnersdorf, Germany; separate tunnels for the different groups and replicates; tunnel size: 18 m × 6 m × 2.5 m (length × width × height); effective crop area: 93.5 m^2; after bee flight of the last day of exposure, the colonies were moved to a monitoring site without flowering crops or intensive agriculture near D-04828 Neualtenbach, Germany, where further assessments were performed.

Test design: Honeybee semi-field test in *Phacelia tanacetifolia*; three treatment groups (control, test item group, reference item); exposure of the bees to control, test item and reference item treatments during flowering of *Phacelia* in separate tunnels; 1 colony/tunnel and 4 tunnels/treatment group; honeybee colonies were introduced to the tunnels 3 days before application; post-application exposure period was 7 days; post-application assessments of mortality on 28 d in dead bee traps and 7 d on gauze sheets; assessment of foraging activity for 7 d on three 1 m^2 plots/tunnel; behavior, colony health, colony strength, general and detailed brood development in marked cells; after the exposure phase, colonies were removed from the tunnels and further brood development was assessed until 28 days after treatment (DAT).

Endpoint: Mortality: daily assessment starting 2 days before to 7 and 28 days after application;
Foraging activity: daily assessment starting 2 days before to 7 and 28 days after application;
Sublethal effects: behavioral changes were monitored daily until test end;
Colony assessments: (food stores, brood status and colony strength): 1 day before and 4, 10, 17, 22 and 28 days after treatment application (DAT) (equal to brood fixing day (BFD) 0, 5, 11, 18, 23 and 29);
Detailed brood status: 1 day before (= BFD 0) and 4 (= BFD 5), 10 (= BFD 11), 17 (= BFD 18) and 22 (= BFD 23) days after the application.

Reference item: Insegar 25 WG (fenoxycarb, 250 g/kg nominal).

Application rates: BAS 500 06 F: 1250 mL/ha; control: tap water (400 L/ha); reference item: 1200 g/ha Insegar 25 WG 8corresponding to 300 g a.s./ha); all substances were applied in 400 L/ha water.

Test conditions: Natural field conditions. Good weather conditions during application; cloud coverage: 20 – 100%; wind: 0 – 0.3 m/s; no precipitation; temperature: 23.7 – 25.1°C; 3.5, 4.0, 2.5 and 7.0 mm precipitation on DAT 1, 4, 5, 6.

Statistics: Descriptive statistics; TUKEY-test ($\alpha = 0.05$) for pre-treatment data evaluation for comparisons between control, test item and reference item treatments.
Post-treatment data evaluation: pair-wise testing for comparisons between treatments (test item or reference item) separately against control, STUDENT-t test (for variance homogeneous data) or WELCH-t test (for variance inhomogeneous data). Mortality and brood termination rate: one-sided greater; brood indices: one-sided smaller. Significance levels of all tests: $\alpha = 0.05$; percentage values of brood termination rate were arcsine-transformed to ensure data homogeneity.

II. RESULTS AND DISCUSSION

The results are summarized in Table 10.3.1.5-1.

Table 10.3.1.5-1: Effects of BAS 500 06 F on honeybee mortality, foraging activity and bee brood under semi-field conditions (tunnel test)

Parameter	Control	BAS 500 06 F [1.25 L/ha]	Reference Item [1.2 kg/ha]
Mean mortality of worker bees / colony / day [n] 1)			
pre-application phase 2)	32.2	33.3	36.1
exposure phase in the tunnels 2)	27.4	29.5	29.0
phase outside the tunnels 3)	9.2	9.5	8.8
overall after application	14.2	15.1	14.4
Mean mortality of pupae / colony / day [n] 1)			
pre-application phase	0.1	0.0	0.0
exposure phase in the tunnels	0.1	0.0	0.1
phase outside the tunnels 3)	0.3	0.1	9.3
overall after application	0.2	0.1	6.8
Mean foraging activity / m^2 colony / day [n] 1)			
pre-application phase	12.5	13.1	12.3
exposure phase in the tunnels	12.5	12.4	12.3
Mean brood termination rate at BFD 22 [%] 1)			
	26.2	27.0	57.8 *
Mean brood index at BFD 22 [%] 1)			
	3.7	3.7	2.1 *
Mean brood compensation index at BFD 22 [%] 1)			
	3.9	4.1	2.4 *

1) Each with 4 tunnels (replicates).
2) Sum of dead honeybees found in dead bee traps and on gauze strips in the tunnels.
3) Dead honeybees found in dead bee traps, only.
* Statistically significantly different (comparing treatment vs. control, STUDENT-t test or WELCH-t test, $\alpha = 0.05$) at post-application period; mortality: one-sided greater; foraging activity: one-sided smaller; brood termination rate: one-sided greater, brood index and brood compensation index: one-sided smaller.
BFD = brood fixing day

Mortality
Adult honeybees: Pre-application mortality was similarly low in control, test item and reference item treatments, indicating well adapted and comparable colonies. No statistically significant differences between the three treatment groups were observed between DAT -2 and DAT 0, as well as at overall comparisons before application (TUKEY-test, two-sided, $\alpha = 0.05$).
No increased mortality in the test item treatment compared to the control was observed at any time during the test; neither directly after application between DAT 0 – DAT 7, nor during post-exposure between DAT 7 – 28, nor at overall comparisons (STUDENT-t or WELCH-t test, one-sided greater, $\alpha = 0.05$). Statistically significant differences (STUDENT-t test, one sided greater, $\alpha = 0.05$) due to very low variability between and within the treatment groups found on DAT 8 only were considered to be not biologically relevant. Exposure of honeybees to the reference item did not increase the number of dead adults.

No increased mortality of honeybee larvae and pupae was observed in the control, test item and reference item treatments on the days before application, indicating comparable, healthy and well adapted colonies. Throughout the test period between DAT 0 – 28, no statistically significant numbers of dead larvae or pupae were found in the control or test item treatment, respectively. Distinctly increased numbers of dead pupae were found between DAT 8 and DAT 22 in the reference item treatment.

Foraging activity
Overall foraging activity before application was 12.5, 13.1 and 12.3 bees/m^2/day in the control, test item and reference item treatments, indicating that the honeybees were equally adapted to the environmental conditions. Shortly before application, foraging activity was average 12.9, 13.4 and 12.3 bees/m^2 in the control, test item and reference item treatments respectively, indicating sufficient exposure of the bees during application. Foraging activity in the test item group was slightly reduced for 1 hour after application before returning to pre-application and control level. During the remaining course of the test, the overall daily means of foraging activity in the test item and reference item treatments were not statistically significantly different from the control and the reference item (STUDENT-t test, one sided-smaller, $\alpha = 0.05$). Overall mean foraging activity in these groups was 97 and 93% compared to the foraging activity shortly before application. Foraging activity in the reference item group was slightly reduced on DAT 0. During the remaining post-application period from DAT 1 – 28, the overall foraging activity of the reference item treatment group was not statistically significantly different from the control (STUDENT-t test, one sided-smaller, $\alpha = 0.05$).

Bee behavior
Exposure of honeybees to the test item did not result in abnormal behavior or intoxication symptoms. Bees were calm and actively foraging nectar and pollen on the treated *Phacelia tanacetifolia* field.

Colony strength
Colony assessment (estimated number of bees/colony) on DAT -1 was similar in the control, test item and reference item treatment groups, respectively. During the post-application period, the development of colony strength was similar in both, the control and the test item treatment group. In contrast, the colony strength of the reference item treatment group was fluctuating strongly in the course of the study and increased only slightly in comparison to the control. The mean number of bees per colony in the three treatment groups one day before application was similar (5709 to 6300 mean number of bees per colony).

Table 10.3.1.5-2: Estimated average number of honeybees/colony:

Treatment group	DAT -1	DAT 4	DAT 10	DAT 17	DAT 22	DAT 28
Control	5709	4922	6722	7959	7959	8156
BAS 500 06 F	5484	5738	6975	8972	9563	8916
Reference item	6300	6075	8466	7931	7144	7481

General brood assessments – brood area
On DAT -1, honeybee queens were in healthy condition actively laying eggs and brood production was at an equal level in all test colonies.
The total mean brood nest area (sum of comb area occupied by eggs, larvae and capped cells) increased comparable during the course of the study in both the control and test item treatment group. The mean comb area of the test item treatment group contained eggs, larvae or pupae at an equal amount as the control.
The total mean brood nest size of the reference item treatment group was on a lower level and decreasing constantly on assessments performed on DAT 4 and DAT 28 in comparison to the control. The effect of the reference item on brood development demonstrated the sensitivity of the test system to detect possible effects on brood development.

Detailed brood assessments in marked cells
Brood termination rate (%)
The mean brood termination rate on BFD 23 was 26.2 and 27.0% in the control and in the test item treatment, respectively, and no significant effect of the test item on brood development was detected (STUDENT-t test, one-sided greater, $\alpha = 0.05$). An increase on the termination rate of 57.8% in the reference item test group, together with an increased pupal mortality, indicated the suitability of the test system to detect possible effects of the test item on brood development.

Brood index
The brood index is a measure for the normal brood development. It is derived by tracking the development of eggs marked at BFD 0 by assigning a developmental stage specific value to each cell at the BFDs. Therefore, the brood index as an indicator for the bee brood development facilitates a comparison between the different treatments. According to the termination rates, the brood indices of the control and the test item treatments showed no statistically significant differences at any assessment day (STUDENT-t test, one-sided smaller, $\alpha = 0.05$). The reference item treatment statistically significantly decreased brood indices at BFD 18 and 23, respectively (STUDENT-t test, one-sided smaller, $\alpha = 0.05$).

Table 10.3.1.5-3: Mean [1] brood-indices [n]:

Treatment group	BFD 0	BFD 5	BFD 11	BFD 18	BFD 23
Control	1.0	2.1	2.9	3.1	3.7
BAS 500 06 F	1.0	1.9	3.0	3.1	3.7
Reference item	1.0	1.7	2.6	1.7 *	2.1 *

1) Mean of n = 4
* Statistically significant differences compared to the control (STUDENT-t test, one-sided smaller, $\alpha = 0.05$).
BFD = brood fixing day

Brood compensation index
The brood compensation index is a measure for the potential of a colony to compensate for lost brood by refilling cells of terminated brood with new eggs. The brood compensation index shows a continuous brood development in the test item and in the control treatment groups. Despite a great loss of brood stages in the reference item group between BFD 0 and BFD 18, several emptied cells were refilled with eggs, and the brood compensation indices were slightly higher than the corresponding brood indices at these assessment days.

Table 10.3.1.5-4: Mean [1] brood compensation-indices [n]:

Treatment group	BFD 0	BFD 5	BFD 11	BFD 18	BFD 23
Control	1.0	2.1	3.1	3.4	3.9
BAS 500 06 F	1.0	1.9	3.2	3.4	4.1
Reference item	1.0	1.7	2.7	1.8 *	2.4 *

[1] Mean of n = 4
* Statistically significant differences compared to the control (STUDENT-t test, one-sided smaller, α = 0.05).
BFD = brood fixing day

III. CONCLUSION

Under semi-field conditions (tunnel test), BAS 500 06 F was applied in a single application at a rate of 1250 ml/ha (equivalent to 250 g a.s./ha) to flowering *Phacelia tanacetifolia* during active foraging conditions. No unacceptable effects on mortality, foraging activity, colony development, colony strength or bee brood were observed after application. Overall, based on the results of this study, BAS 500 06 F does not adversely affect honeybee colonies.

CP 10.3.1.6 Field tests with honeybees

As BAS 500 06 F does not pose an unacceptable risk to honeybees, further tests are not necessary.

CP 10.3.2 Effects on non-target arthropods other than bees

The new representative formulation was not evaluated within the previous Annex I inclusion process. Consequently, no EU agreed endpoints are available for non-target arthropods. Endpoints from new studies with the new representative formulation BAS 500 06 F are used for the risk assessments (see Table 10.3.2-1).

Table 10.3.2-1: Ecotoxicological endpoints for non-target arthropods

Test substance	Test species	EU agreed endpoints	Endpoints used in risk assessment
Tier I			
BAS 500 06 F	*Typhlodromus pyri*	--	LR_{50} = 0.87 L/ha
BAS 500 06 F	*Aphidius rhopalosiphi*	--	LR_{50} = 0.04 L/ha
Tier II			
BAS 500 06 F	*Typhlodromus pyri* extended laboratory test	--	LR_{50} = 2.45 L/ha No unacceptable effects on reproduction up to 2.5 L/ha
BAS 500 06 F	*Aphidius rhopalosiphi* extended laboratory test	--	LR_{50} > 2.5 L/ha No unacceptable effects on reproduction up to 2.5 L/ha
BAS 500 06 F	*Chrysoperla carnea* extended laboratory test	--	LR_{50} = 0.72 L/ha No unacceptable effects on reproduction up to 0.63 L/ha
BAS 500 06 F	*Chrysoperla carnea* aged residue design	--	No unacceptable effects on survival and reproduction after exposure to fresh dried residues at 1.25 L/ha 0 DAT and after exposure to aged residues at 2.5 L/ha 7 DAT
BAS 500 06 F	*Aleochara bilineata* extended laboratory test	--	No unacceptable effects on reproduction up to 3.75 L/ha

Overall summary

Data on BAS 500 06 F and its potential effects on non-target arthropods are evaluated and appropriate risk assessments are provided based on the already registered use pattern in cereals and maize (critical GAP: maximum 2 x 250 g pyraclostrobin per ha).

The endpoints of non-target arthropods to BAS 500 06 F are shown in Table 10.3.2-2.

Table 10.3.2-2: Effects on other arthropod species

Test substance	Species / Life stage	Test type	Endpoint [L/ha]	PER in-field [L/ha]	PER off-field [L/ha] 1)	HQ in-field	HQ off-field
Tier I							
BAS 500 06 F	*T. pyri* protonymphs	Laboratory test, artificial substrate, 2D	LR_{50} = 0.87	2.125	0.005	**2.4**	0.057
	A. rhopalosiphi adults		LR_{50} = 0.04	2.125	0.005	**53**	0.125
Tier II							
BAS 500 06 F	*T. pyri* protonymphs	Extended laboratory test, natural substrate, 2D	LR_{50} = 2.45 L/ha ER_{50} > 2.5 L/ha	2.125	0.005	Endpoint ≥ PER → acceptable risk	
	A. rhopalosiphi adults	Extended laboratory test, natural substrate, 3D	LR_{50} > 2.5 L/ha ER_{50} > 2.5 L/ha	2.125	0.051	Endpoint ≥ PER → acceptable risk	
	C. carnea larvae	Extended laboratory test, natural substrate, 2D	LR_{50} = 0.72 ER_{50} > 0.63	2.125	0.005	Endpoint < PER 2) → further testing necessary	
	C. carnea larvae	Extended laboratory test, natural substrate, 2D	no unacceptable effects on survival and reproduction after exposure to fresh dried residues at 1.25 L/ha 0 DAT and after exposure to aged residues at 2.5 L/ha 7 DAT	2.125	0.005	Endpoint ≥ PER → acceptable risk	
	A. bilineata adults	Aged-residue design, natural substrate, 2D	ER_{50} > 3.75	2.125	0.051	Endpoint ≥ PER → acceptable risk	

PER = predicted environmental rate, HQ = Hazard Quotient
HQ values in **bold** are above the trigger.

1) In the Tier 2 off-field risk assessment, the standard 5-fold uncertainty (correction) factor should be included to the calculation to cover the inter-species variability in sensitivity of off-field non-target arthropod species. However, as additional species are tested, the uncertainty is reduced and no additional safety factor was applied.

2) Endpoint < $PER_{in\text{-}field}$, $PER_{off\text{-}field}$ is covered.

The risk assessment was conducted for the critical application rate in cereals (covering also maize). Based on the calculated $HQ_{in\text{-}field}$ of *T. pyri* and *A. rhopalosiphi*, a potential in-field risk to non-target arthropods cannot be excluded (HQ > 2) at this level of testing. Therefore, higher Tier II tests were carried out with *T. pyri* and *A. rhopalosiphi* and additionally with the foliage-dweller *Chrysoperla carnea* and the soil-dweller *Aleochara bilineata*.

For *T. pyri*, *A. rhopalosiphi* and *A. bilineata* no risk was indicated for in- and off-field habitats. Based on the results of an extended laboratory study with *C. carnea*, a potential risk for in-field habitats cannot be excluded. Therefore, an aged residue study with *C. carnea* was carried out under more realistic conditions (field application techniques instead of laboratory sprayer, aging under rain protected outdoor conditions instead of laboratory conditions and application on a 3-dimensional substrate (i.e. whole plants) instead of 2-dimensional substrate), which resulted in no unacceptable effects on survival and reproduction at DAT 0 up to 1.25 L/ha and DAT 7 up to 2.5 L/ha. Based on the results of the aged residue study, no in-field and off-field risk for *C. carnea* is indicated.

Overall conclusion:

Taking all available data into account, it can be concluded that low risk for non-target arthropods is expected from the application of BAS 500 06 F in cereals and maize. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

Toxicity

The toxicity of BAS 500 06 F to non-target arthropods has been investigated. The testing and risk assessment strategy used follow the approach recommended in the ESCORT 2 guidance document [*Candolfi et al. (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' From the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000*], ESCORT 3 [*Alix, et al. (2012) 'Linking non-target arthropod testing and risk assessment with protection goals'*] and the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002).

The toxicity of BAS 500 06 F to non-target arthropods has been investigated by carrying out Tier I tests on *Aphidius rhopalosiphi* and *Typhlodromus pyri* and Tier II tests on *Aphidius rhopalosiphi, Typhlodromus pyri*, *Chrysoperla carnea* and *Aleochara bilineata.* These species are tested, in accordance with ESCORT 2 and ESCORT 3, as representative non-target arthropods since they have been found to be particularly sensitive species, and therefore can be considered as indicators of potential effects to the most sensitive arthropods in the field. For convenience, the results of these studies are summarized in Table 10.3.2-3. Study summaries are provided below (see M-CP 10.3.2.1).

Table 10.3.2-3: Summary of toxicity data for BAS 500 06 F to non-target arthropods

Species	Exposed life stage	Study type	Application rate [L/ha]	Corrected mortality [1)] [%]	Sublethal effects [%]	Reference (BASF DocID)
Tier I						
Typhlodromus pyri	Protonymphs	Laboratory test using artificial substrate	0.046 0.139 0.417 1.25 3.75	11.23 18.48 34.78 74.64 94.57	n.d.	2007/1035599
			LR_{50} = 0.87 L/ha			
Aphidius rhopalosiphi	Adults	Laboratory test using artificial substrate	0.006 0.019 0.056 0.167 0.25 0.50	16.22 21.62 59.46 100.00 100.00 100.00	n.d.	2007/1035600 + Amendment 2007/1050841
			LR_{50} = 0.040 L/ha			
Tier II						
Typhlodromus pyri	Protonymphs	Extended laboratory test using natural substrate	0.3125 0.625 1.25 2.50 3.75	12.8 20.5 16.7 53.5 67.1	11.7 24.6 41.3 36.8 n.d.	2008/1010712
			LR_{50} = 2.45 L/ha ER_{50} > 2.5 L/ha			
Aphidius rhopalosiphi	Adults	Extended laboratory test using natural substrate	0.07 0.15 0.30 0.60 1.25 2.50	0 0 0 0 10 20	n.d. n.d. n.d. 0.8 8.7 0.8	2008/1010713
			LR_{50} > 2.5 L/ha ER_{50} > 2.5 L/ha			
Chrysoperla carnea	Larvae	Extended laboratory test using natural substrate	0.31 0.63 1.25 2.50 3.75	8.2 44.9 81.6 93.9 100.0	no effects no effects n.d. n.d. n.d.	2008/1032666
			LR_{50} = 0.72 L/ha ER_{50} > 0.63 L/ha			

Species	Exposed life stage	Study type	Application rate [L/ha]	Corrected mortality [1] [%]	Sublethal effects [%]	Reference (BASF DocID)
Chrysoperla carnea	Larvae	Extended laboratory test using natural substrate	DAT 0 bioassay: 1.25 2.50 DAT 7 bioassay: 1.25 2.50 DAT 14 bioassay: 1.25 2.50	 36.2 66.0 6.3 41.7 2.0 10.0	 no effects n.d. no effects no effects no effects no effects	2008/1042190
			no unacceptable effects on survival and reproduction after exposure to fresh dried residues at 1.25 L/ha 0 DAT and after exposure to aged residues at 2.5 L/ha 7 DAT			
Aleochara bilineata	Adults	Extended laboratory test using natural substrate	2.50 3.75	-- --	14.4 10.6	2008/1010700
			ER_{50} > 3.75 L/ha			

n.d. = not determined; DAT = days after treatment
[1] Negative values indicate an increase, positive a decrease relative to the control.

Exposure

Table 10.3.2-4: Critical use pattern of BAS 500 06 F

Crop	Application time (BBCH growth stage)	Number of applications	Interval [d]	Application rate per treatment	
				Pyraclostrobin [g a.s./ha]	BAS 500 06 F [L/ha]
cereals	25 - 69	2	21	250	1.25
maize	30 - 65	1	--	200	1.0

In-field exposure

Non-target arthropods inhabiting the crop can be exposed to residues of BAS 500 06 F by direct contact, either as a result of overspray or through contact with residues on plants and soil or in food items. BAS 500 06 F is applied at a maximum rate of 2 x 1.25 L/ha in cereals (worst-case scenario). Since the cereal scenario is also covering the maize scenario, no specific risk assessment is presented for maize.

The in-field exposure (predicted environmental rate, PER) is calculated according to ESCORT 2 using the following equation:

$PER_{in\text{-}field}$ = Application rate [L/ha] x MAF

The MAF is a generic multiple application factor, which is used to take into account the potential build-up of applied substances between applications based on the application interval, DT_{50} value and number of applications. Default foliar and soil MAF values following multiple applications are given in the ESCORT 2 Guidance Document. For 2 applications, the default MAF of 1.7 (foliar) and 1.9 (soil) is considered, respectively. However, as a pre-emergence or early post-emergence application is not foreseen according to the proposed use pattern, the PER (soil) will not be used in the following risk assessment.

The maximum predicted environmental rate (PER) occurring within the field after application of BAS 500 06 F at the maximum application rate are presented in Table 10.3.2-5.

Table 10.3.2-5: In-field PER values for application of BAS 500 06 F

Substance	Worst-case application rate	$PER_{in\text{-}field}$ (foliar) [L/ha]
BAS 500 06 F	2 x 1.25 L/ha	2.125

Off-field exposure

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods to BAS 500 06 F living in off-field areas will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure via soil residues in off-field areas was not considered. Off-field foliar PER values were calculated from in-field foliar PERs in conjunction with drift values published by the BBA [*90th percentile drift according to BBA (2000): Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden*] as shown in the following equation:

$$PER_{off\text{-}field} = \frac{\text{maximum } PER_{in\text{-}field} \times (\%\ \text{drift}/100)}{\text{vegetation distribution factor}}$$

The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 10 is recommended by ESCORT 2. For 3-dimensional studies, *i.e.* where the spray treatment is applied onto whole plants, the dilution factor of 10 is not used, as any dilution over the 3-dimensional vegetation surface is accounted for in the study design. The correction factor from the ESCORT 2 formula for off-field exposure calculation is considered for in the respective risk assessment.

The drift value for two applications at 1 m distance in field crops is 2.38% of the application rate (82^{th} percentile drift). The drift factor (% drift/100) is therefore 2.38/100 = 0.0238. The resulting PER off-field values are shown in Table 10.3.2-6.

Table 10.3.2-6: Off-field Predicted Environmental Rates (PER)

Study type	Maximum $PER_{in\text{-}field}$ [L/ha]	drift factor [% drift/100]	Vegetation distribution factor	$PER_{off\text{-}field}$ [L/ha]
2D exposure scenario	2.125	0.0238	10	0.005
3D exposure scenario	2.125	0.0238	--	0.051

Risk assessment for other non-target arthropods

The risk to non-target arthropods is assessed using the approach recommended in the published ESCORT 2 document [*Candolfi et al. 2001*] and the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002).

In-field risk assessment (Tier I)

The potential risk of BAS 500 06 F to in-field non-target arthropods was assessed by calculation of the hazard quotient (HQ) using the $PER_{in\text{-}field}$ and the lowest lethal rate (LR_{50}) values according to the following equation:

$$HQ_{in\text{-}field} = \frac{PER_{in\text{-}field}\ [L/ha]}{LR_{50}\ [L/ha]}$$

The HQ trigger for Tier I laboratory studies is 2. The resulting $HQ_{in\text{-}field}$ values are presented, in Table 10.3.2-7.

Table 10.3.2-7: In-field HQs for non-target arthropods

Species	LR_{50} [L/ha]	In-field		Trigger
		PER [L/ha]	HQ	
T. pyri Tier I, 2D exposure scenario	0.87	2.125	**2.4**	2
A. rhopasoliphi Tier I, 2D exposure scenario	0.04	2.125	**53**	

HQ values in **bold** are above the trigger.

Based on the calculated $HQ_{in\text{-}field}$ of *T. pyri* and *A. rhopalosiphi*, a potential in-field risk to non-target arthropods cannot be excluded (HQ > 2) at this level of testing. According to ESCORT 2, a higher tier study with *T. pyri* and *A. rhopalosiphi* on natural substrate is required, as well as testing of one additional species. Besides the higher tier test with *T. pyri* and *A. rhopalosiphi*, extended laboratory studies were carried out with the foliage-dweller *Chrysoperla carnea* and additionally with the soil-dweller *Aleochara bilineata*.

In-field risk assessment (Tier II)

A summary of the Tier II risk assessment is provided in Table 10.3.2-8.

Table 10.3.2-8: In-field Tier II risk assessment for non-target arthropods

Species	Endpoints [L/ha]	$PER_{in\text{-}field}$ [L/ha]	Trigger
T. pyri, 2D exposure scenario, extended laboratory test	LR_{50} = 2.45 ER_{50} > 2.5	2.125	Endpoint ≥ PER → acceptable risk
A. rhopalosiphi, 3D exposure scenario, extended laboratory test	LR_{50} > 2.5 ER_{50} > 2.5	2.125	Endpoint ≥ PER → acceptable risk
C. carnea, 2D exposure scenario, extended laboratory test	LR_{50} = 0.72 ER_{50} > 0.63	2.125	Endpoint < PER → further testing necessary
C. carnea, 3D exposure scenario, aged residue design	No unacceptable effects on survival and reproduction after exposure to fresh dried residues at 1.25 L/ha 0 DAT and after exposure to aged residues at 2.5 L/ha 7 DAT	2.125	Endpoint ≥ PER → acceptable risk
A. bilineata, 2D exposure scenario, extended laboratory test	ER_{50} > 3.75	2.125	Endpoint ≥ PER → acceptable risk

Typhlodromus pyri:
The rate of 2.45 L/ha (no unacceptable effects on survival observed) and the rate of 2.5 L/ha (no unacceptable effects on reproduction observed) exceeded the $PER_{in\text{-}field}$ of 2.125 L/ha. Therefore, no risk or unacceptable effects are expected for *T. pyri* resulting from the intended use of BAS 500 06 F considering in-field habitats.

Aphidius rhopalosiphi:
The rate of 2.5 L/ha, at which no unacceptable effects on survival and reproduction were observed, exceeded $PER_{in\text{-}field}$ of 2.125 L/ha. Therefore, no risk or unacceptable effects are expected for *A. rhopalosiphi* resulting from the intended use of BAS 500 06 F considering in-field habitats.

Chrysoperla carnea:
A potential in-field risk to non-target arthropods cannot be excluded based on the extended laboratory study. The LR_{50} of 0.72 L/ha and the rate of 0.63 L/ha, at which no unacceptable effects on survival and reproduction were observed, did not exceed the in-field foliar predicted environmental rate (PER) of 2.125 L/ha, respectively. Therefore, an aged residue study with *C. carnea* for a higher tier risk assessment was carried out.
The main criterion for the acceptability of effects on arthropods living in-field is defined as the potential for recovery of any affected population, i.e. demonstrating that residual toxicity declines sufficiently rapidly to allow recovery within one year. This is usually done by aged-residue trials exposing the arthropods to residues, which have been aged for increasing time periods. Thus, aged residue trials are used to show the acceptability of the effects by assessing the decline in residual toxicity of a product. In this case, *C. carnea* was used as bio-indicator, because it proved to be the most sensitive species. According to ESCORT 2, the most sensitive test species identified by dose-response studies using natural substrate is selected for aged residue studies. It is anticipated that for less sensitive non-target arthropods the potential re-colonization will be faster.

The aged residue study presents a more realistic exposure scenario, since the application was done on whole bean plants, representing a 3-dimensional structure, whereas the application in the extended laboratory study was done on detached leaves, representing a 2-dimensional structure. Further, application in this study was carried out under more realistic conditions, i.e. using field application techniques instead of laboratory sprayer and aging under rain protected outdoor conditions instead of laboratory conditions.
In this study, two application rates (1.25 and 2.5 L/ha) were tested. No unacceptable effects on survival and reproduction were observed at DAT 0 following the application of 1.25 L/ha BAS 500 06 F, as well as on DAT 7 and DAT 14 following the application of 2.5 L/ha BAS 500 06 F. This application rate is higher than the maximum $PER_{in\text{-}field}$ of 2.125 L/ha. Therefore, no risk or unacceptable effects are expected for *C. carnea* resulting from the intended use of BAS 500 06 F considering in-field habitats.

Aleochara bilineata:
The rate of 3.75 L/ha, at which no unacceptable effects on reproduction were observed, exceeded the foliar $PER_{in\text{-}field}$ of 2.125 L/ha. Therefore, no risk or unacceptable effects are expected for *A. bilineata* resulting from the intended use of BAS 500 06 F considering in-field habitats.

Off-field risk assessment (Tier I)

In order to assess the potential risk of BAS 500 06 F to off-field non-target arthropods, the predicted environmental rate (Table 10.3.2-6) is compared with the toxicity endpoints according to the following formula:

$$HQ_{off\text{-}field} = \frac{PER_{off\text{-}field}\ [L/ha]}{LR_{50}\ [L/ha]} \times \text{Correction factor}$$

The HQ trigger value for Tier I laboratory studies is 2. Furthermore, ESCORT 2 recommends a correction factor of 10 for Tier I data in the off-field risk assessment to account for extrapolation from testing just two representative species to the higher species diversity expected in off-crop areas. The results are presented in Table 10.3.2-9.

Table 10.3.2-9: Off-field HQ values for non-target arthropods

Species	LR_{50} [L/ha]	$PER_{off\text{-}field}$ [L/ha]	Correction factor	$HQ_{off\text{-}field}$	Trigger value
Typhlodromus pyri, Tier I, 2D exposure scenario	0.87	0.005	10	0.06	2
Aphidius rhopalosiphi, Tier I, 2D exposure scenario	0.04	0.005		1.25	

The calculated $HQ_{off\text{-}field}$ values for *T. pyri* and *A. rhopalosiphi* fall below the trigger value, indicating no unacceptable risk to non-target arthropods in off-field areas resulting from exposure to BAS 500 06 F.

Off-field risk assessment (Tier II)

Both $HQ_{off\text{-}field}$ for the standard indicator species *T. pyri* and *A. rhopalosiphi* were below the trigger value of 2. In addition, the lowest endpoint from extended lab testing (no unacceptable effects on survival and reproduction for *Chrysoperla carnea* in aged residue DAT 0 at 1.25 L/ha) provides sufficient margin of safety to the anticipated off-field PER-values. Therefore, no further assessment is considered necessary.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Report: CP 10.3.2.1/1
Sipos K., 2007a
Effect of BAS 500 06 F on the predatory mite (Typhlodromus pyri) in a laboratory trial
2007/1035599
Guidelines: Bluemel et al. (2000)
GLP: yes
(certified by National Institute of Pharmacy, Budapest, Hungary)

Executive Summary

In a worst-case laboratory study, *Typhlodromus pyri* (Acarina: Phytoseiidae) was exposed to dried residues of BAS 500 06 F. The test item was applied to glass plates at application rates of 0.046, 0.139, 0.417, 1.25 and 3.75 L BAS 500 06 F/ha. Additional test units were treated with deionized water as control and with Perfekthion as a reference item. Endpoint of the study was the mortality after 7 days of exposure, including determination of the LR_{50}.

After 7 days of exposure, the mortality in the test item treatments was between 18.33 and 95.00% in comparison to 8.00% in the control. Based on this, the corrected mortality for the different test item rates ranged between 11.23 and 94.57%. A statistically significant difference compared to the control was observed at the test rates of 0.417, 1.25 and 3.75 L BAS 500 06 F/ha.

In a worst-case laboratory study with BAS 500 06 F the LR_{50} for the predatory mite *Typhlodromus pyri* was determined to be 0.87 L/ha BAS 500 06 F in 200 L water/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 200 g/L (202.7 g/L analyzed).

Test species: The predatory mite *Typhlodromus pyri*; protonymphs (not older than 24 h); source: in-house originating from PK- Nützlingszuchten, Germany.

B. STUDY DESIGN

Test design:	The mites were exposed to dried residues on glass plates. Seven treatments (5 test item rates, water treated control and reference item) were tested. For the control 5 replicates were set up, for the test item and the reference item 3 replicates were used. Each replicate contained 20 mites. Assessment of mortality was done 1, 3 and 7 days after application.
Endpoints:	Mortality after exposure over 7 days, including determination of a LR_{50}.
Reference item:	Perfekthion (dimethoate: nominal 400 g/L).
Test rates:	Control, 0.046, 0.139, 0.417, 1.25 and 3.75 L BAS 500 06 F/ha. The reference item was applied at an application rate of 0.015 L/ha. All treatment groups were applied in 200 L water/ha. The substances were sprayed onto glass plates with a laboratory spraying equipment and air dried afterwards.
Test conditions:	Temperature: 23.7°C - 26.4°C; relative humidity: 63% - 89%; photoperiod: 16 h light : 8 h dark; light intensity: 911.0 lux; food: pollen of *Pinus* sp..
Statistics:	Descriptive statistics. Probit analysis for calculation of LR_{50} and Bonferroni t-test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 7 days of exposure, the mortality in the test item treatments was between 18.33 and 95.00% in comparison to 8.00% in the control. Based on this, the corrected mortality for the different test item rates ranged between 11.23 and 94.57%. A statistically significant difference compared to the control was observed at the test rates of 0.417, 1.25 and 3.75 L BAS 500 06 F/ha.
The LR_{50} was calculated to be 0.87 L/ha BAS 500 06 F in 200 L water/ha (Bonferroni t-test, $\alpha = 0.05$). The results are summarized in Table 10.3.2.1-1.

Table 10.3.2.1-1: Effects of BAS 500 06 F on predatory mites (*Typhlodromus pyri*) mortality under worst-case laboratory conditions

Treatment	Rate [1] [L/ha]	Mortality [2] [%]	Corrected mortality [3] [%]
Control	--	8.00	--
BAS 500 06 F	0.046	18.33	11.23
BAS 500 06 F	0.139	25.00	18.48
BAS 500 06 F	0.417	40.00 *	34.78
BAS 500 06 F	1.25	76.67 *	74.64
BAS 500 06 F	3.75	95.00 *	94.57
Endpoint [L/ha BAS 500 06 F]			
LR_{50} (95% CL [4])	0.87 (0.65 - 1.10)		

1) Application rate in 200 L water/ha.
2) Mortality after 7 day exposure to BAS 500 06 F on treated glass plates.
3) Corrected overall mortality according to Abbott.
4) 95% CL means lower and upper 95% confidence limits.
* = statistically significant differences compared to the control (Bonferroni t-test, $\alpha = 0.05$).

100% mortality was observed in the reference item treatment after 7 days of exposure.

III. CONCLUSION

In a worst-case laboratory study with BAS 500 06 F the LR_{50} for the predatory mite *Typhlodromus pyri* was determined to be 0.87 L/ha BAS 500 06 F in 200 L water/ha.

Report:	CP 10.3.2.1/2 Sipos K., 2007b Effect of BAS 500 06 F on the parasitic wasp (Aphidius rhopalosiphi) in a laboratory trial 2007/1035600
Guidelines:	Mead-Briggs M. et al. (2000)
GLP:	yes (certified by National Institute of Pharmacy, Budapest, Hungary)

Report:	CP 10.3.2.1/3 Sipos K., 2007c Amendment to the final report: Effect of BAS 500 06 F on the parasitic wasp (Aphidius rhopalosiphi) in a laboratory trial 2007/1050841
Guidelines:	Mead-Briggs M. et al. (2000)
GLP:	yes (certified by National Institute of Pharmacy, Budapest, Hungary)

Executive Summary

In a worst-case laboratory study, adults of *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) were exposed to dried residues of BAS 500 06 F. The test item was applied to glass plates at application rates of 0.006, 0.019, 0.056, 0.167, 0.25 and 0.5 L BAS 500 06 F/ha. Additional test units were treated with deionized water as control and with Perfekthion as a reference item. Endpoint of the study was the mortality after 48 hours of exposure, including determination of the LR_{50}.

After 48 hours, the mortality in the test item treatments was between 22.50 and 100.00% in comparison to 7.50% in the control. Based on these results the corrected mortality for the different rates ranged between 16.22 and 100.00%. A statistically significant difference compared to the control was observed at the test item rates of 0.056, 0.167, 0.25 and 0.5 L/ha BAS 500 06 F.

In a worst-case laboratory study with BAS 500 06 F, the LR_{50} for the parasitic wasp *Aphidius rhopalosiphi* was determined to be 0.04 L/ha BAS 500 06 F in 200 L water/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 200 g/L (202.7 g/L analyzed).

Test species: The parasitic wasp *Aphidius rhopalosiphi*; adults less than 48 hours old; source: Katz Biotech AG, Welzheim, Germany.

B. STUDY DESIGN

Test design: Exposure of the wasps was reached via air-dried residues on treated glass plates. Eight treatments (6 test item rates, water treated control and a reference item) were set up with 4 replicates each. Each replicate contained 10 wasps. Assessment of mortality was carried out 2, 24 and 48 h after test initiation.

Endpoints: Mortality after exposure over 48 h, including determination of a LR_{50}.

Reference item: Perfekthion (dimethoate: nominal 400 g/L).

Test rates: Control, 0.006, 0.019, 0.056, 0.167, 0.25 and 0.5 L BAS 500 06 F/ha. The reference item was applied at an application rate of 0.3 mL/ha. All treatment groups were applied in 200 L water/ha. The test item was sprayed onto glass plates via laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 18.9°C - 21.3°C; relative humidity: 63% - 81%; photoperiod: 16 h light : 8 h dark; light intensity: 925.0 lux; food: 1:3 v/v solution of honey and water.

Statistics: Descriptive statistics. Probit analysis for calculation of LR_{50} and Dunnett`s Test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 48 hours, the mortality in the test item treatments was between 22.50 and 100.00% in comparison to 7.50% in the control. Based on these results the corrected mortality for the different rates ranged between 16.22 and 100.00%. A statistically significant difference compared to the control was observed at the test item rates of 0.056, 0.167, 0.25 and 0.5 L/ha BAS 500 06 F (Dunnett`s Test, $\alpha = 0.05$). The LR_{50} was calculated to be 0.04 L/ha BAS 500 06 F in 200 L water/ha. The results are summarized in Table 10.3.2.1-2.

Table 10.3.2.1-2: Effects of BAS 500 06 F on parasitic wasps (*Aphidius rhopalosiphi)* mortality under worst-case laboratory conditions

Treatment	Rate [1] [L/ha]	Mortality [2] [%]	Corrected mortality [3] [%]
Control	--	7.50	--
BAS 500 06 F	0.006	22.50	16.22
BAS 500 06 F	0.019	27.50	21.62
BAS 500 06 F	0.056	62.50 *	59.46
BAS 500 06 F	0.167	100.00 *	100.00
BAS 500 06 F	0.250	100.00 *	100.00
BAS 500 06 F	0.500	100.00 *	100.00
	Endpoint [L/ha BAS 500 06 F]		
LR_{50} (95% CL [4])	0.04 (0.03 - 0.05)		

1) Application rate in 200 L water/ha.
2) Mortality: after 48 hours of exposure to BAS 500 06 F on treated glass plates.
3) Corrected mortality according to Abbott (1925).
4) 95% CL means lower and upper 95% confidence limits.
* = Statistically significant differences compared to the control (Dunnett`s Test, $\alpha = 0.05$).

III. CONCLUSION

In a worst-case laboratory study with BAS 500 06 F, the LR_{50} for the parasitic wasp *Aphidius rhopalosiphi* was determined to be 0.04 L/ha BAS 500 06 F in 200 L water/ha.

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Report:	CP 10.3.2.2/1 Vaughan R., 2008a A rate-response extended laboratory test to determine the effects of BAS 500 06 F on the predatory mite, Typhlodromus pyri (Acari: Phytoseiidae) 2008/1010712
Guidelines:	Bluemel et al. (2000)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, The United Kingdom)

Executive Summary

A rate response extended laboratory study was carried out to determine the toxicity of BAS 500 06 F on protonymphs of the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae). For determination of mortality the mites were exposed to fresh residues of BAS 500 06 F on leaf disks from bean plants (*Phaseolus vulgaris*). Application rates were 0.3125, 0.625, 1.25, 2.50 and 3.75 L/ha BAS 500 06 F. A water treated control and a reference were applied to additional leaves. Endpoints were mortality in order to determine a LR_{50} and effects on reproduction for those treatments in which ≤ 60% corrected mortality was observed.

After 7 days of exposure the mortality was 14.0% in the control, compared to 25.0 to 71.7% in the test item treatments. This resulted in corrected mortality rates between 12.8 and 67.1%. The results for mortality differed significantly from the control in the four highest test item treatments.
The mean number of eggs per female was 9.9 in the control. The mean number of eggs produced in the test item treatments was 5.8 to 8.7 eggs/female, resulting in an effect on reproduction of 11.7 to 41.3%. There were no statistically significant reductions in egg production compared to the control at treatment rates up to and including 0.625 L/ha.

The LR_{50} of BAS 500 06 F on *Typhlodromus pyri* under extended laboratory conditions was 2.4524 L/ha. No unacceptable effects on reproduction were observed at treatment rates up to and including 2.5 L/ha BAS 500 06 F in 200 L water/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 200 g/L (202.7 g/L analyzed).

Test species: Predatory mite (*Typhlodromus pyri*), protonymphs (less than 24 h old); source: in-house culture.

B. STUDY DESIGN

Test design: Exposure of the mites was reached via air-dried residues on the treated upper (adaxial) side of leaves of dwarf French bean plants (*Phaseolus vulgaris)*. Seven treatment groups (5 test item rates, control and reference item) with 5 replicates for the control and 3 replicates for the test item and reference item were set up, each with 20 mites. Assessment of mortality was carried out 1 and 7 days after application.
For the reproduction assessment mites from the control and the test item treatments displaying a corrected mortality rate ≤ 60% were sexed, left *in situ* and the number of eggs/female was recorded during the second week. Reproduction capacity of the mites was assessed 7 and 14 days after application.

Endpoints: Mortality after 7 days of exposure; reproduction capacity between day 7 and 14 after application.

Reference item: Perfekthion (dimethoate: nominal 400 g/L).

Test rates: Control, 0.3125, 0.625, 1.25, 2.50 and 3.75 L/ha BAS 500 06 F. The reference item was applied at an application rate of 30 mL/ha. All substances were applied in 200 L water/ha. The substances were sprayed onto the upper (adaxial) surfaces of bean leaf disks via a calibrated laboratory track-sprayer and left air dried afterwards.

Test conditions: Exposure period: temperature: 25°C - 27°C, relative humidity: 65% - 80%. Reproduction period: temperature: 25°C - 26°C, relative humidity: 67% - 74%.
Photoperiod: 16 h light : 8 h dark; light intensity: 900 lux - 1600 lux.
Food: 1:1 mixture of almond (*Prunus* sp.) and apple (*Malus* sp.) pollen.

Statistics: Descriptive statistics. Probit analysis for calculation of LR_{50}, Fishers exact test for mortality data and Dunnett-test for reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

The LR_{50} value was 2.4524 L/ha BAS 500 06 F.

After 7 days of exposure the mortality was 14.0% in the control, compared to 25.0 to 71.7% in the test item treatments. This resulted in corrected mortality rates between 12.8 and 67.1%. The results for mortality differed significantly from the control in the four highest test item treatments (Fishers exact test, $\alpha = 0.05$).
The mean number of eggs per female was 9.9 in the control. The mean number of eggs produced in the test item treatments was 5.8 to 8.7 eggs/female, resulting in an effect on reproduction of 11.7 to 41.3%. There were no statistically significant reductions in egg production compared to the control at treatment rates up to and including 0.625 L/ha (Dunnett-test, $\alpha = 0.05$). The results are summarized in Table 10.3.2.2-1.

Table 10.3.2.2-1: Effects on predatory mites (*Typhlodromus pyri*), exposed to fresh dried residues of BAS 500 06 F in an extended laboratory trial.

Treatment	Rate [1] [L/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Reproduction [4] [eggs/female]	Effect on reproduction [%]
Control	--	14.0	--	9.9	--
BAS 500 06 F	0.3125	25.0	12.8	8.7	11.7
BAS 500 06 F	0.625	31.7 *	20.5	7.5	24.6
BAS 500 06 F	1.25	28.3 *	16.7	5.8 *	41.3
BAS 500 06 F	2.50	60.0 *	53.5	6.2 *	36.8
BAS 500 06 F	3.75	71.7 *	67.1	--	--
Endpoint [L/ha BAS 500 06 F]					
LR_{50} (95% CL[5])	2.452 (1.6105 - 5.3037)				
Effects on reproduction	$ER_{50} > 2.5$				

1) Application rate in 200 L water/ha.
2) Mortality after 7 day exposure to BAS 500 06 F on the treated bean leaves.
3) Corrected overall mortality according to Abbott (1925).
4) Reproduction: mean number of eggs per female from day 7 to 14.
5) 95% CL means lower and upper 95% confidence limits.
* = statistically significant effects compared to the control (Fishers exact-test for mortality, Dunnett-test for reproduction, $\alpha = 0.05$).

The reference item produced a corrected mortality of 84.5% of exposed mites after 7 days.

III. CONCLUSION

The LR_{50} of BAS 500 06 F on *Typhlodromus pyri* under extended laboratory conditions was 2.452 L/ha. No unacceptable effects on reproduction were observed at treatment rates up to and including 2.5 L/ha BAS 500 06 F in 200 L water/ha.

Report:	CP 10.3.2.2/2 Stevens J., 2008a A rate-response extended laboratory test to determine the effects of BAS 500 06 F on the parasitic wasp, Aphidius rhopalosiphi (Hymenoptera, Braconidae) 2008/1010713
Guidelines:	Mead-Briggs M. et al. (in preparation) An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp Aphidius rhopalosiphi (De Stefani-Perez) (Hymenoptera Braconidae)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, The United Kingdom)

Executive Summary

In an extended laboratory study adults of the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera Braconidae) were exposed to dried residues of 0.07, 0.15, 0.30, 0.60, 1.25 and 2.50 L/ha BAS 500 06 F on treated barley seedlings. The mortality of the wasps was assessed 2, 24 and 48 h after treatment. Additionally, for the reproduction assessment 15 females from the control and the 3 highest treatment groups displaying ≤ 60% corrected mortality were transferred individually to pots with untreated, aphid-infested barley plants for 24 h and then removed. The number of parasitized aphid mummies was recorded after 10 days. Additional test units were treated with water as a control and with Perfekthion as reference item.

During the first 3 h of the test 38.7% of the wasps in the control treatment settled on the treated plants compared to 28.7 to 34.0% in the test item treatments. After 24 h and 48 h 43.3% of the wasps in the control treatment settled on the treated plants compared to 35.8 to 41.4% in the test item treatments. A statistically significant difference compared to the control was not observed. After 48 h of exposure no mortality could be detected in the control. Corrected mortalities of 0.0 to 20.0% were observed in the test item treatments. Only the mortality in the 2.50 L/ha treatment rate differed significant from the control. The mean number of mummies per female was 26.1 in the control and ranged from 23.9 to 25.9 in evaluated test item groups. This resulted in effects on reproduction between 0.8 and 8.7%. There were no significant effects on reproduction, relative to the control, at rates up to and including the highest rate of 2.50 L/ha BAS 500 06 F.

The LR_{50} of BAS 500 06 F on *Aphidius rhopalosiphi* under extended laboratory conditions was > 2.5 L/ha. The test item caused no unacceptable effects on reproduction if applied up to and including a rate of 2.5 L/ha BAS 500 06 F in 400 L water/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 200 g/L (202.7 g/L analyzed).

Test species: Parasitic wasp *(Aphidius rhopalosiphi)*, adults, age: less than 48 h; source: in-house culture.

B. STUDY DESIGN

Test design: Exposure of the parasitoids was reached via air-dried residues on treated barley seedlings (*Hordeum vulgare)*. The study included 8 treatment groups (6 test item rates, water treated control, reference item) with 6 replicates per treatment, each containing 5 female wasps. Repellence was assessed after 3, 24 and 48 h. Wasp mortality was assessed 2, 24 and 48 hours after test initiation. At 48 h 15 female wasps from the 3 highest treatment groups displaying ≤ 60% corrected mortality were transferred individually to pots with untreated, aphid-infested barley plants for 24 h and then removed for the reproduction assessment. The number of parasitized aphid mummies was recorded after 10 days. Host: cereal aphids (*Rhopalosiphum padi, Metopolophium dirhodum*).

Endpoints: Mortality after 48 h of exposure; effects on reproduction capacity after additional 24 h.

Reference item: Perfekthion (dimethoate, nominal 400 g/L).

Test rates: Control, 0.07, 0.15, 0.30, 0.60, 1.25 and 2.50 L/ha BAS 500 06 F. The reference item was applied at an application rate of 0.01 L/ha. All treatments were applied in 400 L/ha water. The treatments were sprayed on potted barley seedlings using a calibrated laboratory track sprayer and left air dried afterwards.

Test conditions: Exposure period: temperature: 20°C; relative humidity: 68% - 88%; photoperiod: 16 h light : 8 h dark; light intensity: 2810 lux.
Reproduction period: temperature: 18°C - 22°C; photoperiod: 16 h light : 8 h dark, light intensity: 4590 lux.
Food: fructose-water solution (10%), sprayed onto test plants before application.

Statistics: Descriptive statistics. Fishers exact test for mortality, one-way analysis of variance (ANOVA) for repellence and for reproduction data (α = 0.05).

II. RESULTS AND DISCUSSION

The LR_{50} value was > 2.50 L/ha BAS 500 06 F.

During the first 3 h of the test 38.7% of the wasps in the control treatment settled on the treated plants compared to 28.7 to 34.0% in the test item treatments. After 24 h and 48 h 43.3% of the wasps in the control treatment settled on the treated plants compared to 35.8 to 41.4% in the test item treatments. A statistically significant difference compared to the control was not observed (ANOVA, $\alpha = 0.05$).
After 48 h of exposure no mortality could be detected in the control. Corrected mortalities of 0.0 to 20.0% were observed in the test item treatments. Only the mortality in the 2.50 L/ha treatment rate differed significantly from the control (Fishers exact test, $\alpha = 0.05$).
The mean number of mummies per female was 26.1 in the control and ranged from 23.9 to 25.9 in evaluated test item groups. This resulted in effects on reproduction between 0.8 and 8.7%. There were no significant effects on reproduction, relative to the control, at rates up to and including the highest rate of 2.50 L/ha BAS 500 06 F (ANOVA, $\alpha = 0.05$). The results are summarized in Table 10.3.2.2-2.

Table 10.3.2.2-2: Effects of BAS 500 06 F on parasitoids (*Aphidius rhopalosiphi*) mortality and reproduction under extended laboratory conditions

Treatment	Rate [1)] [L/ha]	Mortality [2)] [%]	Corrected mortality [3)] [%]	Reproduction [4)] [mummies/ female]	Effects on reproduction [%]
Control	--	0.0	0.0	26.1	--
BAS 500 06 F	0.07	0.0	0.0	--	--
BAS 500 06 F	0.15	0.0	0.0	--	--
BAS 500 06 F	0.30	0.0	0.0	--	--
BAS 500 06 F	0.60	0.0	0.0	25.9	0.8
BAS 500 06 F	1.25	10.0	10.0	23.9	8.7
BAS 500 06 F	2.50	20.0 *	20.0	25.9	0.8
Endpoint [L/ha BAS 500 06 F]					
LR_{50}	> 2.5				
Effects on reproduction	ER_{50} > 2.5				

1) Application rate in 400 L water/ha.
2) Mortality: after 48 hours of exposure to BAS 500 06 F on barley seedlings.
3) Corrected mortality according to Abbott (1925).
4) Reproduction: mean number of parasitized aphids/surviving female.
* = Statistically significant differences compared to the control (Fisher's exact test, $\alpha = 0.05$).

The reference item caused 73.3% corrected mortality after 48 h of exposure.

III. CONCLUSION

The LR_{50} of BAS 500 06 F on *Aphidius rhopalosiphi* under extended laboratory conditions was > 2.5 L/ha. The test item caused no unacceptable effects on reproduction if applied up to and including a rate of 2.5 L/ha BAS 500 06 F in 400 L water/ha.

Report:	CP 10.3.2.2/3 Roehlig U., 2008a Effects of BAS 500 06 F on the green lacewing Chrysoperla carnea STEPH. under extended laboratory conditions - Rate-response test 2008/1032666
Guidelines:	Vogt H. et al. (2000) Laboratory method to test effects of plant protection products on larvae of Chrysoperla carnea (Neuroptera Chrysopidae)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

An extended laboratory study was carried out to determine the effects of the fungicide BAS 500 06 F on the green lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae). For determination of the mortality larvae were exposed to fresh, dry residues of BAS 500 06 F on bean leaves at rates of 0.31, 0.63, 1.25, 2.5 and 3.75 L/ha with a water volume of 200 L/ha. Additional test units were treated with deionized water as control and with Perfekthion as reference item. Endpoints of the study were the pre-imaginal mortality and additionally effects on reproduction. Assessment of mortality was carried out regularly until hatching of adult lacewings. Effects on reproduction were assessed by number of eggs produced per female and the hatching rate.

In the water treated control 2.0% mortality was observed. Corrected mortalities between 8.2% and 100.0% were observed in the test item treatments. No statistically significant effect on mortality was determined in the 0.31 L/ha treatment group. In all other test item treatment groups a statistically significant effect on mortality was observed.
In the control as well as in the 0.31 and 0.63 L/ha BAS 500 06 F test item treatments the number of eggs per female per day was ≥ 15 and the hatching rate was ≥ 70%.

In an extended laboratory study with BAS 500 06 F the LR_{50} for *Chrysoperla carnea* was determined to be 0.72 L/ha BAS 500 06 F in 200 L water/ha. No unacceptable effects on reproduction of *Chrysoperla carnea* occurred when BAS 500 06 F was applied at rates up to and including 0.63 L/ha BAS 500 06 F.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 200 g/L (202.7 g/L analyzed).

Test species: Green lacewing (*Chrysoperla carnea*); larvae (2-3 days old); source: in-house.

B. STUDY DESIGN

Test design: Exposure of the lacewings was reached via air-dried residues on excised bean leaves. The study encompassed seven treatment groups (5 test item rates, control, reference item), each with 50 replicates (1 larvae/replicate) per treatment. Condition of exposed individuals was assessed until emergence of adult lacewings. Mortality assessment was carried out regularly until hatching of the adult lacewings. In addition, for the control and the test item groups, in which corrected mortality was < 50% the reproduction performance, i.e. egg deposition and hatching rate, was determined (2 assessments/week, 24 h period each).

Endpoints: Pre-imaginal mortality (mortality of exposed larvae and pupae until hatching of adults) including the determination of a LR_{50}. Reproductive capacity: assessment of number of eggs per female per day and hatching rate.

Reference item: Perfekthion (dimethoate, nominal 400 g/L).

Test rates: Control, 0.31, 0.63, 1.25, 2.5 and 3.75 L/ha BAS 500 06 F. The reference item was applied at an application rate of 40 mL/ha. All substances were applied in 200 L/ha water. The substances were sprayed on bean leaves (*Phaseolus vulgaris*) via calibrated laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 23°C - 27°C; relative humidity: 68% - 73%; photoperiod: 16 hours light : 8 hours dark; light intensity: 2180 lux.
Food: larvae: *Sitotroga cerealella* eggs (UV-sterilized); adults: artificial diet

Statistics: Descriptive statistics. Fishers Exact Binominal Test for pre-imaginal mortality data ($\alpha = 0.05$), Probit analysis for LR_{50}.

II. RESULTS AND DISCUSSION

The LR_{50} value was determined to be 0.72 L/ha BAS 500 06 F.

In the water treated control 2.0% mortality was observed. Corrected mortalities between 8.2% and 100.0% were observed in the test item treatments. No statistically significant effect on mortality was determined in the 0.31 L/ha treatment group. In all other test item treatment groups a statistically significant effect on mortality was observed (Fisher's Exact Binominal Test, $\alpha = 0.05$).
In the control as well as in the 0.31 and 0.63 L/ha BAS 500 06 F test item treatments the number of eggs per female per day was ≥ 15 and the hatching rate was $\geq 70\%$. The results are summarized in Table 10.3.2.2-3.

Table 10.3.2.2-3: Effects on lacewings (*Chrysoperla carnea*) exposed to BAS 500 06 F in an extended laboratory trial

Treatment	Rate [1] [L/ha]	Mortality[2] [%]	Corrected mortality[3] [%]	Mean number [eggs/female/day]	Hatching rate [%]
Control	--	2.0	--	22.8	79.4
BAS 500 06 F	0.31	10.0	8.2	26.7	78.7
BAS 500 06 F	0.63	46.0 *	44.9	23.0	78.1
BAS 500 06 F	1.25	82.0 *	81.6	n.d.	n.d.
BAS 500 06 F	2.5	94.0 *	93.9	n.d.	n.d.
BAS 500 06 F	3.75	100.0 *	100.0	n.d.	n.d.
Endpoint [L/ha BAS 500 06 F]					
LR_{50} (95% CL[4])	0.72 (0.62 - 0.84)				
Effects on reproduction	$ER_{50} > 0.63$				

1) Application rate in 200 L water/ha.
2) Percentage of individuals, which did not reach maturity.
3) Corrected mortality according to Abbott (1925).
4) 95% CL means lower and upper 95% confidence limits.
* = statistically significant differences compared to the control (Fishers exact binominal test, $\alpha = 0.05$).
n.d. = not determined, corrected mortality > 50% compared to the control.

In the reference treatment 75.5% corrected mortality was observed.

III. CONCLUSION

In an extended laboratory study with BAS 500 06 F the LR_{50} for *Chrysoperla carnea* was determined to be 0.72 L/ha BAS 500 06 F in 200 L water/ha. No unacceptable effects on reproduction of *Chrysoperla carnea* occurred when BAS 500 06 F was applied at rates up to and including 0.63 L/ha BAS 500 06 F.

Report:	CP 10.3.2.2/4 Roehlig U., 2008b Effects of BAS 500 06 F on the green lacewing Chrysoperla carnea STEPH. in an extended laboratory test (under semi-field conditions aged residues on bean plants) 2008/1042190
Guidelines:	Vogt et al. (2000)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

An aged residue extended laboratory study was carried out to determine the effects of the fungicide BAS 500 06 F on the green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae). Larvae were exposed to both freshly-dried and field aged residues of BAS 500 06 F on bean leaves at rates of 1.25 and 2.50 L/ha with a water volume of 400 L/ha. Additional test units were treated with deionized water as control and with Perfekthion as reference item. Extended laboratory bioassays were initiated within 1 hour after application (DAT 0) and also at 7 and 14 days after treatment (DAT 7, DAT 14). Endpoints of the study were the pre-imaginal mortality and additionally effects on reproduction. Assessment of mortality was carried out regularly until hatching of adult lacewings. Effects on reproduction were assessed by number of eggs produced per female and the hatching rate.

In the bioassay started at DAT 0 corrected mortality rates of 36.2 and 66.0% were observed at treatment rates of 1.25 and 2.5 L/ha, respectively. In the control as well as in the 1.25 L/ha treatment the number of eggs per female per day was > 15 and the hatching rate was > 70%. In the 2.50 L/ha test item treatment group no reproduction test was carried out, because corrected mortality was > 50%.
In the bioassay started on DAT 7 corrected mortality rates of 6.3 and 41.7% were observed. In the control as well as in both BAS 500 06 F treatments the number of eggs per female per day was > 15 and the hatching rate was > 70%.
In the bioassay started on DAT 14 corrected mortality rates of 2.0 and 10.0% were observed. No statistically significant effects on mortality were determined in both test item groups. In the control as well as in both BAS 500 06 F treatments the number of eggs per female per day was > 15 and the hatching rate was > 70%.

In an aged residue extended laboratory study with BAS 500 06 F no unacceptable effects on survival or reproduction were observed after exposure to freshly dried residues (DAT 0) obtained from a rate of 1.25 L/ha in 400 L water/ha. After exposure to aged residues (DAT 7and DAT 14) no unacceptable effects on survival or reproduction were observed at an application rate of 1.25 and 2.5 L BAS 500 06 F/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 200 g/L (202.7 g/L analyzed).

Test species: Green lacewing (*Chrysoperla carnea*); larvae (2-3 days old); source: in-house.

B. STUDY DESIGN

Test design: Exposure of the lacewings was reached via both, freshly-dried and field aged residues on bean leaves. The study encompassed four treatment groups (2 test item rates, control and reference item), each with 50 replicates (1 larvae/replicate) per treatment. Following treatment, the plants were maintained outdoors but under UV light-permeable rain protection. Bioassays were initiated within 1 hour after application (DAT 0) and also at 7, 14 and 21 days after treatment (DAT 7, DAT 14, DAT 21). The results of the DAT 14 bioassay indicated that the test item had no adverse effects and the bioassay started on DAT 21 was terminated and not reported. For each bioassay exposure lasted until pupae were transferred to oviposition boxes for development of adults. Mortality assessment was carried out regularly until hatching of the adult lacewings. In addition, for the control and the test item groups, in which < 50% corrected pre-imaginal mortality was observed, the reproduction performance, i.e. egg deposition and hatching rate, was determined (2 assessments/week, 24 h period each).

Endpoints: Pre-imaginal mortality (mortality of exposed larvae and pupae until hatching of adults). Reproductive capacity: assessment of number of eggs per female per day and hatching rate.

Reference item: Perfekthion (dimethoate, nominal 400 g/L).

Test rates: Control, 1.25 and 2.50 L/ha BAS 500 06 F. The reference item was applied at an application rate of 0.10 L/ha. All substances were applied in 400 L/ha water. The substances were sprayed on bean leaves (*Phaseolus vulgaris*) via plot-sprayer and air dried afterwards.

Test conditions: Temperature: 23°C - 26°C (DAT 0, DAT 7 and DAT 14); relative humidity: 64% - 88% (DAT 0 and DAT 7), 64% - 84% (DAT 14); light intensity: 2070 lux (DAT 0), 2180 lux (DAT 7) and 2190 lux (DAT 14). Photoperiod: 16 hours light : 8 hours dark; food: larvae: *Sitotroga cerealella* eggs (UV-sterilized), adults: artificial diet.

Statistics: Descriptive statistics. Fishers Exact Binominal Test for pre-imaginal mortality data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

In the bioassay started at DAT 0 corrected mortality rates of 36.2 and 66.0% were observed. Statistically significant effects on mortality were determined in both test item treatments (Fishers Exact Binominal Test, $\alpha = 0.05$). In the control as well as in the 1.25 L/ha treatment the number of eggs per female per day was > 15 and the hatching rate was > 70%. In the 2.50 L/ha test item treatment group no reproduction test was carried out, because corrected mortality was > 50%.
In the bioassay started on DAT 7 corrected mortality rates of 6.3 and 41.7% were observed. Statistically significant effects on mortality were determined only in the 2.5 L/ha treatment. In the control as well as in both BAS 500 06 F treatments the number of eggs per female per day was > 15 and the hatching rate was > 70%.
In the bioassay started on DAT 14 corrected mortality rates of 2.0 and 10.0% were observed. No statistically significant effects on mortality were determined in both test item groups (Fishers Exact Binominal Test, $\alpha = 0.05$). In the control as well as in both BAS 500 06 F treatments the number of eggs per female per day was > 15 and the hatching rate was > 70%. The results are summarized in Table 10.3.2.2-4.

Table 10.3.2.2-4: Effects on lacewings (*Chrysoperla carnea*) exposed to BAS 500 06 F in an aged residue extended laboratory study

Treatment	Rate [1)] [L/ha]	Mortality[2)] [%]	Corrected mortality[3)] [%]	Mean number [eggs/female/day]	Hatching rate [%]
DAT 0 [4)]					
Control	--	6.0	--	25.4	81.0
BAS 500 06 F	1.25	40.0*	36.2	20.8	80.8
BAS 500 06 F	2.50	68.0*	66.0	n.d.	n.d.
DAT 7 [4)]					
Control	--	4.0	--	21.8	78.4
BAS 500 06 F	1.25	10.0	6.3	22.1	79.2
BAS 500 06 F	2.50	44.0*	41.7	20.3	79.5
DAT 14 [4)]					
Control	--	0	--	19.4	79.1
BAS 500 06 F	1.25	2.0	2.0	20.3	79.3
BAS 500 06 F	2.50	10.0	10.0	20.8	78.4

1) Application rate in 400 L water/ha.
2) Percentage of individuals, which did not reach maturity.
3) Corrected mortality according to Abbott (1925).
4) DAT = Days After Treatment (equivalent to days over which residues were aged before bioassay was initiated).
* = statistically significant differences compared to the control (Fishers exact binominal test, $\alpha = 0.05$).
n.d. = no determined, corrected mortality > 50% compared to control.

In the bioassay DAT 0, the reference item caused 85.1% corrected mortality of exposed lacewings.

III. CONCLUSION

In an aged residue extended laboratory study with BAS 500 06 F no unacceptable effects on survival or reproduction were observed after exposure to freshly dried residues (DAT 0) obtained from a rate of 1.25 L/ha in 400 L water/ha. After exposure to aged residues (DAT 7and DAT 14) no unacceptable effects on survival or reproduction were observed at an application rate of 1.25 and 2.5 L BAS 500 06 F/ha.

Report:	CP 10.3.2.2/5 Schmitzer S., 2008a Effects of BAS 500 06 F on the reproduction of rove beetles (Aleochara bilineata) - Extended laboratory study 2008/1010700
Guidelines:	Grimm et al. (2000) A test for evaluating the chronic effects of plant protection products on the rove beetle Aleochara bilineata Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory conditions
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary

An extended laboratory study was performed to determine the effects of the fungicide BAS 500 06 F on the staphylinid beetle *Aleochara bilineata*. The test item was applied to the soil (LUFA 2.1) surface at rates of 2.5 and 3.75 L BAS 500 06 /ha. The study covers the complete life cycle of the beetle: parental generation, mating and oviposition of parental generation, hatching of F1 larvae and parasitization period until emergence of the F1 adults. The endpoint of the study was the effect on reproduction represented by the living offspring hatched from the onion fly pupae, which were added to the natural test soil during the first 3 weeks of the study.

The number of hatched beetles was 550 in the control. In the two test item treatments 471 and 492 beetles hatched. This is corresponding to an effect of 14.4 and 10.6%, respectively, relative to the control. No statistically significant difference compared to control was observed.

In an extended laboratory study with BAS 500 06 F, no unacceptable effects on reproduction of *Aleochara bilineata* occurred after exposure to natural soil treated with 2.5 and 3.75 L BAS 500 06 F/ha in 400 L water/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 200 g/L (202.7 g/L analyzed).

Test species: Staphylinid beetle (*Aleochara bilineata*), adults 2 - 4 days old, source: De Groene Vlieg, Nieuwe Tonge, The Netherlands.

B. STUDY DESIGN

Test design: The test item rates, a control and a reference item were sprayed via laboratory spray applicator on the soil surface. Exposure of the beetles was reached via treated natural soil LUFA 2.1. The beetles were introduced to the test units immediately after treatment. Four replicates per treatment group were set up with 10 female and 10 male beetles each. This study included the complete life cycle of the beetles: parental generation, mating and oviposition of parental generation, hatching of F1 larvae and parasitization period until emergence of the F1 adults. On day 7, 14 and 21 approximately 500 pupae of *Delia antiqua* were dug into the soil to be parasitized by larvae of the beetles. On day 28 adults were separated from the soil and the soil with the pupae was allowed to dry for seven days. On day 35 the pupae were sieved out of the natural soil and transferred into an emergence container. The emergence of the F1-generation of beetles was observed from day 38 - 75.

Endpoints: Effect on reproduction (reproduction capacity).

Reference item: Perfekthion EC (dimethoate, nominal 400 g/L).

Test rates: Control, 2.5 and 3.75 L/ha BAS 500 06 F. The reference item was applied at a rate of 4.4 L/ha. All substances were applied in 400 L water/ha directly on soil surface via laboratory spraying equipment.

Test conditions: Temperature: 18°C - 22°C; relative humidity: 68% - 90%; photoperiod: 16 h light : 8 h dark; light intensity: 520 lux - 1250 lux. Food: frozen midge larvae (*Chironomus* sp.).

Statistics: Descriptive statistics. Student t-test, one-sided smaller, for reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

The number of hatched beetles was 550 in the control. In the two test item treatments 471 and 492 beetles hatched. This is corresponding to an effect of 14.4 and 10.6%, respectively, relative to the control. No statistically significant difference compared to control was observed (Student t-test, one-sided smaller, $\alpha = 0.05$). The results are summarized in Table 10.3.2.2-5.

Table 10.3.2.2-5: Effects on staphylinid beetles (*Aleochara bilineata*) exposed to BAS 500 06 F in an extended laboratory trial

Treatment	Rate [1)] [L/ha]	Reproduction [mean number of emerged beetles ± standard deviation]	Effects on reproduction [2)] [%]
Control	--	550 ± 71	--
BAS 500 06 F	2.5	471 ± 56	14.4
BAS 500 06 F	3.75	492 ± 91	10.6
Endpoint [L/ha BAS 500 06 F]			
ER_{50}	> 3.75		

1) Application rate in 400 L water/ha.
2) Effects on reproduction calculated based on the exact raw data (calculation: (1-Rt/Rc)*100%).

The reference item resulted in a reduction of reproduction of 99.8% compared to the control.

III. CONCLUSION

In an extended laboratory study with BAS 500 06 F, no unacceptable effects on reproduction of *Aleochara bilineata* occurred after exposure to natural soil treated with 2.5 and 3.75 L BAS 500 06 F/ha in 400 L water/ha.

CP 10.3.2.3 Semi-field studies with non-target arthropods

As BAS 500 06 F does not pose an unacceptable risk to non-target arthropods, further tests are not necessary.

CP 10.3.2.4 Field studies with non-target arthropods

As BAS 500 06 F does not pose an unacceptable risk to non-target arthropods, further tests are not necessary.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

As BAS 500 06 F does not pose an unacceptable risk to non-target arthropods, further tests are not necessary.

CP 10.4 Effects on non-target soil meso- and macrofauna

The new representative formulation BAS 500 06 F was not evaluated within the previous Annex I inclusion process. It is an emulsifiable concentrate (EC) containing 200g/L pyraclostrobin.

Endpoints from new studies with the active substance, its metabolites and the new representative formulation BAS 500 06 F are used for the risk assessment on earthworms and other non-target soil meso- and macrofauna (see Table 10.4-1).

Table 10.4-1: Ecotoxicological endpoints for earthworms and other non-target soil meso- and macrofauna

Test substance	Test species	EU agreed endpoints	Endpoints used in risk assessment
Acute			
Pyraclostrobin	*Eisenia fetida*	LC_{50} = 567 mg a.s./kg dry soil $LC_{50\ CORR:}$ = 283 mg a.s./kg dry soil [1)]	--
BF 500-6		LC_{50} > 1000 mg/kg dry soil $LC_{50\ CORR}$ > 500 mg/kg dry soil [1)]	--
BF 500-7		LC_{50} > 1000 mg/kg dry soil $LC_{50\ CORR}$ > 500 mg/kg dry soil [1)]	--
Chronic			
Pyraclostrobin *	*Eisenia fetida*	--	$NOEC_{CORR}$ = 11.6 mg/kg dry soil [1)]
BF 500-6 *		--	$NOEC_{CORR}$ ≥ 160 mg/kg dry soil [1)]
BF 500-7 *		--	$NOEC_{CORR}$ ≥ 160 mg/kg dry soil [1)]
BAS 500 06 F [2)]		--	NOEC = 30.0 mg/kg dry soil (corresponding to 5.75 mg pyraclostrobin/kg dry soil) [3)]
BF 500-6 *[2)]	*Folsomia candida*	--	NOEC ≥ 1000 mg/kg dry soil
BF 500-7 *[2)]		--	NOEC ≥ 800 mg/kg dry soil
BAS 500 06 F [2)]		--	NOEC = 125.0 mg/kg dry soil (corresponding to 23.9 mg pyraclostrobin/kg dry soil) [3)]
BAS 500 06 F [2)]	*Hypoaspis aculeifer*	--	NOEC ≥ 90.0 mg/kg dry soil (corresponding to ≥ 17.3 mg pyraclostrobin/kg dry soil) [3)]
Field studies			
BAS 500 06 F	earthworm field population	--	no unacceptable effects up to and including 6.25 L/ha
BAS 500 06 F	organic matter decomposition	--	no unacceptable effects at 1.25 L/ha equivalent to 0.333 mg a.s./kg dry soil

1) Toxicity endpoint is re-adjusted using a soil factor of 2 to address the organic content of the soil (peat 10%), and the log P_{ow} of the substance is > 2.
2) Test was conducted with only 5% peat in the test substrate.
3) Based on a nominal content of BAS 500 06 F (200.0 g/L pyraclostrobin) and taken into account the density of 1.04 g/cm^3.
* Study summary is presented in M-CA 8.4.

Overall summary

Data on pyraclostrobin, its metabolites and BAS 500 06 F and their potential effects on earthworms and other non-target soil meso- and macrofauna are evaluated and appropriate risk assessments are provided for the formulation and relevant metabolites based on the already registered use pattern in cereals and maize (critical GAP: maximum 2 x 250 g pyraclostrobin per ha).

The endpoints and calculated toxicity/exposure ratios are shown in Table 10.4-2.

Table 10.4-2: Toxicity/exposure ratios for earthworms and other soil non-target macro-organisms

Test substance	Use pattern	Species	Test type	Endpoint [mg/kg dry soil]	PEC [mg/kg dry soil]	TER	TER risk assessment trigger
Pyraclostrobin	2 x 250 g a.s./ha	*Eisenia fetida*	56-d reproduction test	$NOEC_{CORR}$ = 11.6	0.228	51	5
BF 500-6	--	*Eisenia fetida*	56-d reproduction test	$NOEC_{CORR} \geq 160$	0.121	≥ 1322	
BF 500-7	--	*Eisenia fetida*	56-d reproduction test	$NOEC_{CORR} \geq 160$	0.073	≥ 2192	
Pyraclostrobin in BAS 500 06 F	2 x 250 g a.s./ha	*Eisenia fetida*	56-d reproduction test	NOEC = 5.75	0.228	25	
BF 500-6	--	*Folsomia candida*	28-d reproduction test	NOEC ≥ 1000	0.121	≥ 8264	
BF 500-7	--	*Folsomia candida*	28-d reproduction test	NOEC ≥ 800	0.073	≥ 10959	
Pyraclostrobin in BAS 500 06 F	2 x 250 g a.s./ha	*Folsomia candida*	28-d reproduction test	NOEC = 23.9	0.228	105	
Pyraclostrobin in BAS 500 06 F	2 x 250 g a.s./ha	*Hypoaspis aculeifer*	14-d reproduction test	NOEC ≥ 17.3	0.228	≥ 76	

Acute studies on earthworms were performed with pyraclostrobin, the metabolites BF 500-6 and BF 500-7 (see SANCO/1420/2001-final, Monograph 12945/ECCO/BBA/01) and with the formulation BAS 500 06 F. According to Regulation 1107/2009 the tier 1 data requirement for acute toxicity on earthworms has been replaced by a requirement for chronic toxicity (i.e. reproduction) on earthworms. However, from previous submissions according to the former Directive EC 91/414 data on the acute toxicity of BAS 500 06 F to earthworms is available, which is for completeness presented as additional information, although acute endpoints are not used anymore in the current risk assessment (see below for respective chronic studies).

Chronic studies on earthworms were carried out with pyraclostrobin (see M-CA 8.4), the metabolites BF 500-6 and BF 500-7 (see M-CA 8.4), as well as with the formulation BAS 500 06 F.

Furthermore, chronic studies on collembolans were carried out with BF 500-6 and BF 500-7 (see M-CA 8.4) and BAS 500 06 F and a chronic study on soil mites was conducted with BAS 500 06 F. Additionally, a field study with BAS 500 06 F on earthworms has been done (see M-CP 10.4.1.2).

In the risk assessment, all TER values exceeded the trigger value of 5 for chronic exposure.

Moreover, a study with BAS 500 06 F on organic matter breakdown has been carried out, which is presented as additional information in M-CP 10.4.2.2.

Overall conclusion:
It is concluded that the use of BAS 500 06 F in cereals and maize will not pose any unacceptable risks to populations of earthworms or other soil macro-organisms.

CP 10.4.1 Earthworms

Toxicity

Acute and chronic earthworm studies have been carried out with pyraclostrobin, its metabolites BF 500-6 and BF 500-7, as well as with the formulation BAS 500 06 F. Furthermore, an earthworm field study has been carried out with BAS 500 06 F. Further details on the studies with the formulation are given below (M-CP 10.4.1, M-CP 10.4.1.1 and M-CP 10.4.1.2) and on the studies with the active substance and metabolites in M-CA 8.4. The acute endpoints of the study with BAS 500 06 F are presented as additional information. The earthworm endpoints are summarized in Table 10.4.1-1.

For substances with log P_{ow} values > 2 and a high content of organic material in the artificial soil (i.e. 10% peat), the resulting endpoints have to be corrected by a soil factor of 2 (f_{oc}) in the risk assessment in order to address lower contents of organic material in soil. The log P_{ow} for the active substance is > 2 (i.e. 3.99). Therefore, the correction was done for the endpoints obtained from the studies, where the artificial soil contained more than 5% peat.

Table 10.4.1-1: Summary of earthworm endpoints for BAS 500 06 F, pyraclostrobin and metabolites

Test substance	Endpoint	Value [mg/kg dry soil]	Reference (BASF DocID)
Acute toxicity			
Pyraclostrobin	$LC_{50\ CORR}$	283	1999/10708
BF 500-6	$LC_{50\ CORR}$	> 500	1999/11308
BF 500-7	$LC_{50\ CORR}$	> 500	1999/11309
Pyraclostrobin in BAS 500 06	$LC_{50\ CORR}$	37.0 [1)]	2004/1004367
Chronic toxicity			
Pyraclostrobin	$NOEC_{CORR}$	11.6	2014/1000461
BF 500-6	$NOEC_{CORR}$	≥ 160	2013/1003174
BF 500-7	$NOEC_{CORR}$	≥ 160	2013/1224029
Pyraclostrobin in BAS 500 06 F	NOEC	5.75 [1)]	2008/1036409

1) Based on a nominal content of BAS 500 06 F (200.0 g/L pyraclostrobin) and taken into account the density of 1.044 g/cm^3.
2) Test was conducted with only 5% peat in the test substrate.

Exposure

Table 10.4.1-2: Critical use pattern

Crop	Application time (BBCH growth stage)	Number of applications	Interval [d]	Application rate per treatment	
				Pyraclostrobin [g a.s./ha]	BAS 500 06 F [L/ha]
cereals	25 - 69	2	21	250	1.25
maize	30 - 65	1	--	200	1.0

The exposure to soil organisms was estimated by calculating the maximum predicted environmental concentrations in soil (PEC_{soil}). For multiple applications, the worst-case maximum PEC_{soil} will be the one immediately after the final application. The worst-case use pattern of BAS 500 06 F foresees two applications in cereals with a maximum dose rate of 250 g pyraclostrobin/ha (equivalent to 1.25 L BAS 500 06 F/ha).

For details see M-CP 9.1. The resulting maximum PEC_{soil} values are presented in Table 10.4.1-3.

Table 10.4.1-3: PEC_{soil} values for pyraclostrobin and relevant metabolites *

Test substance	$PEC_{soil, max}$ [mg/kg dry soil]	$PEC_{soil, plateau}$ [mg/kg dry soil]	$PEC_{soil, accu}$ [mg/kg dry soil]
Pyraclostrobin	0.228	--	--
BF 500-6	0.065	0.057	0.121
BF 500-7	0.039	0.034	0.073

* Only worst-case scenario is presented.

Risk assessment for earthworms

The potential long-term risk of BAS 500 06 F to earthworms was assessed by calculating long-term TER (TER_{LT}) values by comparing the NOEC values and the maximum instantaneous PEC_{soil} using the following equation:

$$TER_{LT} = \frac{NOEC\ [mg/kg]}{PEC_{soil}\ [mg/kg]}$$

The resulting TER_{LT} values are presented below:

Table 10.4.1-4: Long-term TER values for earthworms

Test substance	NOEC [mg/kg dry soil]	PEC_{soil} [mg/kg dry soil]	TER_{LT}	TER trigger
Pyraclostrobin	11.6	0.228	51	5
BF 500-6	≥ 160	0.121	≥ 1322	
BF 500-7	≥ 160	0.073	≥ 2192	
Pyraclostrobin in BAS 500 06 F	5.75	0.228	25	

The long-term TER values calculated for BAS 500 06 F, pyraclostrobin and the relevant soil metabolites are above the trigger of 5. Therefore, chronic risk for earthworms arising from long-term use of BAS 500 06 F is negligible. However, for additional information, a field study was carried out with BAS 500 06 F.

The test site in this study was a grassland site. At an application scenario of up to 6.25 L BAS 500 06 F/ha (equivalent to 1250 g pyraclostrobin/ha), no unacceptable effects to earthworm populations were observed (for details see M-CP 10.4.1.2). The intended maximum use of BAS 500 06 F, i.e. 2 x 1.25 L/ha (equivalent to 2 x 250 g pyraclostrobin/ha) in cereals is therefore covered by the tested field rate. Thus, it can be concluded that the use of BAS 500 06 F will be of low risk to natural earthworm communities.

Based on the standard risk assessment and on data from the field study, it is concluded that earthworm communities will be at low risk following the use of BAS 500 06 F in cereals and maize according to the recommended use pattern.

Earthworms – acute toxicity

This study was a former requirement under Council Directive 91/414/EEC and SANCO/10329/2002 rev 2. However, under Regulation (EC) 1107/2009 it is not required anymore. Therefore, the study is considered as additional information.

Report:	CP 10.4.1/1 Fleischer G., 2004a Effect of BAS 500 06 F on the mortality of the earthworm Eisena fetida 2004/1004367
Guidelines:	ISO 11268-1 (1993)
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Adult earthworms, of the species *Eisenia fetida*, were exposed to BAS 500 06 F. The test item was mixed into artificial soil at a rate of 197.5, 296.3, 444.4, 666.7 and 1000.0 mg BAS 500 06 F/kg dry soil. For the control treatment, the soil was left untreated. The test soil had an organic content of approximately 10% (as sphagnum peat). The worms were placed on the surface of the soil. Four replicates were prepared for each treatment group and the control, each containing 10 worms. Assessment of mortality was made 7 and 14 days after treatment. Assessment of worm weight was made after 14 days.

After 14 days of exposure no mortality was observed in treatment groups up to 296.3 mg/kg dry soil. High mortality was observed at test concentrations of 444.4 mg/kg dry soil and higher. All treatment groups including the control showed reduction in biomass. Statistically significant differences were determined in the treatment group with the test concentration of 444.4 mg/kg dry soil; surviving worms in this test concentration were shorter and thinner. No other particular behavioral or morphological changes were observed.

In a 14-d toxicity study with BAS 500 06 F on earthworms (*Eisenia fetida)* the LC_{50} was 385.6 mg BAS 500 06 F/kg dry soil. The NOEC related to mortality and biomass was 296.3 mg BAS 500 06 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 200 g/L (202.7 g/L analyzed), density: 1.044 g/cm³.
Test species:	Earthworm (*Eisenia fetida*), adult worms (with clitellum and weight 300 - 600 mg), less than 1 year old; source: in-house culture.

B. STUDY DESIGN

Test design:	14-d exposure in treated artificial soil; different concentrations of the test item were mixed homogeneously into the soil which was filled in glass vessels before the earthworms were introduced on top of the soil; 6 treatment groups (5 test item rates, control); 4 replicates/group with 10 worms each. Earthworm mortality effects were assessed after 7 and 14 d, measurement of weight change as sublethal parameter after 14 d.
Endpoints:	Mortality of earthworms after exposure over 14 days, weight change.
Reference item:	2-chloroacetamide. The effects of the toxic reference item were evaluated in a separate study.
Test concentrations:	Control, 197.5, 296.3, 444.4, 666.7 and 1000.0 mg BAS 500 06 F/kg dry soil.
Test conditions:	Artificial soil according to ISO 11268-1:1993(E) with 10% sphagnum peat; pH 6.1; water content: 28.8 g/100 g dry soil at test initiation, 26.4 g/100 g dry soil at test termination; temperature: 20°C ± 2°C; photoperiod: 16 h light : 8 h dark, light intensity: 400 lux - 800 lux.
Statistics:	Descriptive statistics. Spearman-Karber Estimate for determination of LC_{50} and Bonferroni t-Test for biomass development ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

The LC_{50} derived from the results was 385.63 mg BAS 500 06 F/kg dry soil.

After 14 days of exposure no mortality was observed in treatment groups up to 296.3 mg/kg dry soil. High mortality was observed at test concentrations of 444.4 mg/kg dry soil and higher. All treatment groups including the control showed reduction in biomass. Statistically significant differences (Bonferroni t-Test, $\alpha = 0.05$) were determined in the treatment group with the test concentration of 444.4 mg/kg dry soil, surviving worms in this test concentration were shorter and thinner. No other particular behavioral or morphological changes were observed. The results are summarized in Table 10.4.1-5.

Table 10.4.1-5: Effect of BAS 500 06 F on earthworm (*Eisenia fetida)* mortality and biomass (14 d)

BAS 500 06 F [mg/kg dry soil]	Control	197.5	296.3	444.4	666.7	1000
Mortality [%]	0.00	0.00	0.00	85.00	100.00	100.00
Weight change [%]	-12.87	-8.90	-14.82	-34.02 *	--	--
Endpoints [mg/kg dry soil]						
NOEC	296.3					
LC_{50} (95% CL) [1)]	385.63 (368.71 - 403.32)					

[1)] Median effect concentration calculated using Spearman-Karber Estimate (with 95% Confidence Limits).
* = statistically significant differences compared to the control (Bonferroni t-Test; $\alpha = 0.05$).

III. CONCLUSION

In a 14-d toxicity study with BAS 500 06 F on earthworms (*Eisenia fetida)* the LC_{50} was 385.6 mg BAS 500 06 F/kg dry soil. The NOEC related to mortality and biomass was 296.3 mg BAS 500 06 F/kg dry soil.

CP 10.4.1.1 Earthworms – sub-lethal effects

Report:	CP 10.4.1.1/1 Luehrs U., 2008a Effects of BAS 500 06 F on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5% peat 2008/1036409
Guidelines:	OECD 222, ISO 11268-2 (1998)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary

The effects of BAS 500 06 F on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* were investigated in a 56-day extended laboratory study. Five application rates (3.8, 7.5, 15.0, 30.0 and 60.0 mg BAS 500 06 F/kg dry soil) were incorporated into the soil (5% peat) with four replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. The reference item was tested in a separate study. Assessment of adult worm mortality and biomass development was carried out after 28 days, assessment of reproduction rate (number of juveniles) was carried out after 56 days.

No mortality of parent earthworms was observed in any treatment group. No statistically significant effects on body weight were observed up to the highest test concentration of 60 mg BAS 500 06 F/kg dry soil. The number of juveniles was statistically significantly reduced in the highest test item concentration of 30 mg BAS 500 06 F/kg dry soil. No behavioral abnormalities were observed in any of the treatment groups.

In a 56-day reproduction study with BAS 500 06 F, no statistically significant effects on mortality and growth of earthworms (*Eisenia fetida*) were observed up to a rate of 60 mg BAS 500 06 F/kg dry soil. The NOEC for reproduction was 30 mg BAS 500 06 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. no.: 304 428): 200 g/L (202.7 g/L analyzed), density: 1.044 g/cm³.

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum and weight of 300 - 600 mg), approximately 10 months old; source: in-house culture.

B. STUDY DESIGN

Test design: 56-day test in treated artificial soil according to OECD 222 (5% peat). Different concentrations of the test item are mixed homogeneously into the soil. 6 treatment groups (5 test item rates, control) were set up with 8 replicates for the control and 4 replicates for the test item group, each with 10 worms. Assessment of worm mortality, behavioral effects and weight change was done after 28 days of exposure, after an additional 28 days (56 days after application) the number of offspring was counted.

Endpoints: Mortality, weight change, behavioral effects, reproduction rate.

Reference item: Brabant Carbendazim Flowable (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.

Test rates: Control, 3.8, 7.5, 15.0, 30.0 and 60.0 mg BAS 500 06 F/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with a reduced content of peat of 5%); pH 5.6 - 5.7 at test initiation, pH 6.1 at test termination; water content at test initiation: 49.7% to 54.1% of maximum water holding capacity (WHC), 52.1% to 57.9% of maximum WHC at test termination; temperature: 19°C - 21°C; photoperiod: 16 hours light : 8 hours dark; light intensity: 410 lux - 700 lux; feeding with cattle manure.

Statistics: Descriptive statistics. Dunnett-test for weight changes and reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

No mortality of parent earthworms was observed in any treatment group. No statistically significant effects on body weight were observed up to the highest test concentration of 60 mg BAS 500 06 F/kg dry soil (Dunnett-test, $\alpha = 0.05$). The number of juveniles was statistically significantly reduced in the highest test item concentration of 30 mg BAS 500 06 F/kg dry soil (Dunnett-test, $\alpha = 0.05$). No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in the test item groups was comparable to the control. The results are summarized in Table 10.4.1.1-1.

Table 10.4.1.1-1: Effect of BAS 500 06 F on earthworm (*Eisenia fetida*) in a 56-day reproduction study

BAS 500 06 F [mg/ kg dry soil]	**Control**	**3.8**	**7.5**	**15.0**	**30.0**	**60.0**
Mortality (28 d) [%]	0	0	0	0	0	0
Weight change (28 d) [%]	29.9	35.6	34.0	29.8	27.8	26.7
Number of juveniles (56 d)	234	193	207	224	208	151*
Reproduction (56 d) [% of control]	--	82.6	88.5	95.9	88.7	64.4
	Endpoints [mg BAS 500 06 F/kg dry soil]					
$NOEC_{mortality,\ weight\ change}$ (28 d)	60					
$NOEC_{reproduction}$ (56 d)	30					

* = Statistically significant compared to the control (Dunnett-test, $\alpha = 0.05$).

III. CONCLUSION

In a 56-day reproduction study with BAS 500 06 F, no statistically significant effects on mortality and growth of earthworms (*Eisenia fetida*) were observed up to a rate of 60 mg BAS 500 06 F/kg dry soil. The NOEC for reproduction was 30 mg BAS 500 06 F/kg dry soil.

CP 10.4.1.2 Earthworms – field studies

Report:	CP 10.4.1.2/1 Luehrs U., 2010a Field study to evaluate the effects of BAS 500 06 F on earthworms 2010/1000056
Guidelines:	ISO 11268-3 (1999), Kula et al. (2006) - Technical Recommendations for the Update of the ISO Earthworm Field Test Guideline (ISO 11268-3)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

A grassland site was chosen as a natural habitat of earthworms. The treatments with 1.25 L/ha, 2.5 L/ha and 6.25 L BAS 500 06 F/ha were assigned randomly to the plots within each replicate. Over the experimental period from May 2009 until May 2010 four earthworm samplings were evaluated. Earthworm extraction was achieved using the electrical octet method in combination with hand sorting.
Prior to the treatment, the test site yielded sufficiently high numbers of earthworms of 202.8 individuals per m^2 representing various common earthworm species such as *Aporrectodea caliginosa*, *Octolasion tyrtaeum* and *Lumbricus terrestris*.

No statistically significant differences between BAS 500 06 F treatments and the untreated control could be detected in terms of total earthworm abundance 1.5, 5 and 12 months after application. The total biomass was reduced after 6 weeks with statistically significant differences compared to the control at 1.25 L and 6.25 L BAS 500 06 F/ha. However, total biomass had fully recovered after 5 months of exposure. After one year, exposure biomass was reduced at a rate of 1.25 L BAS 500 06 F/ha, however based on lacking dose-response it was not considered to be treatment related. For the most abundant earthworm species *Lumbricus terrestris* and *Aporrectodea caliginosa* the abundance was slightly lower at 6.25 L BAS 500 06 F/ha after 6 weeks and had recovered after 5 months. At the end of the study (12 month after application) no statistically significant effects were found, i.e. all reductions observed during the study were transient.

Based on the results of this field study, it is concluded that exposure of earthworm populations to rates up to 6.25 L BAS 500 06 F/ha does not cause unacceptable long-term effects.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F): 200 g/L (202.7 g/L analyzed), density: 1.044 g/cm³.

Test species: Naturally occurring population of earthworms comprising all mobile stages (juveniles and adult worms) including epilobous species such as Aporrectodea caliginosa, Aporrectodea limicola, Aporrectodea rosea, Octolasion tyrtaeum and Octolasion cyaneum as well as tanylobous species such as Lumbricus castaneuss and Lumbricus terrestris.

B. STUDY DESIGN

Test site: Grassland site near Klein-Zimmern in municipality Groß-Umstad, Germany. The site had not received chemical applications for the past 5 years and none were applied during the study apart from the test and reference item.

Test design: Randomized block design with 5 treatment groups (control, 3 test item rates and reference item) each with of 4 replicates. A number of 20 plots each 10 m x 10 m, were arranged. The substances in all treatments were applied in a water volume equivalent to 400 L/ha. Application was performed using a plot sprayer with 2.5 m boom width. Natural rainfall within 2 days after application was 23 mm, therefore no additional irrigation.

Endpoints: Total abundance and biomass of earthworms.

Reference item: Luxan Carbendazim-500 FC (carbendazim, 500 g/L).

Test rates: Untreated control; BAS 500 06 F: 1.25 L/ha, 2.5 L/ha, 6.25 L/ha; reference item: 6 kg carbendazim/ha.

Application date: 15.05.2009.

Method of extraction: Earthworm extraction was achieved by using the electrical octet method in combination with hand sorting. 4 samples per plot were taken from the inner area of the plots.

Sampling dates: Pre-sampling: 2 to 3 days before application (12. + 13.05.2009); 1st sampling: approx. 6 weeks after application (24. + 25.06.2009); 2nd sampling: approx. 5 months after application (20. + 21.10.2009); 3rd sampling: approx. 12 months after application (10. + 11.05.2010).

Test conditions:	Natural field conditions; strong clayey silt to strong silty clay (according to DIN 4220), pH 5.9 - 6.2, 2.2 - 2.3% total organic carbon, max. water holding capacity 61.0%.
Air temperature at application day:	13.4°C - 16.6°C, Soil temperature at application day: 14.9°C - 15.6°C.
Analytics:	Analytical verification of pyraclostrobin in 5 soil cores taken from each treated plot using an LC-MC/MS method.
Statistics:	Descriptive statistics; Dunnett's t-test and Bonferroni-Welch t-test for the test item treatment groups, Student t-test for the reference item group ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

No measurable residues of BAS 500 06 F were determined in any of the soil samples of the control plots. In the plots treated with the test item BAS 500 06 F, mean residue values of 58 to 68% (ranging from 44 to 92% of the expected soil concentration) of the application rate were found. All mean recoveries were in the recommended range of 50-150%.

Prior to the treatment, the test site yielded sufficiently high numbers of earthworms of 202.8 individuals per m^2 representing various common earthworm species such as *Aporrectodea caliginosa*, *Octolasion tyrtaeum* and *Lumbricus terrestris*.
Surface monitoring on days 1-3 after application showed that there was no acute primary effect on earthworms by the test item. No alive, moribund and dead earthworms were found on the soil surface in any of the plots treated with BAS 500 06 F and in the control plots.
No statistically significant differences between BAS 500 06 F treatments and the untreated control could be detected in terms of total earthworm abundance 1.5, 5 and 12 months after application. The total biomass was reduced after 6 weeks (maximum 27.7%) with statistically significant differences compared to the control at 1.25 L and 6.25 L BAS 500 06 F/ha (Bonferroni-Welch t-test, $\alpha = 0.05$). However, total biomass had fully recovered after 5 months of exposure. After one year, exposure biomass was reduced at a rate of 1.25 L BAS 500 06 F/ha, which was not considered to be treatment related as no dose relation could be observed. For the most abundant earthworm species *Lumbricus terrestris* and *Aporrectodea caliginosa* the abundance was slightly but not statistically significantly reduced at 6.25 L BAS 500 06 F/ha after 6 weeks and had recovered after 5 months (Dunnett's t-test, $\alpha = 0.05$). The slight but not significant reduction of juvenile earthworms after 6 weeks had recovered after 12 months. Moreover, no statistically significant effects were found at the end of the study. The results are summarized in Table 10.4.1.2-1.

Table 10.4.1.2-1: Summary of total earthworm abundance and biomass in a field study with BAS 500 06 F

Treatment	Pre sampling 12. + 13.05.2009	First sampling 24. + 25.06.2009	Second sampling 20. + 21.10.2009	Third sampling 10. + 11.05.2010
Total earthworm abundance [Ind./m²]				
Control	202.8	261.0	235.0	211.0
1.25 L/ha BAS 500 06 F	195.5	235.5	214.0	185.5
% of control	**96.4**	**90.2**	**91.1**	**87.9**
2.5 L/ha BAS 500 06 F	202.5	218.5	209.0	205.5
% of control	**99.9**	**83.7**	**88.9**	**97.4**
6.25 L/ha BAS 500 06 F	206.8	185.0	188.5	197.0
% of control	**102.0**	**70.9**	**80.2**	**93.4**
Reference item: carbendazim	102.2% of control	38.5% of control *	38.9% of control *	55.0% of control *
Total earthworm biomass [g/m²]				
Control	75.3	80.9	60.9	66.7
1.25 L/ha BAS 500 06 F	65.3	58.9	61.9	46.6
% of control	**86.7**	**72.8 ***	**101.7**	**69.8**
2.5 L/ha BAS 500 06 F	66.4	65.9	60.4	59.3
% of control	**88.2**	**81.5**	**99.2**	**88.9**
6.25 L/ha BAS 500 06 F	78.7	59.0	61.1	56.2
% of control	**104.5**	**73.0 ***	**100.4**	**84.2**
Reference item: carbendazim	101.2% of control	31.6% of control *	47.7% of control *	57.9% of control *

* Statistically significantly different compared to the control (Dunnett's t-test, Bonferroni-Welch t-test or Student t-test, α = 0.05).

The validity criterion for the reference item Luxan Carbendazim-500 FC was fulfilled by reductions > 50% and statistically significant differences in earthworm abundance and biomass at the first and second sampling (Student t-test, α = 0.05).

III. CONCLUSION

Based on the results of this field study, it is concluded that exposure of earthworm populations to rates up to 6.25 L BAS 500 06 F/ha does not cause unacceptable long-term effects.

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

According to SANCO/10329/2002 rev2 final the test on 'sub-lethal effects on collembola or soil mites' is required if *(a)* the $DT_{90, field}$ is between 100 and 365 days and *(b)* the standard HQ for arthropods (*T. pyri* and *A. rhopalosiphi*) is above 2.

Toxicity and Exposure

Since the worst-case $DT_{90, field}$ values of pyraclostrobin and the metabolites BF 500-6 and BF 500-7 exceed 100 days and the standard HQ values for *T. pyri* and *A. rhopalosiphi* exceed the trigger value of 2 (please refer to M-CP 10.3), a test with soil macro-organisms other than earthworms was triggered. Therefore, reproduction studies with BAS 500 06 F as well as with the metabolites BF 500-6 and BF 500-7 were carried out with *Folsomia candida*. Furthermore, a reproduction study with BAS 500 06 F and *Hypoaspis aculeifer* was carried out in order to gain additional information for the risk assessment. The chronic endpoints are summarized in Table 10.4.2-1. In addition, a study on effects of BAS 500 06 F on organic matter breakdown is available and shown in M-CP 10.4.2.2. However, since the assessment on collembolan and soil mites indicated low potential risk and the study type of organic matter break down is currently not mentioned as data requirement under Regulation (EC) 1107/2009, the study is added for completeness and not considered for risk assessment.

Table 10.4.2-1: Summary of chronic endpoints on *Folsomia candida* and *Hypoaspis aculeifer* for BAS 500 06 F

Test substance	Test species	Endpoint	Value [mg/kg dry soil]	Reference (BASF DocID)
BF 500-6 [1)]	*Folsomia candida*	NOEC	≥ 1000	2013/1068054
BF 500-7 [1)]	*Folsomia candida*	NOEC	≥ 800	2013/1224030
Pyraclostrobin in BAS 500 06 F [1)]	*Folsomia candida*	NOEC	23.9 [2)]	2008/1037495
Pyraclostrobin in BAS 500 06 F [1)]	*Hypoaspis aculeifer*	NOEC	≥ 17.3 [3)]	2012/1129444

1) Test was conducted with only 5% peat in the test substrate.
2) Based on a nominal content of BAS 500 06 F (200.0 g/L pyraclostrobin) and taken into account the density of 1.044 g/cm^3.
3) Based on a nominal content of BAS 500 06 F (200.0 g/L pyraclostrobin) and taken into account the density of 1.038 g/cm^3.

Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)

For risk assessment the chronic toxicity data (NOEC for reproduction) are compared to the predicted environmental concentration considering long-term exposure following multi-year use of BAS 500 06 F. The results are presented in Table 10.4.2-2.

Table 10.4.2-2: Long-term TER values for Folsomia candida and Hypoaspis aculeifer

Test substance	Test species	NOEC [mg/kg dry soil]	PEC_{soil} [mg/kg dry soil]	TER_{LT}	TER trigger
BF 500-6	*Folsomia candida*	≥ 1000	0.121	≥ 8264	5
BF 500-7	*Folsomia candida*	≥ 800	0.073	≥ 10959	
Pyraclostrobin in BAS 500 06 F	*Folsomia candida*	23.9	0.228	105	
Pyraclostrobin in BAS 500 06 F	*Hypoaspis aculeifer*	≥ 17.3		≥ 76	

All long-term TER values exceed the Commission Regulation (EU) 546/2011 trigger value of 5, indicating that no unacceptable effects are expected for other non-target soil meso- and macrofauna (other than earthworms) when BAS 500 06 F is applied according to the proposed use pattern.

CP 10.4.2.1 Species level testing

Report: CP 10.4.2.1/1
Friedrich S., 2008b
Effects of BAS 500 06 F on the reproduction of the collembolans Folsomia candida in artificial soil with 5% peat
2008/1037495
Guidelines: ISO 11267 (1999)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of BAS 500 06 F on mortality and reproduction of Collembola *(Folsomia candida)* were investigated in a laboratory study over 28 days. Six application rates (15.7, 31.3, 62.5, 125.0, 250.0 and 500.0 mg BAS 500 06 F/kg dry soil) were incorporated into the soil (5% peat only) with 5 replicates per treatment (each containing 10 juvenile collembolans). An untreated control with 5 replicates was included. Assessment of adult springtail mortality and reproduction rate (number of juveniles) was carried out after 28 days.

A mortality of 4% was observed in the control group compared to 0 - 8% mortality at 15.7, 31.3, 62.5 and 125 mg BAS 500 06 F/kg dry soil. At the concentrations of 250 and 500 mg/kg dry soil statistically significant mortality of 52 and 80% was determined. In the control a mean of 575.4 juveniles was counted. In the treatment groups a mean number of juveniles of 535.8 to 19.0 was counted with statistically significant differences compared to the control for the two highest treatment groups.

In a 28-day collembolan reproduction study with BAS 500 06 F the NOEC based on mortality and reproduction was 125 mg BAS 500 06 F/kg dry soil. The EC_{50} was 189.6 mg BAS 500 06 F/kg dry soil, the LC_{50}.was determined to be 283.8 mg BAS 500 06 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. no. 304 428): 200 g/L (202.7 g/L analyzed), density: 1.044 g/cm³.

Test species: Collembola (*Folsomia candida*), juveniles (10-12 days old); source: in-house culture.

B. STUDY DESIGN

Test design: 28-day test in treated artificial soil according to ISO 11267 (5% peat only); artificial soil was filled in glass vessels after treatment with different concentrations of the test item before collembolans were introduced. 7 treatment groups (6 test item concentrations, control) were set up with 5 replicates for each and each containing 10 juvenile collembolans.
Assessment of adult collembolans mortality and reproduction rate (number of juveniles) was carried out after 28 days.

Endpoints: Mortality, reproduction rate.

Reference item: Betosip (phenmedipham, 114 g/L). The effects of the reference item were investigated in a separate study.

Test rates: Control, 15.7, 31.3, 62.5, 125.0, 250.0 and 500.0 mg BAS 500 06 F/kg dry soil (nominal).

Test conditions: Artificial soil according to ISO 11267 (with reduced content of peat: 5%); pH 6.1 at test initiation, pH 5.9 - 6.0 at test termination; water content at study initiation 59.6% - 59.8% of maximum water holding capacity (WHC) and 58.9% - 59.3% of maximum WHC at test termination; temperature: 20°C - 22°C; photoperiod: 16 h light : 8 h dark; light intensity: 570 lux; food: 2 mg granulated dry yeast at the start of the test and after 14 days.

Statistics: Descriptive statistics. Fisher-exact test for mortality, Welch-t-test for reproduction, Probit analysis for determination of the LC_{50}, Logit analysis for determination of the EC_{50}.

II. RESULTS AND DISCUSSION

A mortality of 4% was observed in the control group compared to 0 - 8% mortality at 15.7, 31.3, 62.5 and 125 mg BAS 500 06 F/kg dry soil. At the concentrations of 250 and 500 mg/kg dry soil a statistically significant mortality of 52 and 80% was determined (Fisher-exact test, $\alpha = 0.05$).
In the control a mean of 575.4 juveniles was counted. In the treatment groups a mean number of juveniles of 535.8 to 19.0 was counted with statistically significant differences compared to the control for the two highest treatment groups (Welch-t-test, $\alpha = 0.05$). The results are summarized in Table 10.4.2.1-1.

Table 10.4.2.1-1: Effect of BAS 500 06 F on Collembola (*Folsomia candida)* in a 28-day reproduction study

BAS 500 06 F [mg/kg dry soil]	**Control**	**15.7**	**31.3**	**62.5**	**125.0**	**250.0**	**500.0**
Mortality (day 28) [%]	4	4	0	2	8	52*	80*
No. of juveniles (day 28)	575.4	535.8	601.8	576.8	579.4	76.6*	19.0*
Reproduction in [%] of control (day 28)	--	93	105	100	101	13	3
Endpoints [mg/kg dry soil]							
$NOEC_{mortality,\ reproduction}$	125						
EC_{50}	189.6						
LC_{50}	283.8						

* = Statistically significant differences compared to the control (Fisher-exact test for mortality; Welch-t-test for reproduction, $\alpha = 0.05$).

III. CONCLUSION

In a 28-day collembolan reproduction study with BAS 500 06 F the NOEC based on mortality and reproduction was 125 mg BAS 500 06 F/kg dry soil. The EC_{50} was 189.6 mg BAS 500 06 F/kg dry soil, the LC_{50}.was determined to be 283.8 mg BAS 500 06 F/kg dry soil.

Report:	CP 10.4.2.1/2 Schulz L., 2012c BAS 500 06 F - Effects of BAS 500 06 F on the reproduction of the predatory mite Hypoaspis aculeifer 2012/1129444
Guidelines:	OECD 226 (2008)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of BAS 500 06 F on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. The test item was mixed into artificial soils at rates of 5.6, 11.3, 22.5, 45.0 and 90.0 mg BAS 500 06 F/kg dry soil. Test item treatments were replicated four times each. As a control treatment, the soil remained untreated in eight replicates. Each treatment contained 10 adult soil mites. Reproduction and mortality assessments of the mites were carried out after 14 days of exposure.

No differences in behavior between mites in the control and the test item treatments could be observed.

Test item treatment groups had mortality rates of between 5.0 - 15.0%. The mortality rate in the control group was 5.0%. The observed mortality rates for adult mites in test item treatment groups were not statistically significantly different from those observed in control groups.

Reproduction rates in test item treatments were not statistically significantly different from reproduction rates in control groups and showed no adverse effects of BAS 500 06 F on reproduction in all tested concentrations.

In a 14-day reproduction study with BAS 500 06 F on predatory soil mites, the NOEC was determined to be 90.0 mg BAS 500 06 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 0003223026; content of a.s.: pyraclostrobin (BAS 500 F, Reg. no. 304 428): 200.0 g/L (nominal); 200.7 g/L (analyzed); density: 1.038 g/cm³.

Test species: *Hypoaspis aculeifer* (Canestrini), adult predatory mite (age difference 3 days); source: in-house culture, originally from Katz Biotech AG, Baruth, Germany.

B. STUDY DESIGN

Test design: 14-day laboratory test on effects of BAS 500 06 F on mortality and reproduction of soil mites. Artificial soil (5% peat) was treated with different concentrations of the test item and filled in glass vessels before predatory mites were introduced on top of the soil; 6 treatment groups (control, 5 test item concentrations); 8 replicates for control and 4 replicates for test item, each with 10 soil mites; assessment of dault mortality and reproduction rate (number of juveniles) after 14 days.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate EC 400 (411.7 g analyzed); trade product: Perfekthion. The effects of the reference item were investigated in a separate study.

Test rates: Untreated control (deionized water), 5.6, 11.3, 22.5, 45 and 90 mg BAS 500 06 F/kg dry soil.

Test conditions: Artificial soil according to OECD 226; pH 5.6 - 5.7 at test initiation, pH 5.2 - 5.3 at test termination; water content at test initiation 50.41% - 51.41% of maximum water holding capacity (WHC) and 48.88 - 50.38% of maximum WHC at test termination; temperature: 19.6°C - 21.4°C; photoperiod: 16 h light : 8 h dark; light intensity: 470 lux. food: cheese mites (*Tyrophagus putrescentiae*).

Statistics: Descriptive statistics; Fisher Exact Binominal Test with Bonferroni Correction for mortality (α = 0.05, one-sided greater), Dunnett-t test for reproduction (α = 0.05, one-sided smaller).

II. RESULTS AND DISCUSSION

Mortality rates of 5.0 to 15.0% were recorded in the test item treatments groups and 5.0% in the control group. The observed mortality rates for adult mites in test item treatment groups was not statistically significantly different from those observed in control groups (Fisher Exact Binominal Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). No differences in behavior between mites in the control and the test item treatments could be observed.
Reproduction rates in the 5.6, 11.3, 22.5, 45.0 and 90.0 mg BAS 500 06 F/kg dry soil were 236.8, 251.8, 273.3, 227.8 and 215.8 juveniles, respectively. BAS 500 06 F caused no statistically significantly differences on the reproduction rates of *Hypoaspis aculeifer* at all tested concentrations (Dunnett-t test, $\alpha = 0.05$, one-sided smaller). The results are summarized in Table 10.4.2.1-2.

Table 10.4.2.1-2: Effects of BAS 500 06 F on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

BAS 500 06 F [mg/kg dry soil]	**Control**	**5.6**	**11.3**	**22.5**	**45**	**90**
Mortality (day 14) [%]	5.0	10.0	7.5	5.0	7.5	15.0
No. of juveniles (day 14)	232.5	236.8	251.8	273.3	227.8	215.8
Reproduction [% of control] (day 14)	100	102	108	118	98	93
Endpoints [mg BAS 500 06 F/kg dry soil]						
$NOEC_{mortality\ and\ reproduction}$	≥ 90					
LC_{50}	> 90					
EC_{50}	> 90					

The reference item dimethoate was tested at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg dry soil. In a separate study (BioChem project no. R 12 10 48 002 S), the EC_{50} (reproduction) for dimethoate was calculated to be 6.87 mg a.s./kg dry soil. The results of the reference item demonstrate the sensitivity of the test system.

III. CONCLUSION

In a 14-day reproduction study with BAS 500 06 F on predatory soil mites, the overall NOEC was determined to be ≥ 90.0 mg BAS 500 06 F/kg dry soil.

CP 10.4.2.2 Higher tier testing

Further studies are not triggered, however a litterbag study on organic matter breakdown is available. This study was a former requirement under Council Directive 91/414/EEC and SANCO/ 10329/2002 rev 2. However, under Regulation (EC) 1107/2009 it is not required anymore. Therefore, the study is considered as additional information.

Report:	CP 10.4.2.2/1 Luehrs U., Schabio S., 2010a Effects of BAS 500 06 F on the breakdown of organic matter in litter bags in the field 2010/1000081
Guidelines:	Roembke et al. (2003), OECD 56 (2006)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The test item BAS 500 06 F was studied for its potential effects on the degradation of buried organic wheat straw compared to a water control after exposure of about 1, 3 and 6 months. The study was set up as randomized block design with an application on soil surface at 1.25 L BAS 500 06 F/ha. An incorporation of a plateau concentration was omitted for this test, because the active substance pyraclostrobin is not persistent (max. $DT_{50,field}$ of 37 days), and therefore does not build up a plateau concentration in soil even after long-term use.

The mass loss of the straw material in the untreated control was 80.5% at the end of the experiment after 179 days. In the treatment group exposing the soil to 1.25 L BAS 500 06 F/ha, the mean mass losses after the exposure phases of about 1, 3 and 6 months were only slightly different from those in the control, resulting in effects of -5.5, +0.2 and -0.1%, respectively. There were no statistically significant differences between this treatment group and the water treated control.

The results of this 6-month field study on an arable field site emphasize that BAS 500 06 F has no treatment-related ecologically relevant effects on the organic matter breakdown following application of 1.25 L BAS 500 06 F/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 500 06 F, batch no. 8265; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 202.7 g/L (nominal: 200.0 g/L); density: 1.044 g/cm^3.
Test species:	Naturally occurring non-target soil organisms.

B. STUDY DESIGN

Test site:	Arable crop site in Rossdorf (Darmstadt-Dieburg), Germany; total size about 1460 m², the site had not received chemical application since May 2008 and none was applied during the study apart from the test item.
Test design:	Randomized block design with two treatments (1 test item groups, water treated control) and six replicates each treatment. Exposure via application of the annual rate on the soil surface. An incorporation of a plateau concentration was omitted for this test because pyraclostrobin is not persistent (max. DT_{50} of 37 days). Within the plots (5 x 6 m plot size) the litter bags were randomly distributed in the upper soil layer (depth of about 5 cm). The treatments were assigned randomly to the plots within each replicate. The substances in all treatments were applied in a water volume equivalent to 400 L/ha. Application was performed using a movable plot sprayer with a distance between nozzles and soil of 0.5 m. Immediately after the application the test site was irrigated with 10.4 mm.
Endpoints:	Mean weight loss of organic matter based on ash-free dry weight per plot per treatment.
Test rates:	Treatment group 1: Untreated control; treatment group 2: 1.25 L BAS 500 06 F/ha (equivalent to 250 g a.s./ha).
Application dates:	Burying of litter bags: 04.05.2009; application: 05.05.2009.
Test conditions:	Natural field conditions; clayey silt/silty loam according to DIN 4220, 1.1% - 1.2% total organic carbon, pH 6.4 - 7.1. Air temperature: 9.6 to 19.6°C, soil temperature: 11.3 - 19.8°C, total precipitation per month: 17.1 - 110.2 L/m².

Litter bags:	Litter bags consisted of mesh material (100% polyester) with a mesh size of about 8 mm. The size of a bag was about 12 x 20 cm. Bags were filled with about 4.0 g (dry weight) of untreated dried wheat straw. Litter-bags were horizontally buried at a depth of about 5 cm, 1 day before application of the annual rate of BAS 500 06 F.
Sampling dates:	1st sampling: 29.05.2009 (after 25 days of exposure). 2nd sampling: 31.07.2009 (after 88 days of exposure). 3rd sampling: 30.10.2009 (after 179 days of exposure).
Sample processing:	Sampling was done at three different time intervals (25, 88 and 179 days of exposure). 8 bags were sampled per replicate per sampling date (48 bags per treatment), immediately transported to the laboratory and stored deep frozen until further processing. The enclosed straw material was cleaned and the bag content was oven-dried at 35°C for 12 hours and weighted. Finally, the straw was ashed at 600°C for 0.5 hours and ash-free weight was determined.
Analytics:	Soil samples were only analyzed for the active substance pyraclostrobin according to the analytical method M432.
Statistics:	Descriptive statistics, Student t test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Recovery rates for the active substance pyraclostrobin ranged from 51 to 92% of the expected soil concentration (mean 75%).

The mass loss of the straw material in the untreated control was 80.5% at the end of the experiment after 179 days. In the treatment group exposing the soil to 1.25 L BAS 500 06 F/ha, the mean mass losses after the exposure phases of about 1, 3 and 6 months were only slightly different from those in the control, resulting in effects of -5.5, +0.2 and -0.1%, respectively. There were no statistically significant differences between this treatment group and the water treated control (Student t-test, $\alpha = 0.05$). Effects on the degradation of buried wheat straw are summarized in Table 10.44.2.2-1.

Table 10.4.4.2.2-1: Mass loss [%] of wheat straw following exposure to BAS 500 06 F

Treatment	Mean mass loss [%]		
	Sampling 1	Sampling 2	Sampling 3
Control	16.9	54.1	80.5
1.25 L BAS 500 06 F/ha, % effect (mass loss)	17.8 (-5.5)	54.0 (+0.2)	80.6 (-0.1)

III. CONCLUSION

The results of this 6-month field study on an arable field site emphasize that BAS 500 06 F has no treatment-related ecologically relevant effects on the organic matter breakdown following application of 1.25 L BAS 500 06 F/ha.

CP 10.5 Effects on soil nitrogen transformation

The new representative formulation BAS 500 06 F was not evaluated within the previous Annex I inclusion process. It is an emulsifiable concentrate (EC) containing 200g/L pyraclostrobin.

The EU agreed endpoints for the soil metabolites of the active substance pyraclostrobin as described in the EU Review Report (SANCO/1420/2001-final, September 2004) plus endpoints from a new study with the new representative formulation BAS 500 06 F are used for the risk assessment on soil micro-organisms (see Table 10.5-1).

Table 10.5-1 Ecotoxicological endpoints for soil micro-organisms

Test substance	Test design [1)]	EU agreed endpoints	Endpoints used in risk assessment
Pyraclostrobin [2)]	C	NOEC = 3.33 mg a.s./kg dry soil, (equivalent to 2.5 kg a.s./ha)	--
	N	NOEC = 3.33 mg a.s./kg dry soil, (equivalent to 2.5 kg a.s./ha)	--
BF 500-6	C	NOEC = 1.0 mg/kg dry soil, equivalent to 0.75 kg/ha	
	N	NOEC = 1.0 mg/kg dry soil, equivalent to 0.75 kg/ha	NOEC = 1.0 mg/kg dry soil, equivalent to 0.75 kg/ha
BF 500-7	C	NOEC = 0.5 mg/kg dry soil, equivalent to 0.375 kg/ha	
	N	NOEC = 0.5 mg/kg dry soil, equivalent to 0.375 kg/ha	NOEC = 0.5 mg/kg dry soil, equivalent to 0.375 kg/ha
BAS 500 06 F	N	--	NOEC = 17.3 mg/kg dry soil (3.33 mg a.s./kg dry soil) [3)], equivalent to 12.5 L/ha

1) C = Carbon transformation, N = Nitrogen transformation.
2) Study was conducted with the pyraclostrobin solo-formulation BAS 500 00 F (250 g pyraclostrobin/L).
3) This study replaces also the old N-transformation study for the active substance pyraclostrobin, which was conducted with the former representative formulation BAS 500 00 F (see above).

Toxicity

The effects of BAS 500 06 F and the relevant metabolites to soil micro-organisms are summarized in Table 10.5-2. Further details on the studies on nitrogen transformation with the formulated product are given in the summary provided at the end of this chapter.

Table 10.5-2: Effects of BAS 500 06 F, BF 500-6 and BF 500-7 to soil micro-organisms

Test substance	Endpoint	NOEC	Reference (BASF DocID)
BF 500-6	Effects on nitrogen and carbon transformation	1.0 mg/kg dry soil	1999/11311 1999/11120
BF 500-7	Effects on nitrogen and carbon transformation	0.5 mg/kg dry soil	1999/11311 1999/11120
BAS 500 06 F	Effects on nitrogen transformation	17.3 mg/kg dry soil (equivalent to 12.5 L/ha)	2012/1129443

Exposure

Table 10.5-3: Critical use pattern of BAS 500 06 F

Crop	Application time (BBCH code)	Number of applications	Interval [d]	Application rate per treatment	
				Pyraclostrobin [g a.s./ha]	BAS 500 06 F [L/ha]
cereals	25 - 69	2	21	250	1.25
maize	30 - 65	1	--	200	1.0

The predicted environmental concentrations of pyraclostrobin and relevant metabolites in soil (PEC_{soil}) were calculated as described in M-CP 9.1. The resulting maximum PEC_{soil} values are presented in Table 10.5-4.

Table 10.5-4: PEC_{soil} values for pyraclostrobin and relevant metabolites *

Test substance	$PEC_{soil, max}$ [mg/kg dry soil]	$PEC_{soil, plateau}$ [mg/kg dry soil]	$PEC_{soil, accu}$ [mg/kg dry soil]
Pyraclostrobin	0.228	--	--
BF 500-6	0.065	0.057	0.121
BF 500-7	0.039	0.034	0.073

* Only worst-case scenario is presented.

Risk assessment for Soil Nitrogen Transformation

BAS 500 06 F had no significant effect on soil micro-organisms at 17.3 mg BAS 500 06 F/kg dry soil, corresponding to 3.33 mg pyraclostrobin/kg dry soil. This is approximately 15-times higher than the maximum $PEC_{soil, max}$ of 0.228 mg pyraclostrobin/kg dry soil. This supports the conclusion that under field conditions, the use of BAS 500 06 F in cereals and maize at the recommended rates poses no unacceptable risk to non-target soil micro-organisms.

Furthermore, the NOEC values for the metabolites of 1.0 mg BF 500-6/kg dry soil and 0.5 mg BF 500-7/kg dry soil are more than 8- and approximately 7-times higher than the maximum $PEC_{soil, accu}$ values of 0.121 mg BF 500-6/kg dry soil and 0.073 mg BF 500-7/kg dry soil, respectively.

It is concluded that the proposed use of BAS 500 06 F in cereals and maize will not pose an unacceptable risk to non-target soil micro-organisms, if applied according to the recommended use pattern.

Report:	CP 10.5/1 Schulz L., 2012b Effects of BAS 500 06 F on the activity of soil microflora (Nitrogen transformation test) 2012/1129443
Guidelines:	OECD 216 (2000)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effect of BAS 500 06 F on nitrogen transformation was tested in loamy sand soil. BAS 500 06 F was applied to samples of the soil at nominal test concentrations of 1.73 and 17.30 mg/kg dry soil. No adverse effects of BAS 500 06 F on nitrogen transformation in soil were observed in the test item concentration of 1.73 mg/kg dry soil after 28 days. Measured deviations of > 25% observed in the 17.30 mg/kg dry soil treatment group 28 days after application demanded extended test duration to 42 days of incubation. Only a small deviation from the control of +22.1% (at test concentration 17.30 mg/kg dry soil) was observed in the time interval of 0 – 42 days.

Based on the results of this study, BAS 500 06 F caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in a loamy sand soil tested up to a concentration of 17.30 mg/kg dry soil after 42 days of incubation.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 0003223026, content of a.s.: pyraclostrobin (BAS 500 F, Reg. no.: 304 428): 200.7 g/L (nominal: 200.0 g/L); density: 1.038 g/cm^3.

Test soil: Biologically active agricultural soil: loamy sand (DIN 4220) / sandy loam (USDA), soil pH 6.5, C_{org} 1.45%, WHC: 33.45 g/100 g dry soil.

B. STUDY DESIGN

Test design: Determination of the N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5%). Three treatment groups were set up (untreated control, two test item concentrations) with three replicates per treatment. Comparison of test item treated soil with a non-treated soil. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen formed from the nitrification process was determined by using an Autoanalyzer (Bran and Luebbe). Sampling scheme: 0, 7, 14, 28 and 42 days after treatment; sub-samples were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO_3-nitrogen production 0, 7, 14, 28 and 42 days after exposure.

Test rates: Control, 1.73 and 17.30 mg BAS 500 06 F/kg dry soil (equivalent to 0.33 and 3.33 mg a.s./kg dry soil, respectively).

Reference item: Dinoterb (purity: 98.0 ± 0.5%). The reference item was tested in a separate study at rates of 6.8, 16.0 and 27.0 mg/kg dry soil.

Test conditions: Water content: approx. 45% of maximum water holding capacity; measured water content: 14.02% – 14.67%; pH 5.8 - 6.2. Soil samples were incubated at 19.2°C – 21.0°C while stored in glass flasks in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of BAS 500 06 F on nitrogen transformation in soil was observed in the test item concentration of 1.73 mg/kg dry soil after 28 days. Only a negligible deviation from the control of +20.1% (at test concentration 1.73 mg/kg dry soil) was observed in the time interval of 0 – 28 days.
Measured deviations of > 25% observed in the 17.30 mg/kg dry soil treatment group 28 days after application demanded extended test duration to 42 days of incubation. Only a negligible deviation from the control of +22.1% (at test concentration 17.30 mg/kg dry soil) was observed in the time interval of 0 – 42 days. The results are summarized below in Table 10.5-5.

Table 10.5-5: Effects of BAS 500 06 F on soil micro-organisms (nitrogen transformation) on days 7, 14, 28 and 42 of incubation

Soil (days)	Control	1.73 mg BAS 500 06 F/kg dry soil		17.30 mg BAS 500 06 F/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
Loamy sand (0 - 7 d)	11.33	12.20	+7.6	17.43	+53.8
Loamy sand (0 - 14 d)	14.77	18.40	+24.6	23.70	+60.5
Loamy sand (0 - 28 d)	24.43	29.33	+20.1	32.87	+34.5
Loamy sand (0 - 42 d)	32.67	--[2)]	--[2)]	39.90	+22.1

1) Based on NO_3-nitrogen production; - = inhibition, + = stimulation.
2) No analysis performed.

In a separate study the reference item Dinoterb produced a stimulation of nitrogen transformation of +40.4, +68.1 and +83.5% at 6.8 mg, 16.0 mg and 27.0 mg/kg dry soil after 28 days incubation, respectively.

III. CONCLUSION

Based on the results of this study, BAS 500 06 F caused no adverse effects (< 25% deviation from control according to OECD 216) on the soil nitrogen transformation (measured as NO3-N production) in a loamy sand soil tested up to a concentration of 17.30 mg/kg dry soil after 42 days of incubation.

CP 10.6 Effects on terrestrial non-target higher plants

The new representative formulation BAS 500 06 F was not evaluated within the previous Annex I inclusion process. It is an emulsifiable concentrate (EC) containing 200g/L pyraclostrobin.

Endpoints from two new studies with the new representative formulation BAS 500 06 F are used for the risk assessment on non-target higher plants (see Table 10.6-1).

Table 10.6-1: Ecotoxicological endpoints for non-target higher plants

Test substance	Test system	Test species	EU agreed endpoints	Endpoints used in risk assessment
BAS 500 06 F	Vegetative vigor, greenhouse	Carrot, lettuce, oilseed rape, cabbage, soybean, tomato, onion, rye grass, oat, maize	--	ER_{50} > 1.25 L/ha
	Seedling emergence, greenhouse	Carrot, lettuce, oilseed rape, cabbage, soybean, tomato, onion, rye grass, oat, maize	--	ER_{50} > 1.25 L/ha

Toxicity

The potential effects of BAS 500 06 F on vegetative vigor and seedling emergence have been tested in two studies. There were no unacceptable effects on vegetative vigor and seedling emergence at rates up to and including 1.25 L BAS 500 06 F/ha for all ten species tested. Further details of the studies are given in M-CP 10.6.2.

Table 10.6-2: Summary of effects on terrestrial non-target plants following exposure to BAS 500 06 F

Test substance	Test species	Test system	ER_{50} plant dry weight, plant height [L/ha]	ER_{50} seedling emergence [L/ha]	Reference (BASF DocID)
BAS 500 06 F	Carrot, lettuce, oilseed rape, cabbage, soybean, tomato, onion, rye grass, oat and maize	Vegetative vigour	> 1.25	--	2012/1115894
		Seedling emergence	--	> 1.25	2012/1115895

Exposure

Table 10.6-3: Critical use pattern

Crop	Application time (BBCH growth stage)	Number of applications	Interval [d]	Application rate per treatment	
				Pyraclostrobin [g a.s./ha]	BAS 500 06 F [L/ha]
cereals	25 - 69	2	21	250	1.25
maize	30 - 65	1	--	200	1.0

Effects on non-target plants are of concern in the off-field environment, where they may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the BBA [*BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden.*] from the spray-drift predictions of Ganzelmeier & Rautmann (2000) [*Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application*]. Only a single application was considered, because factors like plant growth will reduce residues per unit area between multiple applications.
For a single application to field crops, 2.77% of the application rate was assumed to reach areas at 1 m from the edge of the crop (worst-case scenario). The highest single application rate of BAS 500 06 F is 1.25 L product/ha, giving a maximum off-field predicted environmental rate ($PER_{off\text{-}field}$) of 0.0346 L product/ha.

Risk assessment for terrestrial non-target higher plants

BAS 500 06 F is a fungicide and therefore this product is not expected to have any significant herbicidal activity. Profiling studies of the effects on pre- and post-emergence of non-target higher plants were conducted and showed no unacceptable effects on any of the ten species tested at rates up to and including 1.25 L BAS 500 06 F/ha.

According to the Terrestrial Guidance Document [*Anonymous (2002b). Guidance Document on terrestrial ecotoxicology under council directive 91/414/EEC. SANCO/10329/2002. 17 October 2002*], the risk to non-target plants should be considered acceptable if less than 50% effect on all ten species is seen at the maximum single application rate. Less than 50% effect on vegetative vigor and seedling emergence were observed on all tested species at the highest tested rate of 1.25 L BAS 500 06 F/ha. Therefore, it can be concluded that the proposed use of BAS 500 06 F poses no unacceptable risk to non-target plants.

The calculated maximum $PER_{off\text{-}field}$ of 0.0346 L product/ha is by far below the level found to have no effects on the non-target higher plants. Based on the drift values it can be concluded that the use of BAS 500 06 F in cereals and maize poses no unacceptable risk to non-target higher plants, too.

In conclusion, BAS 500 06 F poses no unacceptable risk to terrestrial non-target plants in off-crop areas following the intended use in cereals and maize.

CP 10.6.1 Summary of screening data

Tests on non-target plants have been conducted. The data point is covered by M-CP 10.6.2.

CP 10.6.2 Testing on non-target plants

Report:	CP 10.6.2/1 Stroemel C. et al., 2013a Effect of BAS 500 06 F on vegetative vigour of ten species of terrestial plants under greenhouse conditions 2012/1115894
Guidelines:	OECD 227 July 2006, EPA 850.4150
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

Executive Summary

In a vegetative vigor test, six species of dicotyledonous plants (carrot, lettuce, oilseed rape, cabbage, soybean and tomato) and four species of monocotyledonous plants (onion, rye grass, oat and maize) were exposed to BAS 500 06 F. BAS 500 06 F was applied post-emergence at test rates of 0.078, 0.156, 0.313, 0.625 and 1.25 L BAS 500 06 F/ha. Assessments for phytotoxicity, survival and plant length were done 7, 14 and 21 days after application (DAT). Plant biomass (shoot dry weight) was determined 21 DAT.

After treatment with BAS 500 06 F, carrot, lettuce, onion, ryegrass and oat showed no phytotoxic symptoms. Only slight damage occurred in oilseed rape, cabbage, soybean, tomato and maize. No plant mortality was observed for all tested species after application of BAS 500 06 F up to a rate of 1.25 L/ha. Plant length at 21 DAT was not significantly affected by BAS 500 06 F up to a rate of 1.25 L/ha. No influence of BAS 500 06 F on plant biomass was observed for all tested species, except for cabbage, showing a significantly reduced biomass by 8% at a rate of 1.250 L BAS 500 06 F/ha. In onion, statistically significantly reduced biomass was found at a rate of 0.313 L/ha, but not at higher test rates, indicating that this difference was not related to the test item. In summary, BAS 500 06 F did not cause adverse effects to the tested species.

The NOER based on plant survival, phytotoxicity, plant length and plant dry weight was determined to be ≥ 1.25 L BAS 500 06 F/ha for all tested species except for maize (NOER phytotoxicity: 0.625 L/ha) and cabbage (NOER plant biomass: 0.625 L/ha). Both, ER_{25} and ER_{50} were determined to be > 1.25 L BAS 500 06 F/ha, the highest tested rate in this study.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch No. 0004863761; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 201.0 g/L (nominal 200.0 g/L); density: 1.037 g/cm^3.

Test species: Carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.), oilseed rape (*Brassica napus* L.), cabbage (*Brassica oleracea* L. var. *capitata L. f. alba*), soybean (*Glycine max* L.), tomato (L*ycopersicum esculentum* Mill.), onion (*Allium cepa* L.), ryegrass (*Lolium perenne* L.), oat (*Avena sativa* L.) and maize (*Zea mays* L.).

B. STUDY DESIGN

Test design: 6 treatment groups (one untreated control and 5 treatment rates per species); 4 – 6 replicates per treatment; 5 – 10 plants per pot (depending on plant species); greenhouse cultivation over a 21 days period, dose-response design; application post emergence (BBCH 12 – 14); assessments for plant damage (phytotoxicity), survival and length was done 7, 14 and 21 days after treatment (DAT); shoot dry weight was determined at study termination 21 DAT.

Endpoints: ER_{25}, ER_{50}, NOER.

Test rates: Untreated control, 0.078, 0.156, 0.313, 0.625 and 1.250 L BAS 500 06 F/ha; applied in a water volume of 223 L/ha using a laboratory spray cabin.

Test conditions: Temperatures between 20.5°C and 26.3°C; humidity: 47.8% to 73.3%; photoperiod: ≥ 16 h light; additional light when daylight was less than 10 klux.

Statistics: Descriptive statistics; ANOVA followed by Welch-t test or Dunnett's t-test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

All control plants remained healthy throughout the trial period and no mortality was observed. Carrot, lettuce, onion, ryegrass and oat showed no phytotoxic symptoms at all. Only slight damage occurred in oilseed rape, cabbage, soybean, tomato and maize.
No plant mortality was observed for all tested species after application of BAS 500 06 F up to a rate of 1.25 L/ha. Plant length at 21 DAT was not statistically significantly affected by BAS 500 06 F up to and including a rate of 1.25 L/ha. No influence of BAS 500 06 F on plant biomass was observed for all tested species, except for cabbage, showing a statistically significantly reduced biomass of 8% at a rate of 1.25 L BAS 500 06 F/ha (Dunnett's t-test, $\alpha = 0.05$). In onion, statistically significantly reduced biomass was found at a rate of 0.313 L/ha (Dunnett's t-test, $\alpha = 0.05$) but not at higher test rates, indicating that this difference was not related to the test item. In summary, BAS 500 06 F did not cause adverse effects to the tested species. The results are summarized in Table 10.6.2-1 and Table 10.6.2-2.

Table 10.6.2-1: Effect of BAS 500 06 F on phytotoxicity, plant height and plant dry weight 21 DAT

Treatment [L/ha]	Carrot	Lettuce	Oilseed rape	Cabbage	Soy bean	Tomato	Onion	Rye grass	Oat	Maize
Mean phytotoxicity [1] [% visual damages]										
control	0	0	0	0	0	0	0	0	0	0
0.078	0	0	0	0	0	0	0	0	0	0
0.156	0	0	0	0	0	0	0	0	0	0
0.313	0	0	0	1	0	0	0	0	0	1
0.625	0	0	1	2	1	3	0	0	0	3
1.250	0	0	3	5	7	8	0	0	0	18
Mean plant length [% of control]										
control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.078	100.9	102.1	103.0	96.5	102.3	101.7	102.6	97.9	97.7	102.0
0.156	101.4	101.8	100.2	96.1	106.8	101.2	99.1	98.8	96.8	102.8
0.313	101.5	102.5	101.9	99.4	101.9	102.7	102.8	99.6	102.1	96.8
0.625	98.7	102.1	105.0	95.7	102.8	102.6	102.4	98.2	100.6	102.5
1.250	102.5	101.6	103.2	99.1	94.4	100.2	103.7	101.3	99.6	103.6
Mean plant dry weight [% of control]										
control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.078	104.1	105.0	96.0	94.5	99.9	96.4	98.1	95.5	101.4	106.8
0.156	99.4	110.7	103.4	99.1	101.9	97.7	101.9	95.7	102.7	106.8
0.313	101.0	109.6	104.2	98.7	91.3	96.8	88.5 *	101.9	100.3	95.2
0.625	100.7	106.9	104.8	95.5	96.2	98.7	91.6	96.3	100.2	105.3
1.250	93.1	105.2	104.9	91.6 *	94.6	97.5	94.4	96.0	99.0	105.4

[1] Mean effect of phytotoxic symptoms (chlorosis, necrosis, deformation and stunting) from single plants per treatment and replicates.
* Statistically significant difference compared to the control, Dunnett's t-test ($\alpha = 0.05$).

Table 10.6.2-2: NOER, ER_{25} and ER_{50} of BAS 500 06 F for non-target plants 21 DAA

Endpoint [L/ha]	Carrot	Lettuce	Oilseed rape	Cabbage	Soy bean	Tomato	Onion	Rye grass	Oat	Maize
Phytotoxicity										
NOER	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	0.625
Plant length										
NOER	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250
ER_{25}	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250
ER_{50}	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250
Plant dry weight										
NOER	≥ 1.250	≥ 1.250	≥ 1.250	0.625	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250	≥ 1.250
ER_{25}	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250
ER_{50}	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250

III. CONCLUSION

The NOER based on plant survival, phytotoxicity, plant length and plant dry weight was determined to be ≥ 1.25 L BAS 500 06 F/ha for all tested species except for maize (NOER phytotoxicity: 0.625 L/ha) and cabbage (NOER dry weight: 0.625 L/ha). Both, ER_{25} and ER_{50} were determined to be > 1.25 L BAS 500 06 F/ha, the highest tested rate in this study for all tested species.

Report:	CP 10.6.2/2 Stroemel C. et al., 2012a Effect of BAS 500 06 F on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions 2012/1115895
Guidelines:	OECD 208 (2006), EPA 850.4100
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

Executive Summary

In a seedling emergence test, six species dicotyledonous plants (carrot, lettuce, oilseed rape, cabbage, soybean, tomato) and four species of monocotyledonous plants (onion, ryegrass, oat, maize) were exposed to BAS 500 06 F to evaluate the phytotoxic potential. BAS 500 06 F was applied pre-emergence in a dose-response design at test rates of 0.078, 0.159, 0.313, 0.625 and 1.25 L/ha. Assessments for seedling emergence, phytotoxicity, plant survival and plant length were done 7, 14 and 21 days after application (DAT) (carrot and onion: 14, 21 and 28 days). Plant dry weight was determined at study termination 21 DAT (28 days for carrot and onion).

Based on the results of this study, conducted under greenhouse conditions, it can be concluded that the fungicide BAS 500 06 F did not cause adverse effects to the seedling emergence, plant survival, phytotoxicity, plant length and dry biomass production of the tested plant species.

The NOER based seedling emergence, plant survival, dry weight and height was determined to be 1.25 L BAS 500 06 F/ha for all tested plant species except for onion (0.313 L BAS 500 06 F/ha). For all tested plant species, the ER_{50} was determined to be > 1.25 L BAS 500 06 F/ha, the highest rate tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch No. 0004863761, content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 201.0 g/L (nominal 200.0 g/L); density 1.037 g/cm³.

Test species: Carrot, (*Daucus carota* L.), lettuce (*Lactuca sativa* L.), oilseed rape (*Brassica napus* L.), cabbage (*Brassica oleracea* L. var. *capitata L. f. alba*), soybean (*Glycine max* L.), tomato (L*ycopersicum esculentum* Mill.), onion (*Allium cepa* L.), ryegrass (*Lolium perenne* L.), oat (*Avena sativa* L.) and maize (*Zea mays* L.).

B. STUDY DESIGN

Test design: 6 treatment groups (5 test item rates, control); 4 – 5 replicates per treatment; 7 – 12 plants per pot (depending on plant species); dose-response design under greenhouse conditions; BAS 500 06 F was applied pre-emergence using a laboratory sprayer at a water rate of 223 L/ha for soybean and onion, and 233 L/ha for the remaining test species; assessments for seedling emergence, plant damage (phytotoxicity), survival and plant length were done 7, 14 and 21 days after treatment (DAT) (carrot and onion 14, 21 and 28 days); shoot dry weight was determined at study termination 21 DAT (carrot and onion: 28 DAT).

Endpoints: NOER, ER_{25}, ER_{50}.

Test rates: Untreated control (tap water), 0.078, 0.156, 0.313, 0.625 and 1.250 L BAS 500 06 F/ha for all tested species.

Test conditions: Temperatures between 17.4°C and 28.8°C; humidity: 42.4% to 73.3%; photoperiod: > 16 h light, additional light was automatically applied when outdoor illumination was < 10 klux.

Statistics: Descriptive statistic, ANOVA followed by Welch-t test or Dunnett's-test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

No adverse dose response effects of BAS 500 06 F on seedling emergence, plant survival, phytotoxicity, plant length and biomass (dry weight) were observed on any of the ten species tested.
None of the tested species was adversely affected concerning seedling emergence and plant survival after pre-emergence application of BAS 500 06 F. All species reached a required emergence rate of 50% at 7 DAT except carrot and onion at 14 DAT. The NOER, ER_{25} and ER_{50} for seedling emergence and plant survival were 1.25, > 1.25 and > 1.25 L BAS 500 06 F/ha for all tested species, respectively.
For none of the tested species, reduction in plant length was observed except for onion. Plant length of onions was statistically significantly reduced by 8 and 9% at 0.625 and 1.25 L BAS 500 06 F/ha (Dunnett's t-test, $\alpha = 0.05$). The NOER, ER_{25} and ER_{50} for plant length were 1.25, > 1.25 and > 1.25 L BAS 500 06 F/ha for all tested species except for onion with a NOER of 0.313 L BAS 500 06 F/ha, respectively.
BAS 500 06 F did not adversely affect plant biomass development in all tested species. Statistically significant differences found in onion only occurred at 0.156 and 0.625 L BAS 500 06 F/ha (Dunnett's t-test, $\alpha = 0.05$), but not at the highest test rate and can thus be assumed not to be treatment-related. The NOER, ER_{25} and ER_{50} for plant biomass were 1.25, > 1.25 and > 1.25 L BAS 500 06 F/ha for all tested species, respectively. In summary, BAS 500 06 F did not cause adverse effects to the tested species. The results are summarized in Table 10.6.2-3 and Table 10.6.2-4.

Table 10.6.2-3: Effects of BAS 500 06 F on seedling emergence, plant survival, dry weight and plant length 21 DAT

Treatment [L/ha]	Carrot [1)]	Lettuce	Oilseed rape	Cabbage	Soy bean	Tomato	Onion [1)]	Rye grass	Oat	Maize
Seedling emergence rate [%]										
control	92	91	95	73	94	89	94	88	95	97
0.078	90	89	95	73	91	86	96	90	98	86
0.156	92	89	85	93	86	86	92	92	100	89
0.313	94	86	98	83	80	86	98	90	98	97
0.625	92	86	100	85	94	91	94	85	98	97
1.250	100	94	93	83	83	80	98	100	100	94
Mean plant survival [% of control]										
control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.078	97.7	96.9	100.0	100	97.0	96.8	102.2	102.4	102.6	88.2
0.156	100.0	96.9	89.5	127.6	90.9	96.8	97.8	104.8	105.3	91.2
0.313	102.3	93.8	102.6	113.8	84.8	96.8	102.2	102.4	102.6	100.0
0.625	100.0	93.8	105.3	117.2	100.0	103.2	100.0	97.6	102.6	100.0
1.250	109.1	103.1	97.4	113.8	87.9	90.3	102.2	114.3	100.0	97.1
Mean biomass [% of control]										
control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.078	110.4	89.8	95.4	94.7	97.8	100.9	92.8	116.2	108.9	101.9
0.156	102.6	99.1	95.2	109.7	90.2	99.1	82.0 *	134.4	106.9	101.7
0.313	100.8	97.8	98.2	103.5	93.8	100.7	102.9	108.1	98.9	105.3
0.625	101.4	96.2	98.6	99.7	93.3	96.5	80.5 *	112.9	101.8	101.7
1.250	105.2	92.1	98.2	102.3	92.7	103.2	85.0	132.5	102.4	102.9
Mean plant length [% of control]										
control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
0.078	100.6	93.9	108.6	106.6	104.5	98.5	93.8	103.5	99.0	102.5
0.156	93.8	95.0	106.7	101.0	101.6	101.8	94.2	102.4	100.2	99.7
0.313	96.5	98.2	102.7	101.4	102.9	98.2	101.4	96.9	98.9	99.5
0.625	97.1	97.8	99.7	97.8	96.9	97.0	92.0 *	102.4	100.6	98.4
1.250	96.4	95.6	102.6	96.3	104.4	99.7	91.1 *	98.8	100.0	100.5

1) Carrot and onion were cultivated over 28 days.
* Statistically significantly different compared to the control (Dunnett's-test, $\alpha = 0.05$).

Table 10.6.2-4: NOER, ER_{25} and ER_{50} of BAS 500 06 F for non-target plants 21 DAA

Endpoint [L/ha]	Carrot [1)]	Lettuce	Oilseed rape	Cabbage	Soy bean	Tomato	Onion [1)]	Rye grass	Oat	Maize
Phytotoxicity										
NOER	1.250	1.250	1.250	1.250	1.250	1.250	1.250	1.250	1.250	1.250
Plant length										
NOER	1.250	1.250	1.250	1.250	1.250	1.250	0.313	1.250	1.250	1.250
ER_{25}	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250
ER_{50}	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250
Plant dry weight										
NOER	1.250	1.250	1.250	1.250	1.250	1.250	1.250	1.250	1.250	1.250
ER_{25}	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250
ER_{50}	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250	> 1.250

[1)] Carrot and onion were cultivated over 28 days.

III. CONCLUSION

The NOER based on seedling emergence, plant survival, dry weight and height was determined to be 1.25 L BAS 500 06 F/ha for all tested plant species except for onion (0.313 L BAS 500 06 F/ha). For all tested plant species, the ER_{50} was determined to be > 1.25 L BAS 500 06 F/ha, the highest rate tested.

CP 10.6.3 Extended laboratory studies on non-target plants

Further tests on non-target plants are not triggered.

CP 10.6.4 Semi-field and field tests on non-target plants

Further tests on non-target plants are not triggered.

CP 10.7 Effects on other terrestrial organisms (flora and fauna)

Further studies are not triggered.

CP 10.8 Monitoring data

According to the knowledge of the applicant, there are currently no monitoring studies available, which are assessing ecotoxicological effects of BAS 500 06 F.

BASF
The Chemical Company

BAS 500 06 F

DOCUMENT M-CP, Section 11

LITERATURE DATA

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[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 11 LITERATURE DATA

Based on their trade names, a number of end-use products containing pyraclostrobin were included in the literature search for the active substance. For further information, please refer to M-CA 9.

BAS 500 06 F

DOCUMENT M-CP, Section 12

CLASSIFICATION AND LABELLING OF THE PLANT PROTECTION PRODUCT

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[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 12 CLASSIFICATION AND LABELLING OF THE PLANT PROTECTION PRODUCT

Physico-chemical properties

Table 12-1: Physico-chemical data relevant for classification of BAS 500 06 F

Parameter	Findings (triggered risk phrase)	Reference (BASF DocID)
Explosive properties	The product has no explosive properties.	2004/1004104
Oxidizing properties	The product has no oxidizing properties.	2004/1004104
Flammability	Flash point: 104°C Auto-ignition temperature: 450°C	2004/1010381 2004/1004104
Content of hydrocarbons	> 10 % **(H304 required, R65 not required, see remark below)**	Doc JCP 1.4.1
Viscosity	The kinematic viscosity (at 40°C) was determined to be 12.9 x 10^{-6} m^2/s. Viscosity (at D=100s^{-1}): 31.4 mPa s at 20°C (Flow behaviour: Newtonian) **(see remark below)**	2004/1010381
Surface tension	36.6 mN/m at 0.06%, 33.4 mN/m at 0.8% and 36.3 mN/m undiluted (all at 20°C) **(see remark below)**	2004/1010381

Remarks:

H 304:
A classification with H304 is triggered for BAS 500 06 F by its physical/chemical properties according to Regulation (EC) No. 1272/2008 (CLP):

- hydrocarbon content: > 10 % (w/w)
- kinematic viscosity at 40°C: < 20.5 x 10^{-6} m^2/s

R 65:
Due to the different trigger values a classification with R65 is not required for BAS 500 06 F by its physical/chemical properties (EU Directives 67/548/EEC and 2001/59/EC):

- hydrocarbon content: > 10 % (w/w)
- kinematic viscosity at 40°C: > 7 x 10^{-6} m^2/s
- surface tension: ≥ 33 mN/m

Toxicology

Table 12-2: Toxicological data relevant for classification of BAS 500 06 F

Study type/species	Results	Classification		Reference
		EU Dir. 67/548/EEC 2001/59/EC	Reg. EC 1272/2008 (CLP)	(BASF DocID)
Acute oral toxicity, rat	LD_{50} ~ 500 mg/kg bw	R22	H302	2007/1053390
Acute dermal toxicity, rat	LD_{50} > 5000 mg/kg bw	-	-	2009/1084157
Acute inhalation toxicity, rat	LC_{50} = 4.48 mg/L (both sexes) LC_{50} = 4.78 mg/L (males) LC_{50} = 4.55 mg/L (females)	R20	H332	2009/1122167
Skin irritation, rabbit	Irritating to rabbit skin	R38	H315	2009/1100358
Eye irritation, rabbit	Irritating to rabbit eye	-	H319	2009/1100359
Skin sensitization LLNA assay, mouse Maximization test, guinea pig	Sensitizing	R43	H317	2007/1053391 2009/1018498

Remarks:
A full acute toxicity data package for BAS 500 06 F is available. BAS 500 06 F is moderately toxic by the oral and inhalation route of exposure and non-toxic by the dermal route. It is irritant to the skin and the eye and may cause skin sensitization.
Neither the classification of the active substance nor the classification of individual co-formulants has any further influence on the classification of the product BAS 500 06 F. Detailed information about the co-formulants is given in Documents JCP and JGH.

Ecotoxicology/Environment

Table 12-3: Ecotoxicological data relevant for classification of BAS 500 06 F

Study type/species	Results	Classification		Reference
		EU Dir. 67/548/EEC 2001/59/EC	Reg. EC 1272/2008 (CLP)	(BASF DocID)
Rainbow trout (96 h)	LC_{50} = 0.036 mg/L	R50	H400	2008/1018046
D. magna (48 h)	EC_{50} = 0.065 mg/L	R50	H400	2004/1004393
Green alga (72 h)	E_RC_{50} = 14.2 mg/L	R52	-	2008/1009325
Biodegradation	Not readily biodegradable	R53	H410	1999/10655

Remarks:
Ecotoxicological studies were conducted with BAS 500 06 F as shown in the table above. The statement on biodegradation has been derived from the properties of the active substance (pyraclostrobin is not readily biodegradable). Neither the classification of the active substance nor the classification of individual co-formulants has any further influence on the classification of the product BAS 500 06 F. Detailed information about the co-formulants is given in Documents JCP and JGH.

Label Elements

Table 12-4: Proposed Risk and Safety Phrases according to Directive 67/548/EEC or 1999/45/EC

Pictogram(s)		
Hazard symbol(s):	Xn, N	
Indication(s) of danger:	Harmful, Dangerous for the environment	
Risk phrases:	R20/22	Harmful by inhalation and if swallowed.
	R38	Irritating to skin.
	R43	May cause sensitization by skin contact.
	R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
Safety phrases	S2	Keep out of the reach of children.
	S13	Keep away from food, drink and animal feeding stuffs.
	S20/21	When using do not eat, drink or smoke.
	S24	Avoid contact with skin.
	S29	Do not empty into drains.
	S35	This material and its container must be disposed of in a safe way.
	S37	Wear suitable gloves.
	S46	If swallowed, seek medical advice immediately and show this container or label.
	S57	Use appropriate container to avoid environmental contamination.

Table 12-5: Proposed Hazard and Precautionary Statements according to Globally Harmonized System/Regulation (EC) No 1272/2008 [CLP]

Pictogram(s)		
Signal word	Danger	
Hazard statements	H319	Causes serious eye irritation.
	H315	Causes skin irritation.
	H332	Harmful if inhaled.
	H302	Harmful if swallowed.
	H317	May cause an allergic skin reaction.
	H304	May be fatal if swallowed and enters airways.
	H400	Very toxic to aquatic life.
	H410	Very toxic to aquatic life with long lasting effects.
	EUH401	To avoid risks to human health and the environment, comply with the instructions for use.
Precautionary Statements*		
Prevention	**P280**	Wear protective gloves/clothing.
	P261	Avoid breathing vapours.
	P264	Wash contaminated body parts thoroughly after handling.
	P270	Do not eat, drink or smoke when using this product.
	P271	Use only outdoors or in a well-ventilated area.
	P272	Contaminated work clothing should not be allowed out of the workplace.
Response	**P301 + P310**	IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
	P331	Do NOT induce vomiting.
	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
	P330	Rinse mouth.
	P304 + P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
	P361	Remove/Take off immediately all contaminated clothing.
	P391	Collect spillage.
Storage	-	-
Disposal	**P501**	Dispose of contents/container to hazardous or special waste collection point.

***** P-statements in **bold** are recommended for product label

Table 12-6: Labelling proposed according to Directive 67/548/EEC or 1999/45/EC

Hazard symbol(s):	Xn, N
Indications of danger:	Harmful, Dangerous for the environment
Risk phrases:	R20/22, 38, 43, 50/53
Safety phrases:	S2, 13, 20/21, 24, 29, 35, 37, 46, 57

Table 12-7: Labelling proposed according to GHS (Globally Harmonized System) Regulation (EC) No 1272/2008

Signal Word	Danger
Hazard Statement	H319, H315, H332, H302, H317, H304, H400, H410 EUH401
Precautionary Statements (Prevention):	P280, P261, P264, P270, P271, P272
Precautionary Statements (Response):	P301+P310, P331, P305+P351+P338, P302 + P352, P333+P313, P330, P304+P340, P361, P391
Precautionary Statements (Storage):	-
Precautionary Statements (Disposal):	P501

BAS 500 06 F

DOCUMENT OCP

COMPLETENESS CHECK FORM

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OCP Evaluation Form Plant Protection Product
for use in checking that all test and study reports required in accordance with SANCO/11803 have been provided

Active Substance: Pyraclostrobin Applicant: BASF SE Date: 18/Jul/2014

Preparation: BAS 500 06 F

As the representative formulation has changed from the original dossier to the supplementary dossier, all studies and references for BAS 500 06 F submitted within the supplementary dossier are new and are therefore not individually identified as new.

SANCO/ 11803 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
1	Identity of the plant protection product				
1.1	Applicant	yes			☐
1.2	Producer of the plant protection product and the active substances	yes			☐
1.3	Trade name or proposed trade name and producer's development code number of the plant protection product if appropriate	yes			☐
1.4	Detailed quantitative and qualitative information on the composition of the plant protection product				
1.4.1	Composition of the plant protection product	yes			☐
1.4.2	Information on the active substances	yes			☐
1.4.3	Information on safeners, synergists and co-formulants	yes	Doc J		☐
1.5	Type and code of the plant protection product	yes			☐
1.6	Function	yes			☐
2	Physical and chemical properties of the plant protection product				
2.1	Appearance	yes			☐
2.2	Explosive and oxidising properties	yes			☐
2.3	Flammability and self-heating	yes			☐
2.4	Acidity/alkalinity and pH value	yes			☐
2.5	Viscosity and surface tension	yes			☐
2.6	Relative density and bulk density	yes			☐
2.7	Storage stability and shelf-life: effects of temperature on technical characteristics of the plant protection product	yes			☐
2.8	Technical characteristics of the plant protection product				
2.8.1	Wettability	not relevant	M-CP 2.8.1		☐
2.8.2	Persistent foaming	yes			☐
2.8.3	Suspensibility, spontaneity and dispersion stability	not relevant	M-CP 2.8.3		☐
2.8.4	Degree of dissolution and dilution stability	not relevant	M-CP 2.8.4		☐
2.8.5	Particle size distribution, dust content, attrition and mechanical stability				
2.8.5.1	Particle size distribution	not relevant	M-CP 2.8.5.1		☐
2.8.5.2	Dust content	not relevant	M-CP 2.8.5.2		☐
2.8.5.3	Attrition	not relevant	M-CP 2.8.5.3		☐
2.8.5.4	Hardness and integrity	not relevant	M-CP 2.8.5.4		☐

SANCO/ 11803 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
2.8.6	Emulsifiability, re-emulsifiability, emulsion stability	yes			☐
2.8.7	Flowability, pourability and dustability	not relevant	M-CP 2.8.7		☐
2.9	Physical and chemical compatibility with other products including plant protection products with which its use is to be authorised	yes			☐
2.10	Adherence and distribution to seeds	not relevant	M-CP 2.10		☐
2.11	Other studies	not relevant	M-CP 2.11		☐
3	Data on application				
3.1	Fields of use envisaged	yes			☐
3.2	Effects on harmful organisms	yes			☐
3.3	Details of intended use	yes			☐
3.4	Application rate and concentration of the active substance	yes			☐
3.5	Method of application	yes			☐
3.6	Number and timing of applications and duration of protection	yes			☐
3.7	Necessary waiting periods and other precautions to avoid phytotoxic effects on succeeding crops	yes			☐
3.8	Proposed instructions for use	yes			☐
4	Further information on the plant protection product				
4.1	Safety intervals and other precautions to protect humans, animals and the environment	yes			☐
4.2	Recommended methods and precautions	yes			☐
4.3	Emergency measures in the case of an accident	yes			☐
4.4	Packaging, compatibility of the plant protection product with proposed packaging materials	yes			☐
4.5	Procedures for destruction or decontamination of the plant protection product and its packaging				
4.5.1	Neutralisation procedure	yes			☐
4.5.2	Controlled incineration	yes			☐
5	Analytical methods				
5.1	Methods used for the generation of pre-authorisation data				
5.1.1	Analysis of the plant protection product	yes			☐
5.1.2	Methods for the determination of residues	yes	M-CA 4.1.2		☐
5.2	Methods for post-authorisation control and monitoring purposes	yes	M-CA 4.2		☐
6	Efficacy data				
6.1	Preliminary test	not relevant	not required		☐
6.2	Testing effectiveness	not relevant	not required		☐
6.3	Information on the occurrence or possible occurrence of the development of resistance	not relevant	not requiredl		☐
6.4	Adverse effects on treated crops				
6.4.1	Phytotoxicity to target plants (including different cultivars), or to target plant products	not relevant	not required		☐
6.4.2	Effects on the yield of treated plants or plant products	not relevant	not required		☐
6.4.3	Effects on the quality of plants or plant product	not relevant	not required		☐
6.4.4	Effects on transformation processes	not relevant	not required		☐
6.4.5	Impact on treated plants or plant products to be used for propagation	not relevant	not required		☐

SANCO/ 11803 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
6.5	Observations on other undesirable or unintended side-effects				
6.5.1	Impact on succeeding crops	not relevant	not required		☐
6.5.2	Impact on other plants, including adjacent crops	not relevant	not required		☐
6.5.3	Effects on beneficial and other non-target organisms	not relevant	not required		☐
7	Toxicological studies				
7.1	Acute toxicity				
7.1.1	Oral toxicity	yes			☐
7.1.2	Dermal toxicity	yes			☐
7.1.3	Inhalation toxicity	yes			☐
7.1.4	Skin irritation	yes			☐
7.1.5	Eye irritation	yes			☐
7.1.6	Skin sensitisation	yes			☐
7.1.7	Supplementary studies on the plant protection product	not relevant	M-CP 7.1.7		☐
7.1.8	Supplementary studies for combinations of plant protection products	not relevant	M-CP 7.1.8		☐
7.2	Data on exposure				
7.2.1	Operator exposure				
7.2.1.1	Estimation of operator exposure	yes			☐
7.2.1.2	Measurement of operator exposure	yes			☐
7.2.2	Bystander and resident exposure				
7.2.2.1	Estimation of bystander and resident exposure	yes			☐
7.2.2.2	Measurement of bystander and resident exposure	not relevant	M-CP 7.2.2.2		☐
7.2.3	Worker exposure				
7.2.3.1	Estimation of worker exposure	yes			☐
7.2.3.2	Measurement of worker exposure	not relevant	M-CP 7.2.3.2		☐
7.3	Dermal absorption	yes			☐
7.4	Available toxicological data relating to co-formulant	yes	Doc J		☐
8	Metabolism and residues data	yes	M-CA 6		
9	Fate and behaviour in the environment				
9.1	Fate and behaviour in soil				
9.1.1	Rate of degradation in soil	yes	M-CA 7.1.2		☐
9.1.1.1	Laboratory studies	yes	M-CA 7.1.2.1		☐
9.1.1.2	Field studies	yes	M-CA 7.1.2.2		☐
9.1.2	Mobility in the soil				
9.1.2.1	Laboratory studies	yes	M-CA 7.1.4.1		☐
9.1.2.2	Lysimeter studies	not relevant	M-CP 9.1.2.2		☐
9.1.2.3	Field leaching studies	not relevant	M-CP 9.1.2.3		☐
9.1.3	Estimations of concentrations in soil	yes			☐
9.2	Fate and behaviour in water and sediment				
9.2.1	Aerobic mineralisation in surface water	yes	M-CA 7.2.2.2		☐
9.2.2	Water/sediment study	yes	M-CA 7.2.2.3		☐
9.2.3	Irradiated water/sediment study	yes	M-CA 7.2.2.4		☐
9.2.4	Estimation of concentrations in groundwater				
9.2.4.1	Calculation of concentrations in groundwater	yes			☐
9.2.4.2	Additional field tests	not relevant	M-CP 9.2.4.2		☐

SANCO/ 11803 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
9.2.5	Estimation of concentrations in surface water and sediment	yes			☐
9.3	Fate and behaviour in air				
9.3.1	Route and rate of degradation in air and transport via air	yes	M-CA 7.3		☐
9.4	Estimation of concentrations for other routes of exposure	not relevant	M-CP 9.4		☐
10	Ecotoxicological studies				
10.1	Effects on birds and other terrestrial vertebrates				
10.1.1	Effects on birds				
10.1.1.1	Acute oral toxicity	yes			☐
10.1.1.2	Higher tier data for birds	yes			☐
10.1.2	Effects on terrestrial vertebrates other than birds				
10.1.2.1	Acute oral toxicity to mammals	yes			☐
10.1.2.2	Higher tier data on mammals	yes			☐
10.1.3	Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)	yes			
10.2	Effects on aquatic organisms				
10.2.1	Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes	yes			☐
10.2.2	Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms	not relevant	M-CP 10.2.2		☐
10.2.3	Further testing on aquatic organisms	not relevant	M-CP 10.2.3		☐
10.3	Effects on arthropods				
10.3.1	Effects on bees				
10.3.1.1	Acute toxicity to bees				
10.3.1.1.1	Acute oral toxicity to bees	yes			☐
10.3.1.1.2	Acute contact toxicity to bees	yes			☐
10.3.1.2	Chronic toxicity to bees	not relevant	M-CP 10.3.1.2		☐
10.3.1.3	Effects on honey bee development and other honey bee life stages	not relevant	M-CP 10.3.1.3		☐
10.3.1.4	Sublethal effects	not relevant	M-CP 10.3.1.4		☐
10.3.1.5	Cage and tunnel tests	yes			☐
10.3.1.6	Field tests with honeybees	not relevant	M-CP 10.3.1.6		☐
10.3.2	Effects on non-target arthropods other than bees				
10.3.2.1	Standard laboratory testing for non-target arthropods	yes			☐
10.3.2.2	Extended laboratory testing, aged residue studies with non-target arthropods	yes			☐
10.3.2.3	Semi-field studies with non-target arthropods	not relevant	M-CP 10.3.2.3		☐
10.3.2.4	Field studies with non-target arthropods	not relevant	M-CP 10.3.2.4		☐
10.3.2.5	Other routes of exposure for non-target arthropods	not relevant	M-CP 10.3.2.5		☐
10.4	Effects on non-target soil meso- and macrofauna				
10.4.1	Earthworms				
10.4.1.1	Earthworms – sub-lethal effects	yes			☐

SANCO/ 11803 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
10.4.1.2	Earthworms – field studies	yes			☐
10.4.2	Effects on non-target soil meso- and macrofauna (other than earthworms)				
10.4.2.1	Species level testing	yes			☐
10.4.2.2	Higher tier testing	not relevant	M-CP 10.4.2.2		☐
10.5	Effects on soil nitrogen transformation	yes			☐
10.6	Effects on terrestrial non-target higher plants				
10.6.1	Summary of screening data	yes	M-CP 10.6.2		☐
10.6.2	Testing on non-target plants	yes			☐
10.6.3	Extended laboratory studies on non-target plants	not relevant	M-CP 10.6.3		☐
10.6.4	Semi-field and field tests on non-target plants	not relevant	M-CP 10.6.4		☐
10.7	Effects on other terrestrial organisms (flora and fauna)	yes			☐
10.8	Monitoring data	not relevant	M-CP 10.8		☐
11	Literature data	yes	M-CA 9		☐
12	Classification and labelling	yes			☐

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DOCUMENT I

Other data on the formulants

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All data available for the formulants contained in the product

BAS 516 07 F

are listed in the safety data sheets which are included
in Document H.

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BAS 516 07 F

DOCUMENT L-CP, Section 1

IDENTITY OF THE PLANT PROTECTION PRODUCT

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DOCUMENT L-CP, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE PLANT PROTECTION PRODUCT

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Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 2.1/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.2/1	Bitterlich S.	2005 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Directive 92/69/EC: Annex A.9-A.17) 2005/1011601 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.2/2	Loehr S.	2011 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) 2011/1065573 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.3/1	Bitterlich S.	2005 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Directive 92/69/EC: Annex A.9-A.17) 2005/1011601 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 2.3/2	Loehr S.	2011 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) 2011/1065573 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.4/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.5/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.6/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 2.7/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.7/2	Keller M.	2011 a	Boscalid/Pyraclostrobin 26.7/6.7 % WG - chemical and physical stability of formula BAS 516 07 F when stored at 23°C up to 3 years in commercial packs (HDPE-bottle) 2011/1102466 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.8.1/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.8.2/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 2.8.3/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.8.5.1/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.8.5.2/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.8.5.3/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 2.8.7/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 2.9/1	Schneider K.-H.	2001 a	Physical and chemical compatibility in aqueous tank mixtures of BAS 516 00 F with other products 2001/1001884 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

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DOCUMENT L-CP, Section 3

DATA ON APPLICATION

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DOCUMENT L-CP, Section 4

FURTHER INFORMATION ON THE PLANT PROTECTION PRODUCT

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18-Jul-2014		BASF DocID 2014/1162352 (version 1)
27-Feb-2017	Addition of a reference in 4.2 and 4.3	BASF DocID 2017/1032485 (version 2)

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Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 4.2/1	Anonymous	2015 a	Safety data sheet - Signum 2015/1260817 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 4.2/2	Ohnsorge U.	2001 a	BAS 516 00 F (preliminary designator): Effectiveness of procedures for cleaning application equipment and protective clothing 2001/1001819 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 4.3/1	Anonymous	2015 a	Safety data sheet - Signum 2015/1260817 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 4.4/1	Schreiner B.	2009 a	EU-Performance-Test of BAS 516 07 F, Bottle, rectangular, 1 Kg (PE-HD) 2009/1052799 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 4.4/2	Keller M.	2011 a	Boscalid/Pyraclostrobin 26.7/6.7 % WG - chemical and physical stability of formula BAS 516 07 F when stored at 23°C up to 3 years in commercial packs (HDPE-bottle) 2011/1102466 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

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DOCUMENT L-CP, Section 5

ANALYTICAL METHODS

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Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 5.1.1/1	Ziegler H.	2000 a	The determination of the amounts of active ingredients Reg.No. 300355 and Pyraclostrobin in BAS 516 00 F by HPLC 2000/1014140 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 5.1.1/2	Ziegler H.	2000 b	Development and validation of the analytical method CF-A 598. Determination of Reg.No. 300355 and Pyraclostrobin in water dispersible granules (WG) (BAS 516 00 F) 2000/1012385 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 5.1.1/3	Euler K.	2009 a	Supplement to the development and validation of the analytical method CF-A 598 2009/1050295 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 5.1.1/4	Stegmaier W.	2009 a	Analytical method AM/01120/01e, gas chromatographic determination of dimethyl sulfate in BAS 516 07 F 2009/1105184 BASF SE - GKA Competence Center Analytics, Ludwigshafen, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 5.1.1/5	Stegmaier W.	2012 a	Validation of an analytical method for the determination of dimethyl sulfate in BAS 516 07 F 2012/1213598 BASF SE - GMC Competence Center Analytics, Ludwigshafen, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

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DOCUMENT L-CP, Section 7

TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 7.1.1/1	[redacted]	2008 a	BAS 516 07 F - Acute oral toxicity in rats - Acute toxic class method 2008/1004838 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 7.1.2/1	[redacted]	2001 a	BAS 516 00 F - Acute dermal toxicity study in rats 2001/1003723 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 7.1.3/1	[redacted]	2001 b	BAS 516 00 F - Acute inhalation toxicity study in Wistar rats 4-hour dust exposure 2001/1001824 [redacted]. yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 7.1.4/1	[redacted]	2007 a	BAS 516 07 F: Acute dermal irritation / corrosion in rabbits 2007/1056989 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 7.1.5/1	[redacted]	2007 b	BAS 516 07 F: Acute eye irritation in rabbits 2007/1056988 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 7.1.6/1	[redacted]	2014 a	BAS 516 07 F - Skin sensitisation: Local lymph node assay 2014/1001403 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 7.3/1	Hassler S.	2014 a	Radiolabeled BAS 510 F in BAS 516 07 F - In vitro study to investigate the dermal penetration through human skin 2014/1001401 Harlan Laboratories Ltd., Itingen, Switzerland yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 7.3/2	Hassler S.	2014 b	Radiolabeled BAS 500 F in BAS 516 07 F - In vitro study to investigate the dermal penetration through human skin 2014/1001402 Harlan Laboratories Ltd., Itingen, Switzerland yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

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DOCUMENT L-CP, Section 9

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 9.1.3/1	Kallweit W.	2014 a	Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes 2014/1000685 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 9.1.3/2	Kallweit W.	2014 b	Predicted environmental concentrations of BAS 510 F - Boscalid in soil, groundwater, surface water and sediment following application to potatoes in Europe 2014/1001461 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 9.2.4/1	Kallweit W.	2014 a	Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes 2014/1000685 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 9.2.4/2	Kallweit W.	2014 b	Predicted environmental concentrations of BAS 510 F - Boscalid in soil, groundwater, surface water and sediment following application to potatoes in Europe 2014/1001461 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 9.2.5/1	Kallweit W.	2014 a	Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes 2014/1000685 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCP 9.2.5/2	Kallweit W.	2014 b	Predicted environmental concentrations of BAS 510 F - Boscalid in soil, groundwater, surface water and sediment following application to potatoes in Europe 2014/1001461 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

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DOCUMENT L-CP, Section 10

ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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18-July-2014		BASF DocID 2014/1162358 (version 1)
09-June-2015	The document was amended in order to reflect that 2 studies in M-CP 10 had been moved to other sub-chapters (M-CP 10.4 and M-CP 10.5). New or changed text is marked in yellow.	BASF DocID 2015/1117545 (version 2)

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Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.1.1.2/1	[redacted]	2006 b	BAS 516 07 F - Acute toxicity in the bobwhite quail (Colinus virginianus) after single oral administration (LD50) 2006/1030309 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 10.2.1/1	[redacted]	2000 a	BAS 516 00 F - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a static system (96 hours) 2000/1018726 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCP 10.2.1/2	Janson G.-M.	2007 a	Acute toxicity of BAS 516 07 F to Daphnia magna STRAUS in a 48 hour static test 2007/1008605 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.2.1/3	Janson G.-M.	2009 a	Acute toxicity of BAS 516 07 F to Daphnia magna STRAUS in a 48 hour static test 2009/1117877 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.2.1/4	Hoffmann F.	2007 a	Effect of BAS 516 07 F on the growth of the green alga Pseudokirchneriella subcapitata 2007/1005074 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.1.1.1/1	Kling A.	2001 a	Assessment of side effects of BAS 516 00 F to the honey bee, Apis mellifera L. in the laboratory 2001/1000868 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.3.1.1.2/1	Kling A.	2001 a	Assessment of side effects of BAS 516 00 F to the honey bee, Apis mellifera L. in the laboratory 2001/1000868 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.1.3/1	Johnson R.M. Percel E.G.	2013 a	Effect of a fungicide and spray adjuvant on queen-rearing success in honey bees (Hymenoptera: Apidae) 2013/1416303 <none> no Published	No	No	Not applicable	public
KCP 10.3.2.1/1	Buehler A.	2000 a	Effect of BAS 516 00 F on the ground dwelling predator Poecilus cupreus (Coleoptera, Carabidae) in a laboratory trial 2000/1012478 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.3.2.1/2	Adelberger I.	2001 a	BAS 516 00 F: Toxicity to the predatory mite, Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) in the laboratory 2001/1000880 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.2.1/3	Ufer A.	2001 a	Effect of BAS 516 00 F on the green lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) in a laboratory trial 2001/1001860 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.2.1/4	Schuld M.	2001 a	BAS 516 00 F: Toxicity to the aphid parasitoid, Aphidius rhopalosiphi (Hymenoptera, Braconidae) DESTEFANI-PEREZ in the laboratory 2001/1001864 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.3.2.1/5	Hermann P.	2001 a	BAS 516 00 F: Toxicity to the staphylinid beetle, Aleochara bilineata GYLL. (Coleoptera, Staphylinidae) in the laboratory 2001/1001863 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.2.1/6	Schmitzer S.	2001 a	Effects of BAS 516 00 F on the wolf spider Pardosa spec. in the laboratory 2001/1005964 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.2.2/1	Ufer A.	2001 b	Effect of BAS 516 00 F on the green lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) in an extended laboratory trial 2001/1001874 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.3.2.4/1	Muether J.	2001 a	A field study to evaluate the effects of BAS 516 00 F against predatory mites in damson plum 2001/1000905 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.2.4/2	Gossmann A.	2001 a	Effects of BAS 516 00 F on predatory mites Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) in apple orchards (field experiments) 2001/1001866 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.3.2.4/3	Nienstedt K.M.	2001 a	A field test to determine the effect of BAS 516 00 F on predatory mite populations (Acari: Phytoseiidae) on stone fruit trees (cherry, Prunus avium) 2001/1001870 Springborn Laboratories (Europe) AG, Horn, Switzerland yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.4.1/1	Wachter S.	2001 b	Acute toxicity of BAS 516 00 F on earthworms, Eisenia foetida using an artificial soil test 2001/1001837 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.4.1.1/1	Friedrich S.	2006 a	Sublethal toxicity of BAS 516 07 F to the earthworm Eisenia fetida in artificial soil with 5% peat 2006/1015860 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.4.1.2/1	Hamberger A.	2011 a	Field study to evaluate the effects of BAS 516 07 F on earthworms in Southern Germany 2011/1043488 Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.4.2.1/1	Friedrich S.	2006 c	Effects of BAS 516 07 F on the reproduction of the collembolans Folsomia candida in artificial soil with 5% peat 2006/1015861 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.4.2.1/2	Schulz L.	2014 b	Effects of BAS 516 07 F on the reproduction of the predatory mite Hypoaspis aculeifer 2014/1010834 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.5/1	Wachter S.	2001 a	Assessment of the side effects of BAS 516 00 F on the activity of the soil microflora; nitrogen turnover 2001/1005959 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.5/2	Wachter S.	2001 c	Assessment of the side effects of BAS 516 00 F on the activity of the soil microflora; short-term respiration 2001/1005960 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.6.2/1	Oberwalder C. Schmidt O.	2001 a	BAS 516 00 F: Effects on non-target plants in the greenhouse - A limit test 2001/1005953 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCP 10.6.2/2	Stroemel C. Brockmann A.	2014 a	Effect of BAS 516 07 F on vegetative vigour of ten species of terrestrial plants under greenhouse conditions 2014/1010836 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCP 10.6.2/3	Stroemel C. Brockmann A.	2014 b	Effect of BAS 516 07 F on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions 2014/1010835 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

BASF
The Chemical Company

BAS 516 07 F

DOCUMENT L-CP, Section 11

LITERATURE DATA

Reference List

Compiled by:

[redacted]

BASF SE
Agricultural Center
P.O. Box 120
67114 Limburgerhof
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Telephone [redacted]
Telefax
E-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

There are no references submitted with this section.

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The Chemical Company

BAS 516 07 F

DOCUMENT M-CP, Section 1

IDENTITY OF THE PLANT PROTECTION PRODUCT

Compiled by:

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E-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 1 IDENTITY OF THE PLANT PROTECTION PRODUCT

BAS 516 07 F is a new representative formulation, which has not been evaluated during a previous Annex I inclusion process.

CP 1.1 Applicant

BASF SE
Crop Protection Division
P.O. Box 120
67114 Limburgerhof
Germany

Contact person:

(a) Contact: [redacted]
BASF SE
Agricultural Center
P.O. Box 120
67114 Limburgerhof
Germany
Telephone: [redacted]
Telefax:
E-mail:

(b) Alternative: [redacted]
BASF SE
Agricultural Center
P.O. Box 120
67114 Limburgerhof
Germany
Telephone: [redacted]
Telefax:
E-mail:

CP 1.2 Producer of the plant protection product and the active substances

Manufacturer of BAS 516 07 F (legal entity):

BASF SE
67056 Ludwigshafen
Germany
Crop Protection Division:
P.O. Box 120
67114 Limburgerhof
Germany

Contact person: Please refer to CP 1.1 Applicant.

Location of the manufacturing site of BAS 516 07 F:

Confidential information - data provided in Document J.

Manufacturer of pyraclostrobin and boscalid (legal entity):

BASF SE
67056 Ludwigshafen
Germany
Crop Protection Division:
P.O. Box 120
67114 Limburgerhof
Germany

Contact person: Please refer to CP 1.1 Applicant.

Location of the manufacturing sites of pyraclostrobin and boscalid:

Confidential information - data provided in Document J.

CP 1.3 Trade names or proposed trade name and producer's development code number of the plant protection product if appropriate

Code number: BAS 516 07 F

Trade Names: Aragon, Bellis Drupacee, Gringo, Signum, Signum S, Signum 26.7/6.7 WG, Signum 33 WG, Signum WG, Terminett, Trigramm 26.7/6.7

CP 1.4 Detailed quantitative and qualitative information on the composition of the plant protection product

Pyraclostrobin pure active substance:
Minimum purity: 975 g/kg

Boscalid pure active substance:
Minimum purity: 960 g/kg

CP 1.4.1 Composition of the plant protection product

Pure active substance

content of pure pyraclostrobin:	**67.0 g/kg**	**(6.70% w/w)**
limits:	60.30 – 73.70 g/kg	(6.03 – 7.37% w/w)
content of pure boscalid:	**267.0 g/kg**	**(26.70% w/w)**
limits:	250.98 – 283.02 g/kg	(25.10 – 28.30% w/w)

Technical active substance[1)]

content of technical pyraclostrobin:	**69.79 g/kg**	**(6.98% w/w)**
limits:	62.81 – 76.77 g/kg	(6.28 – 7.68% w/w)
content of technical boscalid:	**273.85 g/kg**	**(27.39% w/w)**
limits:	257.42 – 290.28 g/kg	(25.74 – 29.03% w/w)

[1)] at a minimum purity of technical pyraclostrobin of 97.5% and technical boscalid of 96.0%

Safeners, synergists and co-formulants

Confidential information - data provided in Document J.

CP 1.4.2 Information on the active substances

Type	Name/Code Number
ISO common name	pyraclostrobin
CAS No	175013-18-0
EC No	n.a.
CIPAC No	657
salt, ester anion or cation present	none

Type	Name/Code Number
ISO common name	boscalid
CAS No	188425-85-6
EC No	n.a.
CIPAC No	673
salt, ester anion or cation present	none

CP 1.4.3 Information on safeners, synergists and co-formulants

Confidential information - data provided in Document J.

CP 1.5 Type and code of plant protection product

Nature: Water dispersible granules [Code: WG]

CP 1.6 Function

Fungicide

BASF
The Chemical Company

BAS 516 07 F

DOCUMENT M-CP, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE PLANT PROTECTION PRODUCT

Compiled by:

[redacted]

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67114 Limburgerhof
Germany

Telephone [redacted]
Telefax
E-mail

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 2 PHYSICAL AND CHEMICAL PROPERTIES OF THE PLANT PROTECTION PRODUCT

BAS 516 07 F is a new representative formulation, which has not been evaluated during a previous Annex I inclusion process.

All tests have been performed in GLP-certified laboratories.

Test or study & Data point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CP 2.1 Appearance	Visual assessment and organoleptic determination	BAS 516 07 F 1779 Boscalid: 26.65%; Pyraclostrobin: 6.78%	BAS 516 07 F is a brown fine granule with a smoky odour (remains unchanged after accelerated storage test).	Y	[see 2004/1024836 Kaestel R. 2004 a]
CP 2.2 Explosive and oxidising properties	OECD 113, Differential Scanning Calorimetry (DSC) 92/69/EEC A.14 92/69/EEC A.17 92/69/EEC A.16	BAS 516 07 F 1789 Boscalid: 26.7%; Pyraclostrobin: 6.8%	DSC in a temperature range from 30°C to 500°C yielded two peaks: 1.Peak: Onset: 150°C Peak temperature: 207°C Energy release: 130 J/g 2.Peak: Onset: 335°C Peak temperature: 393°C Energy release: 130 J/g The exothermic decomposition energy is less than 500 J/g. BAS 516 07 F does not react explosively to thermal and mechanical stress or to friction.	Y Y	[see 2005/1011601 Bitterlich S. 2005 a] [see 2011/1065573 Loehr S. 2011 a]

Test or study & Data point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
			A preliminary experiment was conducted to determine if the test item burns at a rate faster than that of the reference sample. From the result of the preliminary test it cannot be concluded that the test substance is without doubt an oxidising substance. Therefore the main test had to be conducted. The highest burning rate determined in the first test and in the repetition tests was 1.4 mm/s. The burning rate of the reference mixture (a barium nitrate/cellulose mixture containing 70% weight of oxidiser) was determined to be 3.6 mm/s.		
CP 2.3 Flammability and self-heating	92/69/EEC A.10 92/69/EEC A.12 92/69/EEC A.16	BAS 516 07 F 1789 Boscalid: 26.7%; Pyraclostrobin: 6.8%	Flammability (solids): The substance in its commercial form is made into a pile. An attempt is then made to ignite the sample under defined conditions and the burning time is measured. Result: Brief burning and rapid extinction was observed. Flammability (in contact with water): It is determined whether the reaction of the substance with water leads to the development of dangerous amounts of highly flammable gases. The substance was tested according to a step by step sequence. In three steps there was no gas evolution. The relative self-ignition temperature is 246°C.	Y Y	[see 2005/1011601 Bitterlich S. 2005 a] [see 2011/1065573 Loehr S. 2011 a]

<table>
<tr><th>Test or study & Data point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td>CP 2.4 Acidity/alkalinity and pH value</td><td>CIPAC MT 75</td><td>BAS 516 07 F
1779
Boscalid: 26.65%;
Pyraclostrobin: 6.78%</td><td>pH value at a concentration of 1% in CIPAC water D and pure water at room temperature:
<table><tr><th>Storage period / Dilution medium</th><th>Initial</th><th>2 weeks at 54°C</th></tr><tr><td>CIPAC water D</td><td>5.2</td><td>5.3</td></tr><tr><td>Pure water</td><td>5.2</td><td>5.4</td></tr></table>pH of CIPAC water D = 6.6
pH of pure water = 5.7</td><td>Y</td><td>[see 2004/1024836 Kaestel R. 2004 a]</td></tr>
<tr><td>CP 2.5 Viscosity and surface tension</td><td>OECD 115
EEC A.5
1.6.1
Plate method</td><td>BAS 516 07 F
1779
Boscalid: 26.65%;
Pyraclostrobin: 6.78%</td><td>Surface tension in pure water at 20°C:
<table><tr><th colspan="3">Surface tension</th></tr><tr><td>Conc.[%]</td><td>0.01</td><td>1.0</td></tr><tr><td>Surface tension [mN/m]</td><td>65.5</td><td>46.0</td></tr></table></td><td>Y</td><td>[see 2004/1024836 Kaestel R. 2004 a]</td></tr>
<tr><td>CP 2.6 Relative density and bulk density</td><td>OECD 109
EEC A.3
1.4.3
CIPAC MT 186</td><td>BAS 516 07 F
1779
Boscalid: 26.65%;
Pyraclostrobin: 6.78%</td><td>The relative density D^{20}_4 was determined to be initial 1.566.
The density at 20°C was determined to be 1.556 g/cm^3.
The bulk density of BAS 516 07 F is 655 g/L (loose) and 754 g/L (tapped).</td><td>Y</td><td>[see 2004/1024836 Kaestel R. 2004 a]</td></tr>
</table>

Test or study & Data point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CP 2.7 Storage Stability and shelf-life: effects of temperature on technical characteristics of the plant protection product	CIPAC MT 46.3 CLI Technical Monograph No. 17	BAS 516 07 F 1779 Boscalid: 26.65%; Pyraclostrobin: 6.78% BAS 516 07 F 08-120050 Boscalid: 26.6%; Pyraclostrobin: 6.9% (data from study 367105_1)	The relative content of the active substances does not decrease >5% during accelerated storage. Thus the product is considered to be stable. Content of pyraclostrobin (HPLC method CF-A598): 6.78% w/w before after storage for 14 days at 54°C Content of boscalid (HPLC method CF-A598): 26.65% w/w before and after storage for 14 days at 54°C Content of Dimethyl sulfate: Not tested. The impurity originates from pyraclostrobin technical, is diluted during the formulation process and reacts with water, resulting in its absence in BAS 516 07 F. Therefore this impurity is not formed during the production process of the formulation. Its content in the formulation does not increase during storage, because it is a molecule with a high energy content. No significant changes of the physical properties: appearance, odour, pH-value, wettability, suspension stability, suspensibility, dry sieve test, wet sieve test, dustiness, attrition resistance and flowability (see corresponding Annex points) were found after the accelerated storage stability test in glass bottles. After storage for 104 weeks at 23°C, the formulation showed good chemical and physical stability: Content of pyraclostrobin (AFL0598/01): 6.9% w/w before and after storage for 2 years at 23°C Content of boscalid (AFL0598/01): 26.6% w/w before and after storage for 2 years at 23°C	Y Y	[see 2004/1024836 Kaestel R. 2004 a] [see 2011/1102466 Keller M. 2011 a]

<table>
<tr><th>Test or study & Data point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
<u>Water content (CIPAC MT 30.2)</u>:
2% before and 2.1% after storage for 2 years at 23°C

<u>Appearance (visual)</u>:
light brown fine granules with moderate smoky odour before and after storage for 2 years at 23°C

<u>pH (CIPAC MT 75):</u>
Concentration of 1%:
<table>
<tr><th>Storage period / Dilution medium</th><th>Initial</th><th>After storage for 2 years at 23°C</th></tr>
<tr><td>CIPAC water D</td><td>5.6</td><td>5.9</td></tr>
<tr><td>Pure water</td><td>5.7</td><td>5.9</td></tr>
</table>
Concentration of 0.01%:
<table>
<tr><th>Storage period / Dilution medium</th><th>Initial</th><th>After storage for 2 years at 23°C</th></tr>
<tr><td>CIPAC water D</td><td>6.0</td><td>6.3</td></tr>
<tr><td>Pure water</td><td>5.9</td><td>5.9</td></tr>
</table>
<u>Persistent Foaming (CIPAC MT 47.2)</u>:
Concentration of 1% in CIPAC water D: 12 mL foam after 1 min before and 8 mL foam after 1 min after storage for 2 years at 23°C

Concentration of 0.01% in CIPAC water D: 7 mL foam after 1 min before and 6 mL foam after 1 min after storage for 2 years at 23°C
</td><td></td><td></td></tr>
</table>

Test or study & Data point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
			Suspensibility (CIPAC MT 184): Concentration of 1% in CIPAC water D: 99% before and after storage for 2 years at 23°C for pyraclostrobin Concentration of 1% in CIPAC water D: 98% before and after storage for 2 years at 23°C for boscalid Concentration of 0.01% in CIPAC water D: 99% before and 98% after storage for 2 years at 23°C for pyraclostrobin Concentration of 0.01% in CIPAC water D: 98% before and after storage for 2 years at 23°C for boscalid Wet Sieve Test (CIPAC MT 185): residue = 0.00% on a 75 µm test sieve before and after storage for 2 years at 23°C Dustiness (CIPAC MT 171): gravimetric collected dust: 0.4 mg before and 0.3 mg after storage for 2 years at 23°C Wettability (CIPAC MT 53.3.1): wetting time without swirling: 0s (in CIPAC water D) before and after storage for 2 years at 23°C Spontaneity of dispersion (CIPAC MT 174): 98% before and after storage for 2 years at 23°C Attrition resistance (CIPAC MT 178): 100% before and after storage for 2 years at 23°C		

<table>
<tr><th>Test or study & Data point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td>CP 2.8 Technical characteristics of the plant protection product</td><td colspan="5"></td></tr>
<tr><td>CP 2.8.1 Wettability</td><td>CIPAC MT 53.3.1</td><td>BAS 516 07 F
1779
Boscalid: 26.65%;
Pyraclostrobin: 6.78%</td><td>Wettability without swirling in CIPAC water D at room temperature:

<table><tr><td colspan="3">Wettability</td></tr><tr><td>Storage period</td><td>Initial</td><td>2 weeks at 54°C</td></tr><tr><td>Wettability [s]</td><td>0</td><td>0</td></tr></table></td><td>Y</td><td>[see 2004/1024836 Kaestel R. 2004 a]</td></tr>
<tr><td>CP 2.8.2 Persistence of foaming</td><td>CIPAC MT 47.2</td><td>BAS 516 07 F
1779
Boscalid: 26.65%;
Pyraclostrobin: 6.78%</td><td>Persistence of foaming determined in CIPAC water D at room temperature:

<u>Initial:</u>
0.01% in CIPAC water D:
3 mL foam after 10 sec.
0 mL foam after 1 min.
0 mL foam after 3 min.
0 mL foam after 12 min.

1.0% in CIPAC water D:
19 mL foam after 10 sec.
7 mL foam after 1 min.
5 mL foam after 3 min.
2 mL foam after 12 min.

<u>After storage for 2 weeks at 54°C:</u>
0.01% in CIPAC water D:
3 mL foam after 10 sec.
0 mL foam after 1 min.
0 mL foam after 3 min.
0 mL foam after 12 min.</td><td>Y</td><td>[see 2004/1024836 Kaestel R. 2004 a]</td></tr>
</table>

<table>
<tr><th>Test or study & Data point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>1.0% in CIPAC water D:
19 mL foam after 10 sec.
7 mL foam after 1 min.
5 mL foam after 3 min.
2 mL foam after 12 min.</td><td></td><td></td></tr>
<tr><td>CP 2.8.3 Suspensibility, spontaneity and dispersion stability</td><td>CIPAC MT 184 (chemical assay)

CIPAC MT 174</td><td>BAS 516 07 F 1779
Boscalid: 26.65%;
Pyraclostrobin: 6.78%</td><td>Concentration: 0.2 % in CIPAC water D:
<table><tr><td rowspan="2">Storage period</td><td colspan="2">Active substance suspensibility[%]</td></tr><tr><td>Initial</td><td>2 weeks at 54°C</td></tr><tr><td>Boscalid</td><td>98</td><td>99</td></tr><tr><td>Pyraclostrobin</td><td>98</td><td>98</td></tr></table>
Concentration: 1.0 % in CIPAC water D:
<table><tr><td rowspan="2">Storage period</td><td colspan="2">Active substance suspensibility[%]</td></tr><tr><td>Initial</td><td>2 weeks at 54°C</td></tr><tr><td>Boscalid</td><td>99</td><td>98</td></tr><tr><td>Pyraclostrobin</td><td>99</td><td>98</td></tr></table>
<u>Initial:</u> 100%
<u>After storage for 14 days at 54 °C:</u> 98%</td><td>Y</td><td>[see 2004/1024836 Kaestel R. 2004 a]</td></tr>
<tr><td>CP 2.8.4 Degree of dissolution and dilution stability</td><td></td><td></td><td>Not applicable for WG formulations</td><td></td><td></td></tr>
</table>

<table>
<tr><th>Test or study & Data point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td>CP 2.8.5 Particle size distribution, dust content, attrition and mechanical stability</td><td colspan="5"></td></tr>
<tr><td>CP 2.8.5.1 Particle size distribution</td><td>CIPAC MT 170</td><td>BAS 516 07 F
1779
Boscalid: 26.65%;
Pyraclostrobin: 6.78%</td><td>Dry sieve test of the substance BAS 516 07 F:
<table><tr><td>Storage period / Mesh sieve[µm]</td><td>Initial
Sum of residues Rx [%]</td><td>2 weeks at 54°C
Sum of residues Rx [%]</td></tr><tr><td>3350</td><td>0</td><td>0</td></tr><tr><td>2000</td><td>0</td><td>0</td></tr><tr><td>1000</td><td>0</td><td>0</td></tr><tr><td>500</td><td>6.6</td><td>7.8</td></tr><tr><td>250</td><td>88.8</td><td>90.2</td></tr><tr><td>125</td><td>99.9</td><td>100.1</td></tr><tr><td>75</td><td>100</td><td>100.1</td></tr><tr><td>X_1 for Rx ≥ 90%</td><td>125µm</td><td>250µm</td></tr><tr><td>X_2 for Rx ≤ 10%</td><td>500µm</td><td>500µm</td></tr><tr><td>Fines ≤ 50µm</td><td>0.1%</td><td>0.1%</td></tr></table></td><td>Y</td><td>[see 2004/1024836 Kaestel R. 2004 a]</td></tr>
<tr><td></td><td>CIPAC MT 185</td><td></td><td>Retention on a 75µm sieve [%w/w]:
0.00 initial and 0.10 after 2 weeks at 54°C.</td><td></td><td></td></tr>
<tr><td></td><td>Laser light diffraction spectrometry</td><td></td><td><table><tr><td></td><td colspan="2">Particle size [µm]</td></tr><tr><td>Storage period</td><td>Initial</td><td>2 weeks at 54°C</td></tr><tr><td>$d_{10\%}$</td><td>0.5</td><td>0.6</td></tr><tr><td>$d_{50\%}$</td><td>1.5</td><td>1.5</td></tr><tr><td>$d_{90\%}$</td><td>4.6</td><td>4.9</td></tr></table></td><td></td><td></td></tr>
</table>

<table>
<tr><th>Test or study & Data point</th><th>Guideline and method</th><th>Test material purity and specification</th><th>Findings</th><th>GLP Y/N</th><th>Reference</th></tr>
<tr><td>CP 2.8.5.2 Dust content</td><td>CIPAC MT 171</td><td>BAS 516 07 F
1779
Boscalid: 26.65%;
Pyraclostrobin: 6.78%</td><td><table><tr><td></td><td colspan="2">Dust content</td></tr><tr><td>Storage period</td><td>Initial</td><td>2 weeks at 54°C</td></tr><tr><td>Dust content [mg]</td><td>7.7</td><td>3.4</td></tr><tr><td>Dust content [%]</td><td>0.03</td><td>0.01</td></tr></table></td><td>Y</td><td>[see 2004/1024836 Kaestel R. 2004 a]</td></tr>
<tr><td>CP 2.8.5.3 Attrition</td><td>CIPAC MT 178.2</td><td>BAS 516 07 F
1779
Boscalid: 26.65%;
Pyraclostrobin: 6.78%</td><td><table><tr><td colspan="3">Attrition resistance (amount of particles retained on the 125 µm sieve): [%]</td></tr><tr><td>Storage period</td><td>Initial</td><td>2 weeks at 54°C</td></tr><tr><td>Attrition resistance</td><td>100</td><td>100</td></tr></table></td><td>Y</td><td>[see 2004/1024836 Kaestel R. 2004 a]</td></tr>
<tr><td>CP 2.8.5.4 Hardness and integrity</td><td></td><td></td><td>Not applicable for WG formulations</td><td></td><td></td></tr>
<tr><td>CP 2.8.6 Emulsifiability, re-emulsifiability, emulsion stability</td><td></td><td></td><td>Not applicable for WG formulations</td><td></td><td></td></tr>
<tr><td>CP 2.8.7 Flowability, pourability and dustability</td><td>CIPAC MT 172</td><td>BAS 516 07 F
1779
Boscalid: 26.65%;
Pyraclostrobin: 6.78%</td><td><table><tr><td colspan="2">Flowability initial [%]
(Passage through a 5 mm sieve)</td></tr><tr><td>spontaneous</td><td>100</td></tr><tr><td>after 5 drops</td><td>100</td></tr><tr><td>after 20 drops</td><td>100</td></tr></table></td><td>Y</td><td>[see 2004/1024836 Kaestel R. 2004 a]</td></tr>
</table>

Test or study & Data point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CP 2.9 Physical and chemical compatibility with other products including other plant protection products with which its use is to be authorised	ASTM method E 1518-93	BAS 516 00 F 2000-1	Test product: BAS 516 00 F (WG) Note: The tested product is the predecessor formulation of BAS 516 07 F. Both products are very similar. Therefore the test is considered to be also valid for BAS 516 07 F. The composition of both products is given in Document J. Tank mix partners: Acrobat Plus (WG), Polyram WG (WG), Decis (EC), Fastac 100 EC (EC), Stratos Ultra (EC) In total five mixtures of BAS 516 00 F with other plant protection products of the types WG and EC were tested. All mixtures were determined to be physically compatible and can be used in spray application. In all mixtures no lumping and no flocculation occurred, but a running agitator should be used in all mixtures. No phase separation and no significant sediment were formed. The mixtures were detected to be homogeneous. In some mixtures it could be useful to add a foam protection agent. There were no indications of any chemical reaction between the mixed products. While performing the tests no significant effect of heating or cooling of the mixtures occurred. No significant screen residues were found or remained in the test vessel. No chemical interactions between BAS 516 00 F and the tank mix partners were observed. Therefore BAS 516 00 F and consequently BAS 516 07 F are apparently chemically compatible with the tested products.	N	[see 2001/1001884 Schneider K.-H. 2001 a]

Test or study & Data point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CP 2.10 Adherence and distribution to seeds			Not applicable for WG formulations		
CP 2.11 Other studies			Not required		

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BAS 516 07 F

DOCUMENT M-CP, Section 3

DATA ON APPLICATION

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-July-2014		BASF DocID 2014/1162340 (version 1)
09-June-2015	The document according to SANCO/2012/11251 (following data point 3.8) was revised in order to a) have the complete information for the representative uses in this document instead of referring to M-CA, section 3 b) shorten table 2 (critical uses only instead of all registered uses). New or changed text is marked in yellow.	BASF DocID 2015/1106132 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 3 DATA ON APPLICATION

BAS 516 07 F is a new representative formulation, which has not been evaluated during a previous Annex I inclusion process.

CP 3.1 Field of use envisaged

Please see below efficacy information according to SANCO/2012/11251 (chapter 3).

CP 3.2 Effects on harmful organisms

Please see below efficacy information according to SANCO/2012/11251 (chapter 8).

CP 3.3 Details of intended use

Please see below Table 2 in the efficacy information according to SANCO/2012/11251 (following chapter 8).

CP 3.4 Application rate and concentration of active substance

Please see below Table 2 in the efficacy information according to SANCO/2012/11251 (following chapter 8).

CP 3.5 Method of application

Please see below efficacy information according to SANCO/2012/11251 (chapter 7).

CP 3.6 Number and timing of applications and duration of protection

Please see below for general information M-CP 3.8 and for detailed information Table 2 (following chapter 8) of the efficacy information given according to SANCO/2012/11251.

BAS 516 07 F is recommended with a spray interval up to 21 days depending on weather conditions, disease pressure and other factors. A sufficient level of protection can be expected for this period.

CP 3.7 Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops

Pyraclostrobin and boscalid have been applied since many years with a several different formulations across a wide range of crops without any reports of phytotoxic effects on succeeding crops. Due to the broad range of crops in which the product has been used, most rotational crop possibilities have been appeared in practice. Therefore no negative impact on succeeding crops is to be expected. Consequently, there is no necessity for restrictions in the choice of following crops, even in the event of crop failure on a field which has been treated with BAS 516 07 F.

CP 3.8 Proposed instructions for use

BAS 516 07 F is a fungicide used in potatoes mainly for the control of Alternaria spp.. Besides potatoes the product is also used against a broad spectrum of diseases in fruits and vegetables.

Time of application

Apply BAS 516 07 F in potatoes at the start of disease attack with Alternaria spp.. Typically the best point in time to start the applications is 6-8 weeks after emergence of the potatoes and the last application should be done latest 3 days before harvest. The recommended spray interval is about 10 – 21 days depending on disease pressure and the general spray program strategy.

Number and rate of application

Apply in potatoes maximum 4 times 0.2 – 0.25 kg BAS 516 07 F in a water volume of 150 – 400 L of water per ha.

On the specific country labels additional detailed guidance for the use of the product e.g. with regard to filling and application, tank cleaning and field use are given considering the local requirements. These instructions will be covered by the dRRs to be submitted for the re-registration of the plant protection product BAS 516 07 F following the renewal of approval for the active substance pyraclostrobin.

Further information can be found in Document C.

Efficacy Information

Active Substance: Pyraclostrobin

Product Code: BAS 516 07 F
67 g pyraclostrobin/kg
267 g boscalid/kg
WG

Applicant: BASF SE
Date: 09/June/2015

Statement

Pyraclostrobin, one of the active substances contained in the plant protection product BAS 516 07 F, has been tested in field development trials which demonstrated efficacious activity against a broad range of fungal diseases. It was included in Annex I of Directive 91/414/EEC on 1 June 2004 (entry into force) under Inclusion Directive 2004/30/EC for the use as fungicide. This use was amended on 22 April 2009 (entry into force) by Inclusion Directive 2009/25/EC to the use as fungicide or plant growth regulator.

BAS 516 07 F is registered in potatoes in many EU countries (for details please see Table 1) based on detailed national assessments of the efficacy data package in compliance with Regulation (EC) No 545/2011 and according to the Uniform Principles as given in Regulation (EC) No 546/2011.

1. INTRODUCTION

This document summarises the information related to the efficacy of the representative formulation BAS 516 07 F containing pyraclostrobin and boscalid as active substances.

2. FUNCTION

BAS 516 07 F is used as a fungicide to control harmful diseases in potatoes and other crops. It shows local systemic and translaminar activity.

3. FIELD OF USE

BAS 516 07 F is used in agriculture in many crops (potatoes, fruits, vegetables) for the control of a broad range of harmful and economically important pathogens.

4. SUPPORTED USES

BAS 516 07 F in potatoes had been identified as representative use to support the renewal of approval for the active substance pyraclostrobin (for details see Tables 1 and 2).

This use is representative because of

- its wide geographical distribution covering the Southern, Central and Northern Zone
- its different application timings during the year
- its agricultural importance in the EU
- its importance in the human diet
- the potato belonging to the solanacea crop group, to which for example also important crops like tomato, eggplant and pepper belong

BAS 500 06 F in cereals and maize had been identified as additional representative uses to support the renewal of approval for the active substance pyraclostrobin (for details see separate dossier for BAS 500 06 F).

5. OVERVIEW OF CURRENT REGISTRATIONS

Current registrations of BAS 516 07 F in the EU for the representative use potatoes are shown in Table 1.
Besides the use of BAS 516 07 F in potatoes, many other uses of pyraclostrobin containing plant protection products are registered covering all EU member states and most of the crops professionally cultivated (further information on these additional uses treated with pyraclostrobin can be found in M-CA 3.5). In addition to various solo formulations other active substances such as boscalid, dimethomorph, dithianon, epoxiconazole, fenpropimorph, fluxapyroxad, folpet and metiram are used in pyraclostrobin containing mixture formulations (further information on these uses can be found in document D2).

6. HARMFUL ORGANISMS CONTROLLED AND CROPS TREATED

Harmful organisms controlled by BAS 516 07 F in potatoes are given in chapter 8 below. An overview on additional harmful organisms controlled by pyraclostrobin and on additional crops treated in the EU with pyraclostrobin-containing formulations can be found in M-CA 3.5.

7. METHOD OF APPLICATION

BAS 516 07 F is applied in potatoes by broadcast foliar spraying using water as carrier. The water volume varies between 150 – 1000 L/ha.

8. MODE OF ACTION - EFFECTS ON HARMFUL ORGANISMS

The **mode of action** for the active substance pyraclostrobin is described below for the use as fungicide and the use as plant growth regulator.

Use as fungicide

Pyraclostrobin belongs to the QoI group of fungicides. The mode of action is the inhibition of mitochondrial respiration resulting from a blockage of the electron transport from ubihydroquinone to cytochrome c by means of a binding to the ubihydroquinone oxidation centre (Qo) of the cytochrome bc1 complex (Complex III). This disrupts the mitochondrial electron transport chain, thus blocking phosphorylation further down in the respiratory chain. In consequence, this leads to a reduction of energy-rich ATP which is required to support a range of essential processes in the fungal cell such as maintenance of membrane potentials and concentration gradients up to DNA, RNA and protein biosynthesis. In the end, the various fungal development processes of spore germination, formation of infection structures, mycelium growth and sporulation are permanently disrupted.

Use as plant growth regulator

In addition to the effects on yield through inhibition of fungal pathogens, pyraclostrobin also delivers a positive effect on yield through influence on the plant metabolism and physiology.
A decrease in ethylene levels has been demonstrated in several lab studies after treatment with pyraclostrobin. This can explain the observed delay in senescence. The reduction in ethylene levels can result in a variety of cellular changes being involved in yield gain (e.g. by an increased and longer photosynthetic performance during the vegetation period of a treated crop):

- enhancement of cytokinin levels resulting in increased chlorophyll concentrations in the leaves
- higher catalase and superoxide dismutase (SOD) activities reducing reactive $O_2^{\cdot}$-levels producing e.g. H_2O_2, resulting in reduced chlorophyll degradation

Furthermore, studies demonstrated that pyraclostrobin increases nitrate reductase, improving nitrogen assimilation and nitrogen use efficiency.

In the following, information on the **foliar uptake** of pyraclostrobin and a description of its **general effects on harmful organisms** are given.

After application to the plant, the active ingredient is taken up via the leaf and then translocated at low rates via the transpiration flow. Due its relatively low mobility, it shows local systemic and translaminar activity. Because of its very high intrinsic activity, pyraclostrobin has been observed to have systemic effects in a number of authorized uses. By that, it can control fungal stages which have already become established in deeper tissue layers. Pyraclostrobin is thus suitable for preventative and curative treatments. Since the vapour pressure of pyraclostrobin is very low, a marked gas phase activity was not observed.

Pyraclostrobin is active against different fungal stages on and in the plant. When applied protectively, pyraclostrobin prevents not only the germination of fungal spores landing on the plant surface but also re-infection, since during these extremely energy-consuming phases fungi react very sensitively to disturbances of their mitochondrial respiratory chain. Due to its ability to penetrate into the leaf and its further translocation as well as its high intrinsic activity, it can also control fungal stages which have already become established in deeper tissue layers.

BAS 516 07 F is a fungicide to control the different Alternaria species (mainly *Alternaria solani* and *Alternaria alternata*) in potatoes.

Furthermore, it controls a broad range of harmful and economically important pathogens in many fruits and vegetables.

Table 1: Supported representative uses for BAS 516 07 F currently registered in the EU

	Representative Uses (for application details see Table 2)				Existing Authorisations								
Crop	Target	Situation of use (e.g. indoor…)	as content & Formulation Type	Application method	Country	Zone	Since	Reg. No.	Product (tradename)	Product application rate per treatment Min and Max	Active substance application rate per treatment Min and Max	Number of treatments per season Min and Max	Active substance Max total dose/ ha Min and Max
Potatoes	*Alternaria spp.*	Outdoor	Pyraclo-strobin 67 g/kg Boscalid 267 g/kg WG	Foliar spray	DK	N	09.06.2006	19-151	BAS 516 07 F (Signum)	0.2 – 0.25 kg product/ha	13-17 g pyraclo-strobin + 53-67 g boscalid/ha	4	54-67 g pyraclo-strobin + 214-267 g boscalid/ha
					EE	N	28.08.2006	307	BAS 516 07 F (Signum)				
					LT	N	22.03.2007	0314F/09	BAS 516 07 F (Signum)				
					LV	N	15.03.2006	269	BAS 516 07 F (Signum)				
					SE	N	24.05.2007	4884	BAS 516 07 F (Signum)				
					AT	C	21.06.2012	3199	BAS 516 07 F (Signum)				
					BE	C	28.07.2005	9429P/B	BAS 516 07 F (Signum)				
							05.03.2009	9726P/B	BAS 516 07 F (Terminett)				
					DE	C	11.07.2006	025483-00	BAS 516 07 F (Signum)				
					NL	C	24.12.2004	12630 N	BAS 516 07 F (Signum)				
					HR	S	29.12.2006	UP/I-320-20/06-01/247	BAS 516 07 F (Signum)				

Table 2: Critical Uses – justification and GAP tables

GAP rev. 1.0, date: 2014-May-09

PPP (code)	**BAS 516 07 F**	**Formulation type:**	**WG**
active substance 1	**Pyraclostrobin**	**Conc. of as 1:**	**67 g/kg**
active substance 2	**Boscalid**	**Conc. of as 2:**	**267 g/kg**
safener	**-**	**Conc. of safener:**	**-**
synergist	**-**	**Conc. of synergist:**	**-**
Applicant:	**BASF SE**	**professional use**	☒
Zone(s):	**EU**	**non professional use**	☐
Verified by MS:	**y/n**		

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	AT, DE	Potatoes	F	*Alternaria* spp.	Spraying	51-89	a) 4 (10d) b) 4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	200-400	3	used as critical GAP in risk assessments
2		Potatoes	F	*Alternaria* spp.	Spraying	41-89	a) 4 (5-10d) b) 4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	150-1000	0-3	used in M-CA 6 for the discussion of residues (reflecting a worst-case scenario based on the most critical parameters of different registered GAPs)

Note: A table showing a detailed overview of the GAPs for all registered EU uses of BAS 500 06 F in potatoes can be found in Document D1.

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The Chemical Company

BAS 516 07 F

DOCUMENT M-CP, Section 4

FURTHER INFORMATION ON THE PLANT PROTECTION PRODUCT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162341 (version 1)
27-Feb-2017	Chapters 4.2 and 4.3 were amended by the MSDS of BAS 516 07 F and the text was updated where necessary (new or changed text is marked in yellow).	BASF DocID 2017/1032483 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 4 FURTHER INFORMATION ON THE PLANT PROTECTION PRODUCT

BAS 516 07 F is a new representative formulation, which has not been evaluated during a previous Annex I inclusion process.

CP 4.1 Safety intervals and other precautions to protect humans, animals and the environment

Pre-harvest interval (in days) for each relevant crop:
For potatoes the application is intended with a pre-harvest interval of 0-3 days.

Re-entry period (in days) for livestock, to areas to be grazed:
Because pyraclostrobin is not intended to be used in areas to be grazed, no re-entry period for livestock has to be defined.

Re-entry period (in hours or days) for man to crops, buildings or spaces treated:
The re-entry assessment is discussed in M-CP 7.2. Re-entry is possible after the spray deposits on the crops have dried given the worker is wearing adequate work clothing.

Withholding period (in days) for animal feeding stuffs:
Due to the favorable residue situation in potatoes with residues consistently below LOQ no withholding period is needed.

Waiting period (in days) between application and handling of treated products:
This is not relevant here since a post-harvest treatment is not intended for potatoes.

Waiting period (in days) between last application and sowing or planting succeeding crops:
No minimum waiting periods needs to be considered for phytotoxicity or for residues in follow-crops.

Information on specific conditions under which the preparation may or may not be used:
Not relevant

CP 4.2 Recommended methods and precautions

Report:	CP 4.2/1 Anonymous, 2015 a Safety data sheet - Signum 2015/1260817
Guidelines:	EEC 1907/2006
GLP:	no

Precautions for safe handling

No special measures necessary if stored and handled correctly. Ensure thorough ventilation of stores and work areas. When using do not eat, drink or smoke. Hands and face should be washed before breaks and at the end of the shift.

Protection against fire and explosion:
Avoid dust formation. Dust can form an explosive mixture with air. Prevent electrostatic charge. Sources of ignition should be kept well clear. Fire extinguishers should be kept handy.

Conditions for safe storage including any incompatibilities

Segregate from foods and animal feeds.
Further information on storage conditions:
Keep away from heat. Protect against moisture. Protect from direct sunlight.

Storage stability:
Storage duration: 60 months
Protect from temperatures above 40 °C.
Changes in the properties of the product may occur if the product is stored above the indicated temperature for extended periods of time.

Exposure controls

Personal protective equipment

Respiratory protection:
Respiratory protection not required.

Hand protection:
Suitable chemical resistant safety gloves (EN 374) with prolonged, direct contact. Recommended is protective index 6 corresponding to > 480 minutes of permeation time according to EN 374, e.g. nitrile rubber (0.4 mm), chloroprene rubber (0.5 mm), butyl rubber (0.7 mm) etc.

Eye protection:
Safety glasses with side-shields (frame goggles), e.g. EN 166

Body protection:
Body protection must be chosen depending on activity and possible exposure, e.g. apron, protecting boots, chemical-protection suit (according to EN 14605 in case of splashes or EN ISO 13982 in case of dust).

General safety and hygiene measures
The statements on personal protective equipment in the instructions for use apply when handling crop-protection agents in final consumer packing. Wearing of closed work clothing is recommended. Store work clothing separately. Keep away from food, drink and animal feeding stuffs.

Transport information

Land transport

ADR

UN number:	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains BOSCALID, PYRACLOSTROBIN)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	Tunnel code: E

RID

UN number:	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains BOSCALID, PYRACLOSTROBIN)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	none known

Inland waterway transport

ADN

UN number:	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains BOSCALID, PYRACLOSTROBIN)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	none known
Transport in inland waterway vessel:	not evaluated

Sea transport

IMDG

UN number:	UN 3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains BOSCALID, PYRACLOSTROBIN)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Marine pollutant:	yes
Special precautions for user:	none known

Air transport

IATA/ICAO

UN number:	UN 3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains BOSCALID, PYRACLOSTROBIN)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	none known

Waste treatment methods

Must be sent to a suitable incineration plant observing local regulations.

Contaminated packaging:
Contaminated packaging should be emptied as far as possible and disposed of in the same manner as the product.

Report: CP 4.2/2
Ohnsorge U., 2001a
BAS 516 00 F (preliminary designator): Effectiveness of procedures for cleaning application equipment and protective clothing
2001/1001819
Guidelines: none
GLP: no

Note 1: This study was erroneously not contained in the application. It needs to be submitted, because BAS 516 07 F is a new representative formulation, which has not been evaluated during a previous Annex I inclusion process.

Note 2: The above described document is based on BAS 516 00 F, the predecessor formulation of BAS 516 07 F. Both products are very similar. Therefore, the document is considered to be also valid for BAS 516 07 F. The composition of both products is given in Document J.

Any surplus spray solution containing BAS 516 07 F should be diluted with water at a ratio of 1:10 and sprayed onto the previously treated area according to the use instructions.

The spray equipment should be cleaned thoroughly immediately after use by draining the system completely and by rinsing spray tank, boom and nozzles two to three times with clean water, so that foam and remainders of BAS 516 07 F will be sufficiently removed. No plant damage can be caused when the equipment is used subsequently for other applications.

Protective clothing will be cleaned effectively when washed with usual laundry detergents.

CP 4.3 Emergency measures in the case of an accident

Report:	CP 4.3/1 Anonymous, 2015 a Safety data sheet - Signum 2015/1260817
Guidelines:	EEC 1907/2006
GLP:	no

First aid measures

Remove contaminated clothing.

If inhaled:
Keep patient calm, remove to fresh air.

On skin contact:
Wash thoroughly with soap and water.

On contact with eyes:
Wash affected eyes for at least 15 minutes under running water with eyelids held open.

On ingestion:
Rinse mouth and then drink plenty of water.

Most important symptoms and effects, both acute and delayed
No significant reactions of the human body to the product known.

Indication of any immediate medical attention and special treatment needed
Treat according to symptoms (decontamination, vital functions).

Fire-fighting measures

Suitable extinguishing media
Dry powder, foam, water spray

Unsuitable extinguishing media for safety reasons
Carbon dioxide

Special hazards arising from the product
The following substances or groups of substances can be released in case of fire:
Carbon monoxide, hydrogen chloride, carbon dioxide, nitrogen oxides, organochloric compounds

Advice for fire-fighters
Special protective equipment:
Wear self-contained breathing apparatus and chemical-protective clothing.
Further information:
Collect contaminated extinguishing water separately, do not allow to reach sewage or effluent systems. Dispose of fire debris and contaminated extinguishing water in accordance with official regulations. In case of fire and/or explosion do not breathe fumes. Keep containers cool by spraying with water if exposed to fire.

Accidental release measures

Personal precautions, protective equipment and emergency procedures
Avoid dust formation. Use personal protective clothing. Avoid contact with skin, eyes and clothing.

Environmental precautions
Do not discharge into subsoil or soil. Do not discharge into drains, surface waters or ground-water.

Methods and material for containment and cleaning up
For small amounts:
Contain with dust binding material and dispose of.
For large amounts:
Sweep/shovel up. Avoid raising dust. Dispose of absorbed material in accordance with regulations. Collect waste in suitable containers, which can be labeled and sealed. Clean contaminated floors and objects thoroughly with water and detergents, observing environmental regulations.

CP 4.4 Packaging, compatibility of the plant protection product with proposed packaging materials

BAS 516 07 F is marketed in mold blown high-density polyethylene containers (HDPE). They are sealed by foil seals, protected by screw caps of polypropylene.

0.5 litre bottle	material:	HDPE
(0.3 kg)	shape/size:	cylindrical / approx. 69 mm diameter x 185.5 mm
	opening:	42 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal
1 litre bottle	material:	HDPE
(0.5kg)	shape/size:	cylindrical / approx. 91 mm diameter x 234 mm
	opening:	42 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal
2 litre bottle	material:	HDPE
(1 kg)	shape/size:	Rectangular, 142 x 97 x 226 mm (LxBxH)
	opening:	42 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal
5 litre container	material:	HDPE
(2.5 kg)	shape/size:	rectangular / approx. 190 mm x 140 mm x 313 mm
	opening:	54 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal
10 litre container	material:	HDPE
(5 kg)	shape/size:	rectangular / approx. 230 mm x 165 mm x 375 mm
	opening:	54 mm inner diameter
	closure:	polypropylene screw cap
	seal:	HF-seal

Report:	CP 4.4/1 Schreiner B., 2009a EU-Performance-Test of BAS 516 07 F, Bottle, rectangular, 1 Kg (PE-HD) 2009/1052799
Guidelines:	none
GLP:	no

ADR-test 6.1.5.3 was performed for drop resistance. In this test, the tightness of the bottles was successfully tested. The leak test (ADR 6.1.5.4), an internal pressure test (hydraulic test, ADR 6.1.5.5) and the test for permeation (ADR 6.1.5.7) were not applicable since the product is a solid. Also the stacking test (ADR 6.1.5.6) was not applicable (bottles are part of combination packaging). Packagings are supplied in UN-approved combination packs (ADR 6.1.4.21).

These packagings meet the requirements for packaging group III as specified by the ADR/RID regulations for the transport of hazardous goods.

Report:	CP 4.4/2 Keller M., 2011a Boscalid/Pyraclostrobin 26.7/6.7 % WG - chemical and physical stability of formula BAS 516 07 F when stored at 23°C up to 3 years in commercial packs (HDPE-bottle) 2011/1102466
Guidelines:	CLI Technical Monograph No. 17,EEC 91/414 Annex III,EPA Product Properties Test Guidelines,OECD Principles of Good Laboratory Practice,GLP Principles of the German Chemikaliengesetz (Chemicals Act)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: In the application this study was only mentioned in section 2.

The study results prove that the original container material and the closure/seal resists to the product BAS 516 07 F. No corrosion of the container could be observed during the storage time.

CP 4.5 Procedures for the destruction or decontamination of the plant protection product and its packaging

CP 4.5.1 Neutralisation procedure

The pH of BAS 516 07 F is in a range between 5.6 and 6.3 in aqueous solution. Therefore, the proposal of a neutralisation procedure is not considered to be necessary. Any spilled product and contaminated soil or water has to be absorbed and disposed according to the use instructions.

CP 4.5.2 Controlled incineration

For purposes of disposal, combustion of BAS 516 07 F in a licensed incinerator is recommended. This method of disposal applies also to contaminated packages, which cannot be cleaned or reused.

Although it is possible to incinerate the product at lower temperatures, combustion at approximately 1100°C with a residence time of about 2 seconds is advised. By doing so, i.e. operating the incinerator according to the conditions laid down in council directive 94/67/EEC resp. directive 2000/76/EC of the European Parliament, one will achieve complete combustion and minimize the formation of undesired by-products in the off-gases.

Users are requested to triple rinse empty primary packages as described in the ECPA "Guidelines for the rinsing of agrochemical containers", 1993.

Pressure rinsing or integrated pressure rinsing of the packaging material achieves a similar or even better result. The rinsing water must be added to the spray liquid.

To minimize waste of packages it is recommended that empty and rinsed containers are delivered to local container collection stations. If these do not exist, empty and rinsed containers must be rendered unusable and disposed according to local regulations.

BASF
The Chemical Company

BAS 516 07 F

DOCUMENT M-CP, Section 5

ANALYTICAL METHODS

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[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 5 ANALYTICAL METHODS

BAS 516 07 F is a new representative formulation, which has not been evaluated during a previous Annex I inclusion process.

CP 5.1 Methods for the generation of pre-authorisation data

CP 5.1.1 Analysis of the plant protection product

(a) Methods for the determination of the active substance and/or variant in the plant protection product

Report:	CP 5.1.1/1 Ziegler H., 2000a The determination of the amounts of active ingredients Reg.No. 300 355 and Pyraclostrobin in BAS 516 00 F by HPLC 2000/1014140
Guidelines:	none
GLP:	no

The active substances of BAS 516 07 F can be quantified using the analytical reversed phase-HPLC method CF-A 598. This method was developed for quantifying pyraclostrobin and boscalid in BAS 516 00 F, but specific chromatograms prove the applicability for BAS 516 07 F as well (BASF DocID 2009/1050295, study summary shown below).
The method CF-A 598 is a reversed phase HPLC method with UV-detection and external calibration to determine boscalid and pyraclostrobin in water dispersible granules (WG).

Chromatographic parameters

Flow rate	1.0 mL/min
Column specifications	Stainless steel column 250 x 4.6 mm, packed with J'sphere ODS-H80 4 µm
Column temperature	Ambient
Detector wavelength	Time [min.] Wavelength [nm] 0 275 6 275 7 225 12 225 13 275 22 275
Injection volume	5 µL
Analytical cycle	Approx. 22 min
Mobile phase	A) 580 ml Acetonitrile, 420 mL water and 771 mg ammonium acetate B) 900 mL Acetonitrile, 100 mL water and 771 mg ammonium acetate
Gradient	Time [min.] A [%] B [%] 0 100 0 12 100 0 13 0 100 18 0 100 20 100 0 22 100 0

Report:	CP 5.1.1/2 Ziegler H., 2000b Development and validation of the analytical method CF-A 598. Determination of Reg.No. 300 355 and Pyraclostrobin in water dispersible granules (WG) (BAS 516 00 F) 2000/1012385
Guidelines:	Appendix 1 to § 19 a Section 1 Chemikaliengesetz of 25th July 1994
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Summary
The active substances of BAS 516 07 F can be quantified using the analytical reversed phase-HPLC method CF-A 598. This method was developed for quantifying pyraclostrobin and boscalid in BAS 516 00 F, but specific chromatograms prove the applicability for BAS 516 07 F as well (BASF DocID 2009/1050295, study summary shown below).

The method CF-A 598 is a reversed phase-HPLC method with UV-detection and external calibration to determine boscalid and pyraclostrobin in water dispersible granules (WG).

Method description

Method Principle:

Method CF-A 598 is applicable for the determination of boscalid and pyraclostrobin in WG formulations, e.g. in BAS 516 07 F. The validation was carried out on BAS 516 00 F, the applicability of this method for BAS 516 07 F is proven by specific chromatograms. The analytes are determined by reversed phase HPLC on a J'sphere ODS-H80 column (250 x 4.6 mm, dp = 4 µm) at room temperature, using external calibration. Injection volume is 5 µL. The separation is achieved by using gradient conditions for the detection and quantification of the actives (flow rate: 1 mL/min). Detection is performed with a HPLC UV detector with variable wavelength adjustment. The mobile phase A consists of 580 mL acetonitrile, 420 mL water and 771 mg ammonium acetate. The mobile phase B consists of 900 mL acetonitrile, 100 mL water and 771 mg ammonium acetate.
The analytes are quantified by comparing the specific response ratios of the samples with those of standards of known quality.

Findings:

The validation data of method CF-A 598 with respect to precision, accuracy, linearity and specificity prove that the method is suitable for the determination of pyraclostrobin and boscalid in WG-formulations, e.g. in BAS 516 00 F or BAS 516 07 F (proved by specific chromatograms as shown in BASF DocID 2009/1050295).

Enforcement analytical methodology:

Specificity: The specificity of the method was demonstrated by spectrometric examination, i.e. by HPLC analysis of the species. It was verified by comparing the retention times of the active ingredients in the preparation with the reference substances, checking the chromatograms of the blank formulation and the technical active ingredients for interference and comparing the UV-spectra of the active ingredient-peaks in the product and the peaks of the reference substances.
There were no indications of interferences. Identical UV-spectra of the active ingredient-peaks in the preparation and the reference substances were measured.

Linearity: BAS 516 00 F was weighed five times in ascending concentrations and analysed in each case in accordance with the present analytical method. The weigh-in weights of this series of concentrations are approximately 0.5 to 1.5 times of the nominal concentrations.

The regression curve data are listed below:

Boscalid:

Slope (m):	70
y axis intercept (b):	4
Correl. coeff. (r^2):	1.0000

Pyraclostrobin:

Slope (m):	71
y axis intercept (b):	1
Correl. coeff. (r^2):	1.0000

Accuracy: The accuracy of the procedure was assessed by preparation and analysis of six samples containing known weights of the analytes, i.e. known amounts of boscalid and pyraclostrobin were added to the blank formulation. The amount of blank formulation was equivalent to the amount in the original formulation BAS 516 00 F.
The average recovery was found to be 100.17% (RSD = 0.399%) for pyraclostrobin and 100.55% (RSD = 0.386%) for boscalid.

Outliers or stragglers could not be determined.

Repeatability: The repeatability (precision) of the method was determined by weighing six samples of BAS 516 00 F (batch 2000-2). Each sample was analysed using method CF-A 598.
Results for the formulation BAS 516 00 F:

Active ingredient	Weight (min, max) [%]	Average [%]	RSD [%]
Pyraclostrobin	6.775 – 6.824	6.80	0.299
Boscalid	27.240 – 27.358	27.29	0.162

It is situated in a range which can be expected by the used equipment and the analytical method in general. The RSD values meet the requirements given by the modified Horwitz equation (Horwitz RSDR * 0.67).
Outliers could not be observed.

Report: CP 5.1.1/3
Euler K., 2009a
Final report: Supplement to the development and validation of the analytical method CF-A 598, Study No. 09L00088
2009/1050295

Guidelines: OECD Principles of Good Laboratory Practice, GLP Principles of the German Chemikaliengesetz (Chemicals Act)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

In order to prove the applicability of CF-A 598 to BAS 516 07 F, specific chromatograms were generated.
The chromatograms demonstrate that the analytical method CF-A 598 is applicable to determine the active ingredients in the formulation BAS 516 07 F.

(b) Methods for determination of relevant impurities identified in the technical material or which may be formed during manufacture of the plant protection product or from degradation of the plant protection product during storage

Boscalid does not contain any impurity of toxicological or ecotoxicological concern.

Pyraclostrobin contains < 1mg/kg dimethyl sulfate. Information on the analytical method for determination of this impurity in the technical material can be found in M-CA 4.1.1. In the following, the method for determination of dimethyl sulfate in BAS 516 07 F is outlined.

Report: CP 5.1.1/4
Stegmaier W., 2009a
Analytical method AM/01120/01e, gas chromatographic determination of dimethyl sulfate in BAS 516 07 F
2009/1105184
Guidelines: none
GLP: no

The GC method AM/01120/01e was developed to determine the impurity dimethyl sulfate in the formulation BAS 516 07 F with quantification by means of standard addition.
The analytical method AM/01120/01e is a GC method equipped with headspace autosampler and a mass spectrometer detector.

Headspace conditions

Temperature of equilibration:	90°C
Duration of equilibration:	60 min
Transfer line temperature:	150°C
Duration of pressurization:	0.5 min
Final headspace pressure:	2 bar
Duration of injection:	0.75 s

GC conditions

Column

Fused silica capillary:	ZB-1701 (Phenomenex)
Length:	30 m
Internal diameter:	0.25 mm
Film thickness:	0.25 µm

Carrier gas

Helium	
Column head pressure:	0.75 bar
Septum purge:	3 mL/min

Temperatures

Oven:	50°C isothermal for 1 min
	50°C → 160°C, 15 K/min
	270°C isothermal for 23 min
Injector:	210°C

Detector

MSD	
Acquisition mode:	SIM
EM offset:	600 V
Resulting EMV:	2247 V
Detection of selected ions:	**m/z = 95.0**, m/z = 96.0, m/z = 125.0
Temperature of source:	230°C
Temperature of quadrupole:	150°C

Report:	CP 5.1.1/5 Stegmaier W., 2012a Validation of an analytical method for the determination of dimethyl sulfate in BAS 516 07 F 2012/1213598
Guidelines:	OECD Principles of Good Laboratory Practice, GLP Principles of the German Chemikaliengesetz (Chemicals Act)
GLP:	yes

Summary
The GC method AM/01120/01e is suitable to determine the impurity dimethyl sulfate in the formulation BAS 516 07 F with quantification by means of standard addition.
The analytical method AM/01120/01e is a GC method equipped with headspace autosampler and a mass spectrometer detector.

Method description

Method Principle:

Method AM/01120/01e is applicable for the determination of the impurity dimethyl sulfate in the WG formulation BAS 516 07 F. The analyte, dissolved in toluene, is determined by headspace gas chromatography utilising a ZB-1701 (Phenomenex) - column (30 m; 0.25 mm id; film thickness 0.25 µm); temperature programmed.
Duration of injection is 0.75 s at a transfer line temperature of 150°C and the final headspace pressure is 2 bar.
The column head pressure is 0.75 bar and carrier gas is helium.
Detection is performed with a MSD (detection of selected ions: m/z = 95.0, 96.0, 125.0).
Quantification is by means of standard addition.

Findings:

The validation data of method AM/01120/01e with respect to precision, accuracy, linearity and specificity prove that the method is suitable for the determination of the impurity dimethyl sulfate in the WG-formulation BAS 516 07 F.

Enforcement analytical methodology:

Specificity/ selectivity: Peaks in the gas chromatogram are assigned to the analyte dimethyl sulfate by comparison of retention times and by the selection of characteristic ions in MS-detection. Constituents of the sample which coelute with the analyte and which yield the same ions as selected for dimethyl sulfate give rise to excessively high mass fractions.

No dimethyl sulfate could be detected in toluene (solvent blank).

Linearity: Linearity was proved for standard additions to the test item (with influence of active agents) and to BAS 516 AA F (without influence of active agents).

Linearity of calibration for standard addition to the test item: Calibration was performed on 6 concentration levels in the range of 0.248 µg/g and 9.78 µg/g.
The calibration points for 0.473 µg/g and 1.00 µg/g were not considered for evaluation. Although they cannot be regarded as outliers according to Dixon's test it is obvious that they do not fit on the regression line through the other calibration points.
Maybe this can be traced back to the fact that calibrations were run on different days and the MSD response altered in between.
The regression curve data are listed below:

Dimethyl sulfate:

Slope:	350894
y axis intercept (b):	9200
Correl. coeff. (R^2):	0.9998 (4 calibration levels)

Linearity of calibration for standard addition to BAS 516 AA F: Calibration was performed on 6 concentration levels in the range of 0.253 µg/g and 9.77 µg/g.
The regression curve data are listed below:

Dimethyl sulfate:

Slope:	625976
y axis intercept (b):	-31405
Correl. coeff. (R^2):	0.9984

Mass fraction of dimethyl sulfate in the test item:

The test item was analyzed without fortification of the analyte.
Dimethyl sulfate could not be detected.

Accuracy:

Accuracy was verified twice. Fortifications of approx. 1µg/g dimethyl sulfate to the test item and to BAS 516 AA F were determined with mean recoveries of 122% (test item) and 103% (BAS 516 AA F).

Precision, repeatability:

Due to the poor solubility of the test item in toluene it was not possible to prepare a homogeneous fortified solution which could have been divided in 6 subsamples in order to test precision. Alternatively precision had to be deduced from the standard deviation of the recoveries of the 6 samples spiked with approx. 1µg/g each.
Determinations 5 and 6 were not considered for evaluation.
Although they cannot be regarded as outliers according to Dixon's test it is obvious that they are far away from the other values.

Results for the spiked test item:

Mean recovery [%]	Standard deviation [%]	Relative standard deviation [%]	Measurement uncertainty [%]
122	4.8	3.9	11.7

Results for spiked BAS 516 AA F:

Mean recovery [%]	Standard deviation [%]	Relative standard deviation [%]	Measurement uncertainty [%]
103	4.1	4.0	12.0

As shown by fortification tests, the analysis of the relevant impurity dimethyl sulfate in the end-product BAS 516 07 F is possible. The fortifications could be detected.

Based on the production process of the formulation BAS 516 07 F in which the active ingredients are mixed with the formulants in the presence of water to get a spray-dryable suspension, the traces of dimethyl sulfate (remaining in the technical active substance pyraclostrobin) react with water.
The analytical method proves that dimethyl sulfate is not present in the end-use product BAS 516 07 F.

(c) Methods for the determination of relevant co-formulants or components of co-formulants, where required by the national competent authorities

Currently not required by EU legislation

CP 5.1.2 Methods for the determination of residues

Methods for the determination of residues are submitted and summarized in M-CA 4.1.2.

CP 5.2 Methods for post-authorisation control and monitoring purposes

Methods for the determination of residues in or on plants, plant products, processed food commodities, food and feed of plant and animal origin

Monitoring methods for plant and animal matrices are discussed in M-CA 4.2.

Methods for the determination of residues in body fluids and tissues

Monitoring methods for body fluids are discussed in M-CA 4.2.

Methods for the determination of residues in soil

Monitoring methods for soil are discussed in M-CA 4.2.

Methods for the determination of residues in water

Monitoring methods for water are discussed in M-CA 4.2.

Methods for the determination of residues in air, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

As pyraclostrobin has a very low vapour pressure of 2.6 x 10^{-8} Pa (for details see M-CA 2.2), it is considered to be non-volatile. Consequently, inhalation exposure of operators, workers, residents or bystanders to vapours of pyraclostrobin is negligible. Further information can be found in M-CP 7.2.

BASF
The Chemical Company

BAS 516 07 F

DOCUMENT M-CP, Section 7

TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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18-Jul-2014		BASF DocID 2014/1162343 (version 1)
27-Feb-2017	7.1.7 was amended to better address literature data and CP 7.3/2 to better characterize the test material. New or changed text is marked in yellow.	BASF DocID 2017/1032484 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 7 TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

BAS 516 07 F is a new representative formulation, which has not been evaluated during previous Annex I inclusion processes.

This document reviews the toxicological studies for BAS 516 07 F, an WG formulation containing 67 g/kg pyraclostrobin and 667 g/kg boscalid. In addition, a full risk assessment is provided, which shows that BAS 516 07 F is safe for operators, workers, bystanders and residents (for details please see M-CP 7.2).

CP 7.1 Acute toxicity

The acute toxicity of BAS 516 07 F is assessed using the studies listed below in Table 7.1-1. Some of the studies have been conducted with BAS 516 00 F, a formulation that is very similar to BAS 516 07 F. Therefore, studies performed with BAS 516 00 F are considered to be also valid for BAS 516 07 F. Information on the detailed composition of BAS 516 00 F and BAS 516 07 F can be found in the confidential part of the dossier (Document J).

BAS 516 07 F is of low toxicity by the oral, dermal and inhalation route of exposure. It is non-irritant to the skin and the eye and does not cause skin sensitization.

Table 7.1-1: Summary of acute toxicity studies relevant for BAS 516 07 F

Type of study	Test substance	Result classification	Reference (BASF DocID)
Oral route - rat	BAS 516 07 F	LD_{50} > 2000 mg/kg bw EU classification: not required CLP classification: not required	CP 7.1.1/1 (2008/1004838)
Dermal route - rat	BAS 516 00 F	LD_{50} > 2000 mg/kg bw EU classification: not required CLP classification: not required	CP 7.1.2/1 (2001/1003723)
Inhalation route - rat	BAS 516 00 F	LC_{50} > 5.6 mg/L (both sexes) EU classification: not required CLP classification: not required	CP 7.1.3/1 (2001/1001824)
Skin irritation - rabbit	BAS 516 07 F	Non-irritating to rabbit skin EU classification: not required CLP classification: not required	CP 7.1.4/1 (2007/1056989)
Eye irritation - rabbit	BAS 516 07 F	Non-irritating to rabbit eye EU classification: not required CLP classification: not required	CP 7.1.5/1 (2007/1056988)
Skin sensitization - LLNA-Assay	BAS 516 07 F	Non-sensitizing EU classification: not required CLP classification: not required	CP 7.1.6/1 (2014/1001403)

CP 7.1.1 Oral toxicity

Report: CP 7.1.1/1
[redacted] 2008a
Acute oral toxicity in rats - Acute toxic class method
2008/1004838
Guidelines: EEC 2004/73 B.1 tris, OECD 423, EPA 870.1100, JMAFF No 12 Nosan No 8147
GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

Executive Summary

In an acute oral toxicity study two groups of each 3 fasted female Sprague Dawley rats were administered single doses of 2000 mg/kg bw of BAS 516 07 F. The test substance was administered undiluted at a volume of 10 mL/kg. The observation period was 14 days. All animals were subjected to necropsy.

Administration of 2000 mg/kg bw of BAS 516 07 F resulted in no unscheduled mortality during the study. Accordingly, the oral LD_{50} was higher than 2000 mg/kg bw.

oral LD_{50} > 2000 mg/kg bw

All animals showed piloerection and hypoactivity (3/6 animals) on day 1 after administration. At necropsy, no apparent abnormalities were observed in any animal.

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 516 07 F is not to be classified for acute oral toxicity.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 516 07 F

Description:	brown granules
Density:	not given
Lot/Batch #:	1789
Purity/content:	Pyraclostrobin (= BAS 500 F): 6.84% Boscalid (= BAS 510 F): 26.7%
Stability of test compound:	Stable: expiry date 21-Mar-2009

2. Vehicle and/or positive control: 0.5% methylcellulose in bidistilled water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley Rj:SD(IOPS Han)
Sex:	female
Age:	approx. 8 weeks
Weight at dosing (mean):	212 ± 15 g
Source:	Janvier, Le Genest-Saint-Isle, France
Acclimation period:	At least 5 days
Diet:	SsniffR/M-H pelleted diet (SSNIFF Spezialdiäten GmbH, Soest, Germany), ad libitum
Water:	drinking water filtered by a FG Millipore membrane (0.22 micron), ad libitum
Housing:	three animals in cages with stainless steel lid

Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	approx. 12 cycles/hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 17-Jan-2008 to 13-Feb-2008

2. Animal assignment and treatment:
Groups of 3 female rats received a single dose of 2000 mg/kg bw. After the first assay, as no mortality occurred, the results were confirmed in three other females at the dose level of 2000 mg/kg bw. BAS 516 07 F was administered by oral gavage under a volume of 10 mL/kg bw. Prior to administration the animals were deprived of food for approximately 18 hours, however had free access to water. Animals were observed for clinical signs and mortality several times on the day of administration, and at least once each workday for the remainder of the 14-day observation period. Body weights were recorded at day 0 (prior to dosing), and then on days 7 and 14. Necropsy with gross-pathology examination was performed on the last day of the observation period. The animals were sacrificed by CO_2-inhalation.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed after administration of 2000 mg/kg bw in two independent experiments (see Table 7.1.1-1).

Table 7.1.1-1: Mortality of rats administered BAS 516 07 F by the oral route

Sex	Dose [mg/kg bw]	No. of administration	Cumulative mortality	Time of deaths
Female	2000	1st	0/3	-
	2000	2nd	0/3	-

h = hour after administration, d1 = day after administration

B. CLINICAL OBSERVATIONS

Piloerection was observed in all animals until 2 hours after administration. In three of six animals hypoactivity was seen at least until six hours after administration. The type, incidence and duration of clinical signs are given in Table 7.1.1-2.

Table 7.1.1-2: Clinical signs in rats administered BAS 516 07 F by the oral route (incidence / duration)

Dose [mg/kg bw]	2000	2000
Total number of animals	**3**	**3**
Administration	**1st**	**2nd**
- Piloerection	3 / h0 – h2	3 / h0 – d1
- Hypoactivity		3 / h0 – d1

h = hour; - not observed

C. BODY WEIGHT

Compared to historical control animals, a slightly lower body weight gain was noted in 2/6 animals between day 1 and day 8, which returned to normal thereafter. The body weight gain of the other animals was not affected by the test item.

D. NECROPSY

Macroscopic examination of the main organs of the animals revealed no apparent abnormalities.

III. CONCLUSION

Under the conditions of this study the oral LD_{50} in rats for BAS 516 07 F was determined to be higher than 2000 mg/kg bw.

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 516 07 F is not to be classified for acute oral toxicity.

CP 7.1.2 Dermal toxicity

Report: CP 7.1.2/1
[redacted] 2001a
BAS 516 00 F - Acute dermal toxicity study in rats
2001/1003723
Guidelines: OECD 402, EEC 92/69 B 3, EPA 870.1200
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an acute dermal toxicity study groups of 5 male and 5 female Wistar rats were administered a single dose of 2000 mg/kg bw of BAS 516 00 F to the clipped skin under semi-occlusive conditions for 24 hours. The formulation was applied diluted in 0.5% aqueous carboxymethylcellulose (4.0 mL/kg bw). The animals were observed for 14 days after administration. Based on the absence of mortality in this study the acute dermal LD_{50} was determined to be

dermal LD_{50} > 2000 mg/kg bw

No clinical observations of systemic toxicity or local signs were made. At necropsy no deviations from normal morphology were observed. Due to the similar composition of BAS 516 00 F and BAS 516 07 F, the study performed with BAS 516 00 F is considered to be valid for BAS 516 07 F, too.

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 516 07 F is not to be classified for acute dermal toxicity.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 516 00 F

Description:	solid / brown
Lot/Batch:	2000-2
Purity:	nominal concentration: BAS 500 F (pyraclostrobin): 6.7% (w/w); BAS 510 F (boscalid): 26.7% (w/w); Analytical concentration: BAS 500 F (pyraclostrobin): 6.6% (w/w); BAS 510 F (boscalid): 27.9% (w/w)
Stability of test compound:	The homogeneity of the test substance was confirmed by analysis. The stability of the test substance in the vehicle was confirmed by analysis over a time period of 4 hours.

2. Vehicle and/or positive control: 0.5% carboxymethylcellulose in bi-distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar (Crl:WI (GLX/BRL/HAN)IGS BR)
Sex:	Male and female (nulliparous and non-pregnant)
Age:	Young adult animals, males about 8-12 weeks, females about 14-18 weeks
Weight at dosing:	Mean of dose groups: 247 g for males and 220 g for females
Source:	Charles River Laboratories, D-97633 Sulzfeld, FRG
Acclimation period:	At least one week
Diet:	Kliba-Labordiät Provimi Kliba SA, CH-4303 Kaiseraugst, Switzerland, ad libitum
Water:	Tap water ad libitum
Housing:	Stainless steel wire mesh cages, Type DK-III (Becker & Co., Castrop-Rauxel, FRG)

Environmental conditions

Temperature:	20 - 24°C
Humidity:	30 - 70%, relative
Air changes:	Fully air-conditioned rooms
Photo period:	12 h light / 12 h dark (6.00 a.m. – 6.00 p.m. / 6.00 p.m. – 6.00 a.m.)

B. STUDY DESIGN AND METHODS

1. Dates of work: 03-Aug-2000 (date of administration) to 17-Aug-2000 (date of necropsy)

2. Animal assignment and treatment:
Single administration of the test substance preparation in 0.5% carboxymethylcellulose in aqua bidest to five male and five female Wistar rats for 24 hours under semi-occlusive dressing at a dose level of 2000 mg/kg bw at a volume of 4 mL/kg bw. The application was performed to the clipped epidermis (dorsal and dorsolateral parts of the trunk) and covered by a semi-occlusive dressing for 24 hours. The application area was about 40 cm^2 (corresponding to at least 10% of the body surface area). The animals were observed for 14 days. Animals were checked for clinical signs of toxicity several times on the day of administration, and at least once each workday for the remainder of the study. A check for any dead or moribund animals was made twice each workday and once on Saturday, Sunday and on public holidays. Body weights were recorded at day 0 (prior to dosing), weekly thereafter and at the end of the study. Individual readings of skin findings were performed according to the Draize scheme 30-60 minutes after removal of the semi-occlusive dressing, weekly thereafter and at the end of the study. Necropsy with gross-pathology examination was performed on the last day of the observation period after killing by CO_2-inhalation.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred during the 14 days post application observation period.

B. CLINICAL OBSERVATIONS
No clinical signs of systemic toxicity and local effects were observed.

C. BODY WEIGHT
The mean body weights of all animals increased throughout the study period.

D. NECROPSY
No macroscopic pathological abnormalities were detected in the animals at the termination of the study.

III. CONCLUSION

The dermal LD_{50} of BAS 516 00 F was determined to be greater than 2000 mg/kg body weight for male and female animals. Due to the similar composition of BAS 516 00 F and BAS 516 07 F, the study performed with BAS 516 00 F is considered to be valid for BAS 516 07 F, too. Information on the detailed composition of BAS 516 00 F and BAS 516 07 F can be found in the confidential part of the dossier (Document J).

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 516 07 F is not to be classified for acute dermal toxicity.

CP 7.1.3 Inhalation toxicity

Report: CP 7.1.3/1
[redacted] 2001b
BAS 516 00 F - Acute inhalation toxicity study in Wistar rats 4-hour dust exposure
2001/1001824
Guidelines: OECD 403, EEC 93/21, EPA 870.1300, EEC 92/69 B 2
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an acute inhalation toxicity study, groups of 5 male and 5 female Wistar rats were exposed to dust aerosol of BAS 516 00 F at an actual concentration of 5.57 ± 0.16 mg/L for 4 hours. The animals were observed for 14 days after exposure. Based on the absence of mortality in this study the acute inhalation LC_{50} was determined to be

LC_{50} (both sexes combined) > 5.6 mg/L

Clinical signs of toxicity in animals exposed to the test substance comprised attempts to escape, visually accelerated respiration, squatting posture and smeared fur, but animals appeared normal from post exposure day 7 onwards. No gross pathological abnormalities were detected in the animals that were necropsied at termination of the study.
The mass mean aerodynamic diameters (MMAD) were 3.1 and 4.1 µm with geometric standard deviations (GSD) of 2.6. 37 to 49% of the particles had a diameter of ≤ 3.0 µm and thus may have reached the alveolar space of the lung.

Due to the similar composition of BAS 516 00 F and BAS 516 07 F, the study performed with BAS 516 00 F is considered to be valid for BAS 516 07 F, too.

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 516 07 F is not to be classified for acute inhalation toxicity.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 516 00 F

Description:	solid / brown
Lot/Batch:	2000-2
Purity:	Nominal concentration: BAS 500 F (pyraclostrobin): 6.7% (w/w); BAS 510 F (boscalid): 26.7% (w/w) Analytical concentration: BAS 500 F (pyraclostrobin): 6.6% (w/w); BAS 510 F (boscalid): 27.9% (w/w)
Stability of test compound:	The homogeneity of the test substance was confirmed by analysis.

2. Vehicle and/or positive control: Exposure as dust aerosol

3. Test animals:

Species:	Rat
Strain:	Wistar (Crl:WI (GLX/BRL/HAN)IGS BR)
Sex:	Male and female (nulliparous and non-pregnant)
Age:	Young adult animals, about 8-9 weeks old
Weight at day of exposure:	Mean of dose groups: 241 g for males and 165 g for females
Source:	Charles River Laboratories, D-97633 Sulzfeld, FRG
Acclimation period:	At least one week
Diet:	Kliba-Labordiät Provimi Kliba SA, CH-4303 Kaiseraugst, Switzerland, ad libitum
Water:	Tap water ad libitum
Housing:	Stainless steel wire mesh cages, Type DK-III (Becker & Co., Castrop-Rauxel, FRG)

Environmental conditions

Temperature:	20-24°C
Humidity:	30-70%, relative
Air changes:	Fully air-conditioned rooms
Photo period:	12 h light / 12 h dark (6.00 a.m. – 6.00 p.m. / 6.00 p.m. – 6.00 a.m.)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 13-Jun-2000 to 27-Jun-2000

2. Animal assignment and treatment:
Five male and five female Wistar rats were exposed to a dust aerosol of the test material for four hours in a head/nose inhalation system at a mean analytical concentration of 5.6 mg/L. The animals were randomly selected from a pool of animals.

3. Clinical examination:
Animals were observed for clinical signs of toxicity several times on the day of administration, and at least once each workday for the remainder of the study. A check for any dead or moribund animals was made twice each workday and once on Saturday, Sunday and on public holidays. Body weights were recorded at day 0 (prior to exposure), weekly thereafter and at the end of the study.

4. Pathology:
Necropsy with gross-pathology examination was performed on the last day of the observation period after killing by CO_2-inhalation.

5. Statistics/Calculations:
The statistical evaluation of the concentration-response relationship was carried out using a computer program. Depending on the data of the concentration-response relationship obtained by the experiment, this program is used to estimate the LC_{50} or to perform a Probit analysis[1].
The calculation of the particle size distribution was carried out in the inhalation laboratory on basis of mathematical methods for evaluating particle measurements[2].

[1] FINNEY, D.J. (1971): "Probit Analysis" Cambridge University Press, pp. 1 - 50

[2] DIN 661 41: Darstellung von Korngrößenverteilungen, DIN 661 61: Partikelgrößenanalyse (Beuth-Vertrieb GmbH, Berlin 30, FRG und Köln 1, FRG)

6. Generation of the test atmosphere / chamber description:
After des-agglomeration of the test substance the inhalation atmosphere was generated by means of a dosing wheel dust generator combined with a dust pre-chamber. From the pre-chamber the dust was passed into the inhalation system by means of compressed air.
The exposure system was located inside an exhaust cabin in an air-conditioned laboratory. A supply airflow (compressed air) of 1.5 m^3/h was used for the exposure. The exhaust airflow was set to 1.35 m^3/h. An air change of about 27 times per hour can be calculated by dividing the supply air flow by the volume of the inhalation system. The lower amount of exhaust air, which was adjusted by means of a separate exhaust air system, achieved a positive pressure inside the exposure system. This ensured that the mixture of test substance and air was not diluted with laboratory air in the breathing zones of the animals.
The animals were exposed to the inhalation atmosphere for 4 hours plus an equilibration time for the inhalation system of about 10 minutes.

7. Analytical investigation:

The flows of supply and exhaust air were adjusted and continuously measured with a flow meter. Air flows, the temperature and the humidity in the exposure system measured were at about 1-hour intervals.
The oxygen content in the inhalation system was not measured. The air change was judged to be sufficient to prevent oxygen depletion by the breathing of the animals, and the concentrations of the test substance used could not have a substantial influence on oxygen partial pressure.
The nominal concentration was calculated from the amount of substance dosed and the supply airflow.
The sampling for determination of the actual dust aerosol concentration was performed with following equipment and procedure:

- Vacuum pump (Millipore)
- Filtration equipment with probe, internal diameter: 7 mm (Millipore)
- Filter: MN 85/90BF (d = 4.7 cm)
- Sampling position: immediately adjacent to the animals' noses
- Sampling flow: 1 L/min
- Sampling velocity: 1.25 m/s
- Sampling frequency: 4 samples at about hourly intervals
- Sample volume: 2 L

Gravimetric determination of the inhalation atmosphere concentration was done with a balance (Mettler AT 250). Pre-weighed filters were placed into the filtration equipment. By means of the vacuum pump metered volumes of the dust were drawn through the filter. The dust concentration in mg/L was calculated from the difference between the pre-weighed of the filter and the weight of the filter after sampling, with reference to the sample volume of the inhalation atmosphere.
The particle size analysis was done using a Stack Sampler Mark III (Andersen) and a vacuum pump (Millipore). The sampling probe had an internal diameter of 6.9 mm.
Before sampling, the impactor was assembled with pre-weighed glass-fiber collecting discs, and a backup particle filter. The impactor was connected to the vacuum pump. Two samples were taken from the breathing zone of the animals starting not earlier than 30 minutes after the beginning of the exposure. The sample volume was 6 L. After sampling the impactor was taken apart. The collecting disc and the backup particle filter were re-weighed. Additionally, material absorbed on the walls of the impactor and the sampling probe (wall losses) were also determined quantitatively.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred at the tested concentration of 5.6 mg/L during the study period of 14 days. Therefore, the study satisfied the criteria of a limit test.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity consisted of attempts to escape, visually accelerated respiration, squatting posture and smeared fur. The animals appeared normal from post exposure day 7 onwards. The maximum incidence and duration of the observations are indicated in Table 7.1.3-1.

Table 7.1.3-1: Clinical signs in rats exposed for 4 hours to a liquid aerosol of BAS 516 00 F (incidence/duration)

Dose [mg/L]	5.6	5.6
Sex	**Males**	**Females**
- Total number of animals	5	5
- Respiration, visually accelerated	≤ 2h – d3	≤ 2h – d3
- Attempts to escape	≤ 1h	≤ 1h
- Squatting posture	d0 – d6	d0 – d6
- Fur, smeared	d0 – d3	d0 – d3

C. BODY WEIGHT

Body weight development in the male animals was slightly depressed in the first post exposure week, but recovered in the second. The female animals showed a slight reduction of body weight development throughout the post observation period.

D. NECROPSY

In the animals examined at the end of the post exposure observation period, no gross pathological abnormalities were detected.

E. ATMOSPHERE ANALYSIS

The exposure conditions are summarized in Table 7.1.3-2.

Table 7.1.3-2: Exposure conditions

Supply air [m³/h]	Exhaust air [m³/h]	Substance flow (g/h)	Temp. [°C]	Relative humidity [%]
1.5	1.35	511.44	22.9	5.5

The low relative humidity resulted from the need to use compressed air for dust generation. This deviation from the guideline recommendations (30 - 70% relative humidity; especially low humidity in dust) are considered not to be of influence for the test results, because of the relatively short exposure time.

The results of the concentration measurements (mean of 4 measurements) are presented in Table 7.1.3-3.

Table 7.1.3-3: Measurement of concentrations (4 hourly measurements)

Mean concentration [mg/L]	Standard deviation	Nominal concentration [mg/L]
5.6	0.16	341.0

The measurements of particle-size distribution revealed mass mean aerodynamic diameters (MMAD) of 3.1 to 4.1 µm with geometric standard deviation of 2.6 (see Table 7.1.3-4).

37 and 49% of the particles had a diameter of ≤ 3.0 µm and thus may have reached the alveolar space of the lung.

Table 7.1.3-4: Particle size measurements

Sample	% ≤ 3 µm	MMAD [µm]	Geometric standard deviation
1	49	3.1	2.6
2	37	4.1	2.6

III. CONCLUSION

The inhalation LC_{50} was determined to be > 5.6 mg/L (4 h) for male and female rats. Due to the similar composition of BAS 516 00 F and BAS 516 07 F, the study performed with BAS 516 00 F is considered to be valid for BAS 516 07 F, too. Information on the detailed composition of BAS 516 00 F and BAS 516 07 F can be found in the confidential part of the dossier (Document J).

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 516 07 F is not to be classified for acute inhalation toxicity.

CP 7.1.4 Skin irritation

Report: CP 7.1.4/1
[redacted]2007a
BAS 516 07 F: Acute dermal irritation / corrosion in rabbits
2007/1056989

Guidelines: OECD 404, EEC 2004/73 B.4, EPA 870.2500, JMAFF No 12 Nosan No 8147

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a primary dermal irritation study the skin irritation/corrosion potential of BAS 516 07 F was tested. An area of 6.25 cm² of clipped skin of 3 New Zealand White rabbits was exposed to 0.5 g of the test-substance for 4 hours under semi-occlusive conditions. The rabbits were observed for 7 days after removal of the dressings. Signs of skin irritation were scored using the Draize scheme.

Administration of BAS 516 07 F to the skin of rabbits caused up to grade 2 (slight to moderate) erythema and no edema. The cutaneous reaction was fully reversible in all animals within 7 days after removal of the patch. The overall 24 to 72 hour skin irritation scores were 1.1 for erythema and 0.0 for edema.

Based on the results of the study no classification as skin irritant is warranted for BAS 516 07 F according to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 516 07 F

Description:	solid
Lot/Batch #:	11041
Purity/content:	Pyraclostrobin (BAS 500 F): 6.5%; boscalid (BAS 510 F): 26.9%
Stability of test compound:	The stability was guaranteed for the duration of the study

2. Vehicle and/or positive control: none

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, A 1077 INRA (SPF)
Sex:	male and female
Age:	6 – 7 months
Weight at dosing (mean):	3.83 – 4.00 kg
Source:	Centre Logo S.A., 01540 Vonnas, France
Acclimation period:	At least 5 days
Diet:	Kliba Laboratory Diet, Provimi Kliba SA, Kaiseraugst, Basel, Switzerland (approx. 130 g/animal/day)
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages with grating, floor area 3000 cm²
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 23-Oct-2007 to 12-Nov-2007

2. In-vitro pre-test:
No in-vitro pre-test was performed.

3. Animal assignment and treatment:
The potential of BAS 516 07 F to cause acute dermal irritation or corrosion was assessed by a single topical application of 0.5 mg of minimally moistened test substance for 4 hours to the intact skin of two male and one female New Zealand White rabbits.

At least 24 hours before treatment, the dorsolateral part of the trunk of the animals was clipped. Only animals without obvious signs of skin irritation were used in the study. A dose of 0.5 mg minimally moistened with doubly distilled water was applied to a 2.5 x 2.5 cm test patch and applied to the flank for 4 hours. The gauze patch was held in place by means of an adhesive semi-occlusive dressing. After a 4 hour exposure period the patch was removed and the application area was washed with Lutrol® (PEG 400) and Lutrol®/water (1:1).
The cutaneous reactions were assessed immediately after removal of the patch, approximately 1, 24, 48 and 72 hours as well as 7 days after removal of the patch.
Body weights were measured shortly prior to application and after the last reading. The animals were checked for mortality, morbidity and clinical signs twice on working days and once on weekends and public holidays.

II. RESULTS

Administration of BAS 516 07 F to the skin of rabbits caused up to moderate erythema (grade 2). No edema were observed (see Table 7.1.4-1). The cutaneous reactions were reversible latest within 7 days after removal of the patch.

Table 7.1.4-1: Individual and mean skin irritation scores after 4 hour dermal application of BAS 516 07 F

Readings	Animal	Erythema	Edema	Additional findings
0 hour	01 (♂)	2	0	
	02 (♀)	2	0	
	03 (♂)	2	0	
1 hour	01	2	0	
	02	2	0	
	03	2	0	
24 hours	01	1	0	
	02	2	0	
	03	2	0	
48 hours	01	1	0	
	02	1	0	
	03	1	0	
72 hours	01	0	0	SD
	02	1	0	
	03	1	0	
7 days	02	0	0	
	03	0	0	
Individual means 24 – 72 hours	01	0.7	0.0	
	02	1.3	0.0	
	03	1.3	0.0	
Group mean 24 - 72 hours		1.1	0.0	

SD : Study discontinued because the animal was free of findings

III. CONCLUSION

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 516 07 F is not to be classified for skin irritation.

CP 7.1.5 Eye irritation

Report: CP 7.1.5/1
[redacted] 2007b
BAS 516 07 F: Acute eye irritation in rabbits
2007/1056988
Guidelines: OECD 405, EEC 2004/73 B.5, EPA 870.2400, JMAFF No 12 Nosan No 8147
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a primary eye irritation study, the eye irritation/corrosion potential of BAS 516 07 F was determined by instillation of 0.1 mL (about 47 mg) of the test substance into the conjunctival sac of the right eye of three rabbits (stepwise procedure starting with one animal and supplementing two additional animals). The left eye, which remained untreated, served as a control. The eyes were rinsed 24 hours after administration of the test substance.
The ocular reactions were assessed approximately 1, 24, 48 and 72 hours after the administration of the test substance.
Signs of ocular irritation consisted of up to grade 2 conjunctival redness and up to grade 2 chemosis with slight to moderate discharge. No effects on the cornea or iris were observed. All signs of ocular irritation were reversible within 72 hours. The mean scores calculated for all animals over 24, 48 and 72 hours were 0.0 for corneal opacity and for iris lesions, 0.9 for redness of the conjunctiva and 0.2 for chemosis.

Based on the results of the study no classification as eye irritant is warranted for BAS 516 07 F according to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 516 07 F

Description:	solid
Lot/Batch #:	11041
Purity/content:	Pyraclostrobin (BAS 500 F): 6.5%; Boscalid (BAS 510 F): 26.9%
Stability of test compound:	The stability was guaranteed for the duration of the study

2. Vehicle and/or positive control: none

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, A 1077 INRA (SPF)
Sex:	male and female
Age:	6 – 7 months
Weight at dosing (mean):	3.08 – 3.38 kg
Source:	Centre Logo S.A., 01540 Vonnas, France
Acclimation period:	At least 5 days
Diet:	Kliba Laboratory Diet, Provimi Kliba SA, Kaiseraugst, Basel, Switzerland (approx. 130 g/animal/day)
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages with grating, floor area 3000 cm²
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 24-Sep-2007 to 12-Nov-2007

2. In-vitro pre-test:
No in-vitro pre-test was performed.

3. Animal assignment and treatment:
The potential of BAS 516 07 F to cause acute eye irritation was assessed by instillation of 0.1 mL bulk volume (about 47 mg of the milled test substance) into the conjunctival sac of the right eye. The left eye, which remained untreated, served as a control. The eyes were rinsed with tap water 24 hours after administration of the test substance.
The ocular reactions were assessed approximately 1, 24, 48 and 72 hours after the administration of the test substance.
Body weights were measured shortly prior to application and after the last reading. The animals were checked for mortality, morbidity and clinical signs twice on working days and once on weekends and public holidays.

II. RESULTS AND DISCUSSION

Moderate conjunctival redness (grade 2) was noted in all animals 1 hour after application and persisted in two animals until the 24-hour reading. Slight conjunctival redness (grade 1) was observed in one animal 24 hours after application and in all animals at the 48-hour reading. Slight or moderate conjunctival chemosis (grade 1 or 2) was noted in all animals 1 hour after application. Slight conjunctival chemosis persisted in two animals until 24 hours. Slight or moderate discharge (grade 1 or 2) was noted in all animals only 1 hour after application. In addition, injected scleral vessels in a circumscribed area were noted during the observation period. The ocular reactions were reversible in all animals within 72 hours after application.
Mean scores calculated for each animal over 24, 48 and 72 hours were 0.0 for corneal opacity and for iris lesions, 0.7, 1.0 and 1.0 for redness of the conjunctiva and 0.0, 0.3 and 0.3 for chemosis, respectively (see Table 7.1.5-1).

Table 7.1.5-1: Individual and mean eye irritation scores after ocular application of BAS 516 07 F

Readings	Animal	Cornea		Iris	Conjunctiva			Additional findings
		Opacity	Area involved		Redness	Chemosis	Discharge	
1 h	01 (♂)	0	0	0	2	1	2	48
	02 (♀)	0	0	0	2	2	1	48
	03 (♂)	0	0	0	2	1	2	48
24 h	01	0	0	0	1	0	0	48
	02	0	0	0	2	1	0	48
	03	0	0	0	2	1	0	48
48 h	01	0	0	0	1	0	0	48
	02	0	0	0	1	0	0	48
	03	0	0	0	1	0	0	48
72 h	01	0	0	0	0	0	0	
	02	0	0	0	0	0	0	
	06	0		0	0	0	0	
Individual means 24 - 72 h	01	0.0		0.0	0.7	0.0		
	02	0.0		0.0	1.0	0.3		
	03	0.0		0.0	1.0	0.3		
Overall mean 24 - 72 h	all	0.0		0.0	0.9	0.2		

48 = scleral vessels injected, circumscribed area

III. CONCLUSION

According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 516 07 F is not to be classified for eye irritation.

CP 7.1.6 Skin sensitization

Report:	CP 7.1.6/1 [redacted] 2014a BAS 516 07 F - Skin sensitisation: Local lymph node assay 2014/1001403
Guidelines:	OECD 429 (2010), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.42
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

For the determination of potential sensitizing properties of the formulation BAS 516 07 F the Murine Local Lymph Node Assay (LLNA) was conducted.
Groups of 5 female mice were treated with three different concentrations of the test substance (5, 10 and 25% (w/w) in DMSO) or with the vehicle alone for three consecutive days.
A skin sensitizing effect for BAS 516 07 F is not considered since Stimulation Indices (S.I.) of 1.86, 2.55 and 2.68 were determined with BAS 516 07 F at concentrations of 5, 10 and 25% (w/w) in DMSO, respectively. A slight dose response was observed, but an EC3 could not be calculated.
A statistically significant increase in lymph node weight and – cell count was obtained in all dose groups in comparison to the vehicle control group. The cut-off value for a positive response regarding the lymph node cell count index (1.55) was exceeded in the mid and high dose groups. However, this was not considered as biological relevant as the S.I.s determined for all concentrations did not exceed the threshold value of 3.
Erythema score 1-2 was observed in all test substance groups. A statistically significant increase in ear weights was observed in all treated groups compared to the vehicle control. The cut-off value of 1.1 for the ear weight index for a positive response regarding ear skin irritation was reached in all animals treated with the test item. The threshold value of 25% increase in ear weights for excessive local skin irritation mentioned in OECD guideline 429 was exceeded in the low and high dose group. The increased ear weights were attributed to irritant properties of the test item.
No signs of systemic toxicity and no mortalities were observed.

Based on the results of this study it is concluded that BAS 516 07 F does not have sensitizing properties in the Murine Local Lymph Node Assay under the test conditions chosen. According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 516 07 F is not to be classified as skin sensitizer.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 516 07 F

Description:	Brown solid
Lot/Batch #:	12-000110
Purity/content:	Pyraclostrobin, BAS 500 F: 6.7% Boscalid, BAS 510 F: 27.1%
Stability of test compound:	Stable: Expiry date 01.03.2015

2. Vehicle/ positive control: DMSO

3. Test animals:

Species:	Mouse
Strain:	CBA/ CaOlaHsd
Sex:	Female
Age:	8 to 10 weeks
Weight at dosing:	20.4 g ± 0.8 g
Source:	Harlan Laboratories B.V., Postbus 6174, 5960 AD Horst / The Netherlands
Acclimation period:	at least 5 days
Diet:	2018C Teklad Global 18% protein rodent diet (certified), ad libitum
Water:	tap water, ad libitum
Housing:	group housing in Makrolon Type II (pre-test) / III (main study), with wire mesh top
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	45 - 65%
Air changes:	not indicated
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm.

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 15-Jan-2014 to 18-Feb-2014

2. Animal assignment and treatment:
The skin sensitizing potential of BAS 516 07 F was assessed using the Murine Local Lymph Node Assay. To determine the highest non-irritant test concentration that at the same time did not induce signs of systemic toxicity, a pre-test was performed in two animals with test item concentrations of 10 and 25% (highest concentration, which could be technically achieved) applied once daily each on three consecutive days. At the tested concentrations the animals did not show any signs of systemic toxicity. The animal treated with 10 and 25% test item concentration showed erythema of the ear skin (score 1 on days 1 and 6, score 2 on day 5 after application). Increase in ear weights was 11.1 and 15.3%, respectively, compared to historical vehicle data. Both animals showed substance residues during the observation period, thus the increase in ear weights was partially attributed to the residues.

Thus, the test item in the main study was assayed at 5, 10 and 25% and female mice were randomly allocated to groups of 5 animals.
The groups were treated either with

- the vehicle (DMSO),
- a 5% (w/w) dilution in DMSO,
- a 10% (w/w) dilution in DMSO, or
- a 25% (w/w) dilution in DMSO.

The application solutions were prepared individually and shortly before treatment.

3. Analysis of treatment solutions:
A concentration control and homogeneity analysis of all three doses was performed as a separate study that confirmed that test substance preparation concentrations were adequate and homogenous.

4. Clinical observation:
Mortality was checked at least once daily from experimental start to necropsy. Clinical signs (local irritation at the application site or systemic toxicity) were recorded at least once daily. Especially the treatment sites were observed carefully.

5. Body weights:
Body weights were determined prior to the first application and prior to treatment with radioactive ^{3}H-methyl thymidine (^{3}HTdR).

6. Treatment of animals:
Each test group was treated with the item preparations by topical application to the dorsal surface of each ear at a volume of 25 µL per ear for three consecutive days. A further group of mice (control animals) was treated with an equivalent volume of the relevant vehicle alone. Five days after the first topical application, 20.2 µCi of ^{3}H-methyl thymidine in 250 µL of phosphate-buffered saline were injected into the tail vein of each mouse.

7. Terminal procedures:

Approximately 5 hours after ^{3}H-thymidine injection the animals were euthanised by using CO_2, which was, after harvesting of the lymph nodes, followed by cervical dislocation to ensure death. The draining lymph nodes were rapidly excised and pooled per animal (2 nodes per animal). Single cell suspensions in phosphate buffered saline of pooled lymph node cells were prepared by gentle mechanical disaggregation through stainless steel gauze (200 µm mesh size). After washing two times with phosphate buffered saline the lymph node cells were resuspended in 5% trichloroacetic acid and incubated at approximately +4°C for at least 18 hours for precipitation of macromolecules. The precipitates were then resuspended in 5% trichloroacetic acid and transferred to scintillation vials. The level of ^{3}HTdR incorporation was then measured in a β-scintillation counter. The β-scintillation counter expresses ^{3}HTdR incorporation as the number of radioactive disintegrations per minute.
After excision, the lymph nodes were pooled per animal and weighed immediately. Furthermore, the lymph node cell count was determined for each animal. For this, the volume of the cell suspensions was adjusted to an equal final volume and vortexed. Subsequently, individual cell counts were determined using a cell counter. After the lymph nodes had been excised, both ears of mice were punched at the apical area using a biopsy punch (Ø 8 mm corresponding to 0.5 cm^2) and immediately weighed (pooled per animal).

8. Calculations:

The stimulation indices of cell count, ^{3}H-thymidine incorporation, lymph node weight, and ear weight were calculated as the ratio of the test group values for these parameters divided by those of the vehicle control group.

9. Data evaluation and interpretation:

The lymph node cell count and the ^{3}H-thymidine incorporation into the lymph node cells as well as to a certain extent lymph node weight are used to determine the potential sensitizing properties of a test article. Because not only sensitization but also irritation of the ear skin by the test substance may induce lymph node responses, the weight of ear punches taken from the area of test-substance application is determined as a parameter for inflammatory ear swelling as an indicator for the irritant action of the test substance.

Stimulation indices of >1.55 for cell count and/or of ≥ 3 for ^{3}H-thymidine incorporation compared to the concurrent vehicle control group are generally considered as indicative for a sensitizing potential of a test substance. If applicable, the EC (estimated concentration) leading to the respective SI values were calculated by linear or semi-logarithmical regression.

If the increase in cell count, ^{3}H-thymidine incorporation and/or lymph node weight is accompanied by a biologically relevant increase in ear weights (cut-off: 1.1) it cannot be ruled out that the lymph node response was caused by irritation and not by skin sensitization. Depending on the magnitude of lymph node response, based on expert judgment, the evaluation of the sensitizing potential may be modified or additional studies might be necessary.

10. Positive controls:
A concurrent positive control (reliability check) with a known sensitizer was not included into this study. Studies using the positive control substance alpha-hexylcinnamaldehyde are regularly performed in the laboratory in order to show that the test system is able to detect sensitizing compounds under the test conditions chosen.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

No deaths and no signs of systemic toxicity occurred during the study period.

B. BODY WEIGHTS

The expected body weight gain was generally observed in the course of the study.

C. STIMULATION INDICES

In this study, Stimulation Indices (S.I.) of 1.86, 2.55 and 2.68 were determined with test item concentrations of 5, 10 and 25%, respectively. A slight dose response was observed but an EC3 value could not be calculated since all S.I.'s were below the threshold value of 3. A statistically significant increase in DPM values was not observed in any of the dose groups in comparison to the vehicle group.

A statistically significant increase in lymph node weights and –cell count was obtained in all dose groups in comparison to the vehicle control group. Moreover, the cut-off value for a positive response regarding the lymph node cell count index (1.55) was exceeded in the mid and high dose groups (Table 7.1.6-1). This was considered as not biologically relevant as the S.I.s determined for all concentrations did not exceed the threshold value of 3.

Animals treated with 5% of the test item showed a very slight erythema of the ear skin (score: 1 on days 2-4). Animals treated with 10% of the test item showed an erythema of the ear skin as well (score: 1 on days 2-5). Animals treated with the high dose (25%) of the test item showed an erythema of the ear skin (score: 2 on days 2 and 3, score: 1 on days 4 and 5).

A statistically significant increase in ear weights was observed in all treated groups in comparison to the vehicle control group. The cut-off value (1.1) of the ear weight index for a positive response regarding ear skin irritation was exceeded in all test item treated groups. The threshold value of 25% increase in ear weights for excessive local skin irritation mentioned in OECD guideline 429 was exceeded in the low and high dose group, thus indicating the irritant properties of the test item.

Based on the above-mentioned findings regarding ear skin irritation, an influence of irritation on lymphocyte proliferation cannot be excluded. Details of the test group mean values and stimulation indices are summarized in Table 7.1.6-1.

Table 7.1.6-1: Stimulation indices for cell counts, ^{3}H-thymidine incorporation, lymph node, and ear weight in mice after treatment with BAS 516 07 F

Test Group	Treatment	Parameter evaluated	Stimulation index (S.I.)[1]
		Cell count [counts x10^6/lymph node pair]	
1	vehicle (DMSO)	13.3 ± 2.9	1.00
2	5% (w/w) in DMSO	19.1 ± 2.0*	1.44
3	10% (w/w) in DMSO	23.3 ± 4.0*	1.76
4	25% (w/w) in DMSO	26.4 ± 1.8*	1.99
		^{3}H-Thymidine incorporation [DPM/lymph node pair]	
1	vehicle (DMSO)	1853.1 ± 743.4	1.00
2	5% (w/w) in DMSO	3455.3 ± 602.3	1.86
3	10% (w/w) in DMSO	4724.3 ± 2453.9	2.55
4	25% (w/w) in DMSO	4966.9 ± 2532.6	2.68
		Lymph node weight [mg/lymph node pair]	
1	vehicle (DMSO)	6.7 ± 1.5	1.00
2	5% (w/w) in DMSO	8.4 ± 1.1*	1.26
3	10% (w/w) in DMSO	10.2 ± 0.9*	1.52
4	25% (w/w) in DMSO	10.6 ± 1.1*	1.58
		Ear weight [mg/animal]	
1	vehicle (DMSO)	27.5 ± 1.7	1.00
2	5% (w/w) in DMSO	34.4 ± 4.8*	1.25
3	10% (w/w) in DMSO	32.1 ± 2.0*	1.17
4	25% (w/w) in DMSO	37.3 ± 1.7*	1.35

[1] test group x / test group 1 (vehicle control)

* Mean cell count, lymph node weight and ear weight for the group was according to the ANOVA (Dunnett-test) significantly higher than the corresponding control value. The p value for the analysis was $p < 0.05$.

D. POSITIVE CONTROL

The sensitivity of mice and the reliability of experimental techniques are assessed regularly in the test laboratory using a known sensitizer. Positive results were consistently obtained over the years. Results of the positive control studies are presented in Table 7.1.6-2.

Table 7.1.6-2: Positive control LLNA studies performed

Positive control Substance	Date	Concentration / Vehicle	S.I. values
Alpha-hexylcinnamaldehyde	October 2013	25% in acetone:olive oil (4+1, v/v)	5.8
	April 2013		5.9
	October 2012		5.7
	April 2012		3.7
	February 2012		4.7
	January 2012		10.8
	January 2012		7.1
	December 2011		5.9
	November 2011		5.3
	October 2011		6.1

III. CONCLUSION

Based on the results of this study it is concluded that BAS 516 07 F does not have sensitizing properties in the Murine Local Lymph Node Assay under the test conditions chosen. According to the 28th ATP of Council Directive 67/548/EEC (Commission Directive 2001/59/EC) and Regulation (EC) No 1272/2008 (CLP), BAS 516 07 F is not to be classified as skin sensitizer.

CP 7.1.7 Supplementary studies on the plant protection product

No supplementary studies on the plant protection product were deemed necessary and thus none were conducted. However, a publication of Cayir et al. 2012 on the genotoxicity of pyraclostrobin, boscalid and **BAS 516 07 F** has been evaluated and is discussed in M-CA 5.4.

CP 7.1.8 Supplementary studies for combinations of plant protection product

No studies for combinations of BAS 516 07 F with other plant protection products product were deemed necessary and thus none were conducted.

CP 7.2 Data on exposure

Exposure assessments and risk evaluations for operators, workers, bystanders and residents are presented below for the new representative formulation BAS 516 07 F, which has not been evaluated during a previous Annex I inclusion process.

CP 7.2.1 Operator exposure

The plant protection product BAS 516 07 F is already registered for the use as fungicide in the representative crop potatoes. Information on the critical use pattern relevant for operator exposure is summarized in Table 7.2.1-1.
The formulation is a water dispersable granule (WG) containing 67 g/kg pyraclostrobin and 267 g/kg boscalid. It is mainly commercialized in 1, 2.5 and 5 kg containers.

Table 7.2.1-1: Summary of critical use pattern

Crop (indoor / field)	**Application rate** (g as/ha)		**Minimum spray dilution** (L/ha)	**Application equipment**	**Number of applications**
Potatoes (field)	Pyraclostrobin	17	200	Tractor mounted boom sprayers	4
	Boscalid	67			

The critical use pattern has been defined based on the already registered uses in potatoes (for details see M-CP 3 Table 2). The GAP registered in DE was used as basis for the risk assessment.

Estimations of potential operator exposure have been undertaken for BAS 516 07 F using the intended use shown above and the following predictive models:

- Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection), Mitteilungen aus der Biologischen Bundesanstalt, Heft 277, Berlin 1992 ("German model").

- Revised UK POE Model, as available on http://www.pesticides.gov.uk/uploadedfiles/Web_Assets/PSD/UK_POEM1.xls [UK Predictive Operator Exposure Model (POEM): Estimation of Exposure and Absorption of Pesticides by Spray Operators, Scientific subcommittee on Pesticides and British Agrochemical association Joint Medical Panel Report (UK MAFF), 1986 and the Predictive Operator Exposure Model (POEM) V 7 of 2008, (UK MAFF), 1992 ("UK Model").

Risk assessment for operator

The toxicological reference values (Acceptable Operator Exposure Level) for pyraclostrobin and boscalid as well as dermal absorption values appropriate for BAS 516 07 F, which were used in the operator risk assessment are shown in Table 7.2.1-2.

Table 7.2.1-2: Endpoints for pyraclostrobin and boscalid used in operator risk assessment

Endpoint	Value	Reference
Pyraclostrobin		
Dermal penetration - Concentrate - Spray dilutions	0.2% 5%	BASF DocID 2014/1001402 (for details see M-CP 7.3 of this dossier)
AOELsystemic	0.015 mg/kg bw/day	EU Review Report SANCO/1420/2001-Final, 8. September 2004
Boscalid		
Dermal penetration - Concentrate - Spray dilutions	0.5% 4%	BASF DocID 2014/1001401 (for details see M-CP 7.3 of this dossier)
AOELsystemic	0.1 mg/kg bw/day	EU Review Report SANCO/3919/2007-rev.5, 21. January 2008

The estimated operator exposure to pyraclostrobin and boscalid for the use of BAS 516 07 F in potatoes without using PPE is shown in Table 7.2.1-3 below. The values reflecting this use including PPE can be found in chapter 7.2.1.1.

Table 7.2.1-3: Estimated operator exposure to pyraclostrobin and boscalid in BAS 516 07 F

Model data	Level of PPE	Active substance	Total absorbed dose (mg/kg bw/day)[1]	% of AOEL[2]	Reference in Appendix
outdoor tractor operated boom sprayer application with hydraulic nozzles to field crops 0.25 kg BAS 516 07 F/ha corresponding to 17 g pyraclostrobin and 67 g boscalid per ha					
German Model - 20 ha/day - 70 kg operator	None	Pyraclostrobin	0.0006	3.7	**7.2-1**
		Boscalid	0. 002	1.9	**7.2-2**
UK POEM - 30 ha/day - 60 kg operator		Pyraclostrobin	0.003	23	**7.2-3**
		Boscalid	0. 0117	12	**7.2-4**

[1] systemic exposure based on dermal absorption: 0.2% for mixing/loading and 5% for application for pyraclostrobin as well as 0.5% for mixing/loading and 4% for application for boscalid
[2] based on a systemic AOEL of 0.015 and of 0.1 mg/kg bw/day for pyraclostrobin and boscalid, respectively

Results and discussion
Following the operator exposure and risk evaluation based on models a safe use could be shown for both the BBA model and UK POEM estimates.

In conclusion operators are considered to be at acceptable risk when exposed to pyraclostrobin and boscalid under the use conditions of BAS 516 07 F in potatoes.

CP 7.2.1.1 Estimation of operator exposure

BAS 516 07 F is applied in field crops (potatoes), which is professional use only. The relevant application scenario is outdoor tractor operated boom sprayer with hydraulic nozzles. Risk assessments are presented based on the BBA model and the UK POEM with consideration of the following input parameters.

Table 7.2.1.1-1: German model input parameters for tractor mounted boom sprayer application in potatoes

Application method:	tractor-mounted boom sprayers with hydraulic nozzles, field crop	
Treated area:	20 ha/day	
Max. dose rate:		0.25 kg BAS 516 07 F/ha
	corresponding to:	0.017 kg pyraclostrobin/ha 0.067 kg boscalid/ha
Operator body weight:	70 kg	

Table 7.2.1.1-2: UK POEM input parameters for tractor mounted boom sprayer application in potatoes

Application method:	Tractor-mounted/trailed boom sprayer: hydraulic nozzles	
Treated area:	30 ha/day*	
Max. dose rate:		0.25 kg BAS 516 07 F/ha
	corresponding to:	0.017 kg pyraclostrobin/ha 0.067 kg boscalid/ha
Spray volume:	200 L/ha	
Duration:	6 h	
Operator body weight:	60 kg	
*Default value for row crops		

Estimation of operator exposure without personal protective equipment

Exposure predictions for operators using no protective equipment (PPE) are summarized Table 7.2.1.1-3.

Table 7.2.1.1-3: Estimated operator exposure to pyraclostrobin and boscalid in BAS 516 07 F without using PPE

Model data	Level of PPE	Active substance	Total absorbed dose (mg/kg bw/day)[1]	% of AOEL[2]	Reference in Appendix
outdoor tractor operated boom sprayer application with hydraulic nozzles to field crops 0.25 kg BAS 516 07 F/ha corresponding to 17 g pyraclostrobin per ha and 67 g boscalid/ha					
German Model - 20 ha/day - 70 kg operator	None	Pyraclostrobin	0.0006	3.7	**7.2-1**
		Boscalid	0. 002	1.9	**7.2-2**
UK POEM - 30 ha/day - 60 kg operator		Pyraclostrobin	0.003	23	**7.2-3**
		Boscalid	0. 0117	12	**7.2-4**

[1] Systemic exposure based on dermal absorption: 0.2% for mixing/loading and 5% for application for pyraclostrobin as well as 0.5% for mixing/loading and 4% for application for boscalid

[2] based on a systemic AOEL of 0.015 and of 0.1 mg/kg bw/day for pyraclostrobin and boscalid, respectively

A safe use could be shown for operators without personal protective equipment with any of the models chosen. Detailed information is presented in appendix 7.2-1 to 7.2-4.

Estimation of operator exposure <u>with personal protective equipment</u>

Although a safe use was already shown for operators without personal protective equipment, exposure predictions for operators using protective equipment are summarized Table 7.2.1.1-4 for completeness.

Table 7.2.1.1-4: Estimated operator exposure to pyraclostrobin and boscalid in BAS 516 07 F with using PPE

Model data	Level of PPE	Active substance	Total absorbed dose (mg/kg bw/day)[1]	% of AOEL[2]	Reference in Appendix
outdoor tractor operated boom sprayer application with hydraulic nozzles to field crops 0.25 kg BAS 516 07 F/ha corresponding to 17 g pyraclostrobin per ha and 67 g boscalid/ha					
German Model - 20 ha/day - 70 kg operator	Gloves during mixing/loading and gloves, coverall and sturdy footwear during application	Pyraclostrobin	0.00001	0.5	**7.2-5**
		Boscalid	0.00028	0.3	**7.2-6**
UK POEM - 30 ha/day - 60 kg operator	Gloves during mixing/loading and gloves during application	Pyraclostrobin	0.001	6	**7.2-7**
		Boscalid	0. 003	3	**7.2-8**

[1] Systemic exposure based on dermal absorption: 0.2% for mixing/loading and 5% for application for pyraclostrobin as well as 0.5% for mixing/loading and 4% for application for boscalid

[2] based on a systemic AOEL of 0.015 and of 0.1 mg/kg bw/day for pyraclostrobin and boscalid, respectively

A safe use could be shown for operators with personal protective equipment. Detailed information is presented in appendix 7.2-5 to 7.2-8.

CP 7.2.1.2 Measurement of operator exposure

Since the risk assessments performed indicate that the health-based limit value (AOEL) will not be exceeded under practical conditions of use for pyraclostrobin and boscalid, further refinement of exposure predictions is not necessary and has thus not been performed.

CP 7.2.2 Bystander and resident exposure

The plant protection product BAS 516 07 F is already registered for the use as fungicide in the representative crop potatoes. Information on the formulation and the critical use pattern relevant for the bystander and resident risk assessment can be found in chapter 7.2.1. The critical GAP is summarized in Table 7.2.1-1.

Exposure assessments and risk evaluations for bystanders and residents for the representative formulation BAS 516 07 F are presented below. According to the EU requirements they have been based on the following model:

- Martin S. et al. (2008): Guidance for Exposure and Risk Evaluation for Bystanders and Residents exposed to Plant Protection Products during and after Application. J. Verbr. Lebensm., drift distances adopted according to the recommendation published by the German BVL: B. Nolting (2012) Bekanntmachung ueber Mindestabstaende, die bei der Anwendung von Pflanzenschutzmitteln zum Schutz von Umstehenden und Anwohnern einzuhalten sind. Bundesanzeiger 2012 No. 4 Pp. 75-76 for the bystander and resident.

Risk assessment for bystander and resident

A summary of the bystander risk assessment is provided in Table 7.2.2-1.

Table 7.2.2-1: Summary of bystander exposure during application of BAS 516 07 F in potatoes

	Active Substance			
	Pyraclostrobin		Boscalid	
	AOEL = 0.015 mg/kg bw/day		AOEL = 0.1 mg/kg bw/day	
	Adults	Children	Adults	Children
Total systemic exposure (mg/kg bw/day)	0.00004	0.000031	0.0001243	0.000101
% of AOEL	0.26	0.21	0.12	0.10

A summary of the risk assessment for residents is provided in Table 7.2.2-2.

Table 7.2.2-2: Summary of residential exposure during application of BAS 516 07 F in potatoes

	Active Substance			
	Pyraclostrobin		Boscalid	
	AOEL = 0.015 mg/kg bw/day		AOEL = 0.1 mg/kg bw/day	
	Adults	Children	Adults	Children
Total systemic exposure (mg/kg bw/day)	0.000005	0.000013	0.000016	0.000043
% of AOEL	**0.033**	**0.09**	**0.02**	**0.04**

Conclusion
It is concluded that there is no unacceptable risk to bystanders or residents after accidental exposure to BAS 516 07 F.

CP 7.2.2.1 Estimation of bystander and resident exposure

Bystanders and residents are not involved in application or handling of plant protection products or the professional handling of treated crops. Therefore, exposure differs significantly from operator exposure levels. The exposure assessment presented below is based on the German guidance paper for evaluation of bystander and resident exposure (Martin S. et al., 2008) with adopted drift distances (B. Nolting, 2012).

A. Bystander exposure

The presence of bystanders is incidental within or directly adjacent to an area where plant protection products are applied. A situation in which bystander exposure could occur would be a person walking alongside an area being treated at the same time. Under these conditions the bystander would never walk directly next to the outer spraying nozzle. A distance of some meters from the spraying device can always be expected. It can further be assumed that any bystander, as soon as becoming aware of an exposure will leave the spraying area. Therefore, bystander exposure is of short duration, typically a matter of minutes. Thus, the exposure duration of 5 minutes is assumed.

Bystander exposure results from spray drift that deposits on the body surface or passes the breathing zone. Assuming that bystanders wear only light clothing (i.e. short-sleeved shirt and shorts), the exposed, uncovered body surface of an adult (head, face, neck front and back, forearms, half upper arms, hands, lower half of thighs, lower legs and feet) is amounting to about 1 m². For children the exposed body surface with the same level of clothing amounts to 0.21 m².

For the scenario of professional agricultural use it is assumed that the bystander is located at a distance of 1 m, downwind from the spraying source. The extent of spray drift and the consequent deposition depends on the plant protection product application rate, the particular crop being treated and the method of application. Measurements of spray drift following different crop/equipment combinations are available from Rautmann D. et al., 2001: New basic drift values in the authorisation procedure for plant protection products. In: Forster, B. and Streloke, M. (eds.), Workshop on Risk Assessment and Risk Mitigation Measures (WORMM). 27–29 September 1999, Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, booklet 383, 2001; actual version of 27th March 2006: Rautmann, D., 2006: Aktuelle Abdrifteckwerte (Current Drift Values). http://www.jki.bund.de/cln_045/nn_926124/SharedDocs/10_FA/Publikationen/Pflanzenschutzgeraete/abdrifteckwerte_xls.html.
Thus, for the corresponding application scenario in cereals the proposed drift value at 1 m distance of 2.77% (90th percentile value) is used for estimations of dermal exposure. However, the drift deposition data by Rautmann D. et al., 2001 cannot easily be transposed into airborne concentrations and consequent inhalation exposure values. Therefore, to ensure a conservative evaluation, measured inhalation exposure data for the unprotected operator during spray applications (Lundehn J.R. et al., 1992, German BBA model) are used for the bystander inhalation exposure estimation.

The parameters used for bystander exposure calculations are summarized in Table 7.2.2.1-1.

Table 7.2.2.1-1: Parameters used for bystander exposure calculations

	Parameter	Pyraclostrobin	Boscalid
AR	Maximum application rate (kg a.s./ha)	0.017	0.067
ar	Maximum application rate (mg a.s./m²)	1.7	6.7
DA	Dermal absorption (%)	5	4
IA	Inhalation absorption (%)	100	
		Default values	
D	Drift at 1 meter distance for field crop (% of application rate)	2.77	
TB	Exposure duration bystander (minutes)	5	
TO	Exposure duration operator (hours)	6	
TF	Exposure duration factor (TB / TO) [1]	0.0139	
A	Area treated (ha/day)	50	
		Adults	Children
BSA	Exposed body surface (m²)	1	0.21
IA	Specific inhalation exposure (mg a.s./kg a.s. handled) [2]	0.001	0.00057
BW	Bystander body weight (kg)	60	16.15

[1] Since the German model values are based on an application period of 6 hours/day, adjustment to 5 minutes is required for exposure calculations.
[2] based on geometric mean values proposed by the German BBA Model (Lundehn et al., 1992) and inhalation rates of 1.74 and 1.0 m³/h for adults and children, respectively

Bystander exposure for adults and children is estimated according the following equations:

Systemic dermal exposure $$SDE_B = \frac{ar \times D \times BSA \times DA}{BW}$$

Systemic inhalation exposure $$SIE_B = \frac{I_A \times AR \times A \times TF \times IA}{BW}$$

Total systemic exposure $$SE_B = SDE_B + SI_B$$

Assessment

For the exposure of a bystander passing by the field treated with BAS 516 07 F the systemic exposure to pyraclostrobin and boscalid was assessed based on 5 minutes exposure applying generic spray drift deposits of 2.77% of the application rate at 1 meter distance and air borne spray concentrations of 0.001 and 0.00057 mg/kg a.s./person for adults and children, respectively.

The result of the bystander exposure estimation is presented in Table 7.2.2.1-2. Details of the estimations are presented in Appendix 7.2-9 (for pyraclostrobin) and in Appendix 7.2-10 (for boscalid).

Table 7.2.2.1-2: Estimated bystander exposure to pyraclostrobin and boscalid in % of the AOEL

	Active Substance			
	Pyraclostrobin		**Boscalid**	
	AOEL = 0.015 mg/kg bw/day		**AOEL = 0.1 mg/kg bw/day**	
	Adults	**Children**	**Adults**	**Children**
Systemic exposure via dermal route (mg/kg bw/day)	0.00004	0.000031	0.000124	0.0001
Systemic exposure via inhalation (mg/kg bw/day)	0.0000001	0.0000002	0.0000003	0.000001
Total systemic exposure (mg/kg bw/day)	**0.00004**	**0.000031**	**0.0001243**	**0.000101**
% of AOEL	**0.26**	**0.21**	**0.12**	**0.10**

[1] according to the German model for bystander and resident exposure assessment; Martin S. et al. (2008)
[2] based on a systemic AOEL of 0.015 and 0.1 mg/kg bw/day for pyraclostrobin and boscalid, respectively

The estimates result for adults in 0.26 and 0.12% of the AOEL for pyraclostrobin and boscalid, respectively. For children the corresponding estimates are 0.21% of the AOEL for pyraclostrobin and 0.1% for boscalid. Thus, the exposure of adult and child bystanders passing by a field treated with BAS 516 07 F is considered to be safe.

In conclusion, bystanders are not considered to be at risk when exposed to spray drift of pyraclostrobin and boscalid under the use conditions of BAS 516 07 F in potatoes.

B. Resident exposure

Residents are persons who live, work or attend any institution adjacent to an area that has been treated with a plant protection product. Possible situations are persons who are standing, working, or sitting in a garden in the vicinity of the application. They may be exposed to the plant protection products mainly via the dermal route from spray drift deposits and by inhalation of vapour drift depending on the vapour pressure of the active substances. For infants and toddlers oral exposure via hand-to-mouth transfer or object-to-mouse transfer has to be considered, too.
As for the bystander it can be assumed that residents are unlikely to take actions to avoid or control exposure, and they wear only light clothing and no protective equipment. In addition, as conservative approach it is assumed that residents are located directly downwind of the centre of the treatment area from the point of spray emission caused by professional agricultural uses.
The field crop application is here considered to represent the worst case scenario for which a distance of 1 m from the spraying device is taken into consideration.

It can be assumed that the exposure duration of residents being in a garden is longer than the exposure duration of bystanders. Therefore, the default exposure duration of 2 hours is adopted for risk evaluations.

According to the German model for bystander and resident exposure assessments, inhalation exposure has only to be considered for semi-volatile and volatile active substances (vapour pressures of $1 \times 10^{-5} - 5 \times 10^{-3}$ Pa and $> 5 \times 10^{-3}$ Pa, respectively). As pyraclostrobin has a vapour pressure of 2.6×10^{-8} Pa it is considered non-volatile. The same applies to boscalid having a vapour pressure of 7.2×10^{-7} Pa. Consequently, inhalation exposure to vapours is therefore not taken into account.

For assessment of the oral exposure of children and toddlers in a first-tier approach the default values proposed by the German model for bystander and resident exposure assessments are used (Martin S. et al. (2008) as derived from a US-EPA policy paper). Table 7.2.2.1-3 summarizes the parameters used for resident exposure of adults and children.

Table 7.2.2.1-3: Parameters used for resident exposure estimations

	Parameter	Pyraclostrobin	Boscalid
	Vapour pressure (Pa)	2.6×10^{-8}	7.2×10^{-7}
	Volatility	non-volatile	non-volatile
	Maximum application rate (kg a.s./ha)[1]	0.017	0.067
	Maximum number of applications	4	4
AR	Application rate relevant for resident (kg a.s./ha)	0.034	0.134
Ar	Maximum application rate (mg a.s./cm²)	3.4	13.4
DA	Dermal absorption (%)	5	4
IA	Inhalation absorption (%)	100	
OA	Oral absorption (%)	50	44
ACV	Airborne concentration of vapour (mg/m³) [2]	-	-
		Default values	
D	Drift (%) at 1 m distance - 90th percentile values[3] for field crop	2.77	
H	Duration (hours)	2	
		Adults	Children
TTR	Turf transferable residues hand (%)	5	5
TC	Transfer coefficient (cm²/hour)	7300	2600
IR	Inhalation rate (m³/day)	16.57	8.31
SE	Salivation extraction factor (%)		50
SA	Surface area of hands (cm²)		20
Freq	Frequency of hand-to-mouth events (events/hour)		20
DFR	Dislodgeable foliar residue object to mouth (%)		20
IgR	Ingestion rate for mouthing of grass (cm²)		25
BW	Resident body weight (kg)	60	16.15

[1] As BAS 516 07 F will be applied 4 times per season at maximum, the double application rate is considered relevant for residential exposure according to the German model for bystander and resident exposure assessment.
[2] Since pyraclostrobin and boscalid are non-volatile, i.e. the vapour pressures are 2.6 x 10-8 and 7.2 x 10-7 Pa, the ACV value is 0.0 mg/m³.
[3] 90th percentile of drift recommended for one application, 82nd percentile of drift for double application rate recommended for more than one application according to Martin S. et al. (2008)

Resident exposure for adults and children is estimated according the following equations:

Systemic dermal exposure $$SDE_R = \frac{AR \times D \times TTR \times TC \times H \times DA}{BW}$$

Systemic inhalation exposure $$SIE_R = \frac{AC \times IR \times IA}{BW}$$

Systemic exposure due to hand-to-mouth transfer (children only) $$SOE_H = \frac{AR \times D \times TTR \times SE \times SA \times F \times H \times OA}{BW}$$

Systemic exposure due to mouthing (children only) $$SOE_O = \frac{AR \times D \times DFR \times IgR \times OA}{BW}$$

Total systemic exposure (adults) $$SE_R = SDE_R + SIE_R$$

Total systemic exposure (children) $$SE_R = SDE_R + SIE_R + SOE_H + SOE_O$$

The resulting predicted exposures are summarized below.

Assessment

For the exposure of a resident located next to a field treated with BAS 516 07 F the systemic exposure to pyraclostrobin and boscalid was assessed based on 2 hour exposure to spray drifts via dermal and oral route. Exposure to airborne concentration of vapour for non-volatile compounds such as pyraclostrobin and boscalid is not considered.

The results of the resident exposure calculations are summarized in Table 7.2.2.1-4 below. Details of the estimations are presented presented in Appendix 7.2-11 (for pyraclostrobin) and in Appendix 7.2-12 (for boscalid).

Table 7.2.1.1-4: Estimated resident exposure to pyraclostrobin and boscalid in % of the AOEL

	Active Substance			
	Pyraclostrobin		**Boscalid**	
	AOEL = 0.015 mg/kg bw/day		**AOEL = 0.1 mg/kg bw/day**	
	Adults	**Children**	**Adults**	**Children**
Systemic exposure via dermal route (mg/kg bw/day)	0.000005	0.000007	0.000016	0.000021
Systemic exposure via inhalation (mg/kg bw/day)	-	-	-	-
Systemic exposure via oral route (mg/kg bw/day)	-	0.000006	-	0.000022
Total systemic exposure (mg/kg bw/day)	0.000005	0.000013	0.000016	0.000043
% of AOEL	**0.033**	**0.09**	**0.02**	**0.04**

[1] based on the German model for bystander and resident exposure assessment; Martin S. et al. (2008)
[2] based on a systemic AOEL of 0.015 and 0.1 mg/kg bw/day for pyraclostrobin and boscalid, respectively

The predicted resident exposure for adults is 0.033 and 0.02% of the AOEL for pyraclostrobin and boscalid, respectively. For children the estimated exposure is 0.09% of the AOEL for pyraclostrobin and 0.04% of the AOEL for boscalid.

In conclusion, residents are not considered to be at risk when exposed to spray drift of pyraclostrobin and boscalid under the use conditions of BAS 516 07 F in potatoes.

CP 7.2.2.2 Measurement of bystander and resident exposure

Since the risk assessment performed indicates that the health-based limit values (AOEL) will not be exceeded under practical conditions of use, studies to provide field data on bystander or residential exposure to BAS 516 07 F were not considered to be necessary and were thus not performed.

CP 7.2.3 Worker exposure

The plant protection product BAS 516 07 F is already registered for the use as fungicide in the representative crop potatoes. Information on the formulation and the critical use pattern relevant for the re-entry worker risk assessment can be found in chapter 7.2.1. The critical GAP is summarized in Table 7.2.1-1.

Exposure assessments and risk evaluations for re-entry workers for the representative formulation BAS 516 07 F are presented below. Estimations of potential worker exposure have been undertaken applying the following guidance for exposure prediction:

- EUROPOEM - Re-entry exposure model final draft adopted by more specific US EPA agricultural transfer coefficients

Risk assessment for worker

A summary of the worker risk assessment is provided in Table 7.2.3-1.

Table 7.2.3-1: Summary of re-entry worker exposure following application of BAS 516 07 F without protective equipment (standard assessment)

	Active ingredient	Estimated worker exposure (mg/kg bw/day)	% of AOEL[1]	Reference in Appendix
2 hour/day scouting and crop inspection in potatoes	pyraclostrobin	0.0005	3.3	7.2-13
	boscalid	0.0016	1.6	7.2-14

[1] based on a systemic AOEL of 0.015 and 0.1 mg/kg bw/day for pyraclostrobin and boscalid, respectively

Conclusion
It is concluded that there is no unacceptable risk anticipated for the worker wearing adequate working clothing when re-entering potatoes treated with BAS 516 07 F after the spray dilute has dried.

CP 7.2.3.1 Estimation of worker exposure

Worker exposure to pyraclostrobin and boscalid for the product BAS 516 07 F

BAS 516 07 F will be used as a fungicde in potatoes during growth stages BBCH 51-89 with a maximum of four applications per season. Thus, the considered reasonable worst case for the maximum applied amount of product per season is 1kg/ha corresponding to 0.067 kg pyraclostrobin and 0.267 kg boscalid per ha.

Hand operations in potatoes, which may result in re-entry exposure do not belong to standard growing procedures after the application of the product. Exposure scenarios one may think of as a worst case is scouting and crop inspection. These operations are considered to be of limited duration and of limited direct contact to the treated plants. For these operations a working period of 2 hours per day is considered as a reasonable approach.

Exposure Estimation Models used

The exposure estimation of the re-entry worker presented below is based on the:

- EUROPOEM - Re-entry exposure model final draft adopted by more specific US EPA agricultural transfer coefficients

The default exposure assessment is based on the following assumptions:

- Re-entry exposure is predominantly via the dermal route (contact with the foliage)
- Residues on the foliage depend on:
  - application rate
  - the crop habitat (total size of foliage compared to surface area, Leaf Area Index (LAI))
- Transfer of residues from the foliage to clothes or skin of workers is more or less independent of the product applied, but depend mainly on the intensity of contact with foliage (work activity).
- Activities with a similar pattern can be grouped and generic Transfer Coefficients (TC) can be used for one group.
- Based on the EUROPOEM proposal an Dislodgeable Foliar Residue (DFR) default value of 3 µg a.s./cm² is taken into account.

It is reasonably presumed that workers re-enter the treated crop after the spray has dried.

For pyraclostrobin an application rate of 0.067 kg a.s./ha and for boscalid the application rate of 0.267 kg a.s./ha is taken into account.

Within the EU, there are no commonly accepted specific transfer coefficients (TC) available to assess scouting and crop inspection activities in potatoes. Whereas the RMS widely accepted a TC of 1500 cm²/hour for crop inspection, the EFSA draft guidance on operator, bystander, resident and worker exposure assessment has proposed a 1100 cm²/hour for crop inspection activities instead.
Following the US EPA agricultural transfer coefficient, a TC of 1500 cm²/hour based on Central value from ARF021 -- scouting dry peas (range 486 to 2760) has been used in this evaluation as the more conservative approach.

In the following Table 7.2.3.1-1 the parameters used in the re-entry worker risk assessment are presented.

Table 7.2.3.1-1: Parameters used for the worker risk assessment

	Parameter	**Pyraclostrobin**	**Boscalid**	
MR	Application rate considered for default worker exposure	0.067	0.267	kg a.s./ha
DF	Dermal absorption[1]	5	4	%
DFR	Default dislodgeable foliar residue	3		µg/cm² x kg a.s. applied
		Default values		
BW	Re-entry worker body weight	60		kg
TC	Transfer coefficient[2]	1,500		cm²/h x person
A	Working period	2		h/day
TR	Transmission to skin for unprotected worker	1.00		(factor, equal to 100%)

[1] The given values for dermal absorption of pyraclostrobin and boscalid represent the estimates for spray dilutes. Exposure during re-entry occurs to dry residues for which it is adequate to expect a very low dermal absorption. Therefore, it is considered to be a conservative approach to use the dermal absorption values determined for the liquid spray dilutes.
[2] A TC of 1500 cm²/h x person was used (according to US EPA agricultural transfer coefficients) for activities like scouting and irrigation in potatoes based on recommendations for Central value from ARF021 -- scouting dry peas (range 486 to 2760).

Based on the assumptions and consideration made above, worker exposure is calculated as follows:

External dermal exposure ED_W

$$ED_W = MR \times A \times DFR \times TC \times TR$$

Total systemic exposure SE_W

$$SE_W = \frac{ED_W}{BW} \times DF$$

Estimatimation of worker exposure without personal protective equipment

The results of the re-entry worker risk assessment without PPE is presented in Table 7.2.3.1-2 below, details are given in Appendix 7.2-13 (for pyraclostrobin) and in Appendix 7.2-14 (for boscalid).

Table 7.2.3.1-2: Estimated worker exposure to pyraclostrobin and boscalid following application of BAS 516 07 F in potatoes (standard assessment)

		Exposure parameter	
Active substance	**AOEL (mg/kg bw/day)**	**Absorbed dose (mg/kg bw/day)**	**% of AOEL**
unprotected worker[1]			
Pyraclostrobin	0.015	0.0005	3.3
Boscalid	0.1	0.0016	1.6

[1] worker wearing working clothing

The predicted exposure to pyraclostrobin is 3.3% of the AOEL and to boscalid is 1.6% of the AOEL. Thus, for a worker performing post-treatment activities (potatoes: scouting and crop inspection) without gloves or protective clothing for 2 hours, the estimated worker exposure levels for pyraclostrobin and boscalid are within the acceptable range.

It is concluded that there is no unacceptable risk anticipated for the worker wearing adequate working clothing when re-entering potatoes treated with BAS 516 07 F after the spray dilute has dried.

CP 7.2.3.2 Measurement of worker exposure

Since the risk assessment performed indicates that the health-based limit value (AOEL) will not be exceeded under practical conditions of use, studies to provide field data on worker exposure to pyraclostrobin and boscalid in BAS 516 07 F were not considered to be necessary and were thus not performed.

Appendices for section 7.2

Appendix 7.2-1: Pyraclostrobin: BBA model estimations for tractor mounted boom sprayer application without PPE

Product:	BAS 516 07 F	**Formulation type:**	WG
Active ingredient:	pyraclostrobin	**Concentration:**	67 g/kg
AOELsys:	0.02 mg/kg bw/day	**Assessment factor:**	100
Maximum Rate:	0.017 kg a.i. per ha	**Area treated per day:**	20 ha
Amount of a.i. handled / day:	0.3 kg a.i. per day	**Dermal absorption (M/L):**	0.2%
Application technique	tractor-mounted boom sprayers with hydraulic nozzles, field crop	**Dermal absorption (Spray):**	5.0%
Personal protective equipment:	None		

$D_{M(H)}$ =	2	mg / person x kg a.i.	x	0.3	=	0.6800	mg / person / day
$D_{A(H)}$ =	0.38	mg / person x kg a.i.	x	0.3	=	0.1292	mg / person / day
$D_{A(B)}$ =	1.6	mg / person x kg a.i.	x	0.3	=	0.5440	mg / person / day
$D_{A(C)}$ =	0.06	mg / person x kg a.i.	x	0.3	=	0.0204	mg / person / day
I_M =	0.008	mg / person x kg a.i.	x	0.3	=	0.0027	mg / person / day
I_A =	0.001	mg / person x kg a.i.	x	0.3	=	0.0003	mg / person / day

			External exposure		Abs. factor		Systemic exposure	
Inhalation	mix/load	I_M =	0.0027	x	100%	=	0.0027	mg / person / day
	spray	I_A =	0.0003	x	100%	=	0.0003	mg / person / day
Dermal	mix/load	D_M =	0.6800	x	0.2%	=	0.0014	mg / person / day
	spray	D_A =	0.6936	x	5.0%	=	0.0347	mg / person / day

Total exposure (assuming person weighing 70 kg):	=	**0.0391**	**mg / person / day**
Total exposure (mg/kg bw/day):	=	**0.0006**	**mg/kg bw/day**
Total exposure (% AOEL):	=	**3.7%**	

Appendix 7.2-2: Boscalid: BBA model estimations for tractor mounted boom sprayer application without PPE

Product:	BAS 516 07 F	**Formulation type:**	WG
Active ingredient:	Boscalid	**Concentration:**	267.0 g/kg
AOELsys:	0.100 mg/kg bw/day	**Assessment factor:**	100
Maximum Rate:	0.067 kg a.i. per ha	**Area treated per day:**	20 ha
Amount of a.i. handled / day:	1.34 kg a.i. per day	**Dermal absorption (M/L):**	0.5%
Application technique	tractor-mounted boom sprayers with hydraulic nozzles, field crop	**Dermal absorption (Spray):**	4.0%
Personal protective equipment:	None		

$D_{M(H)}$ =	2	mg / person x kg a.i.	x 1.3	=	2.68000	mg / person / day
$D_{A(H)}$ =	0.38	mg / person x kg a.i.	x 1.3	=	0.50920	mg / person / day
$D_{A(B)}$ =	1.6	mg / person x kg a.i.	x 1.3	=	2.14400	mg / person / day
$D_{A(C)}$ =	0.06	mg / person x kg a.i.	x 1.3	=	0.08040	mg / person / day
I_M =	0.008	mg / person x kg a.i.	x 1.3	=	0.01072	mg / person / day
I_A =	0.001	mg / person x kg a.i.	x 1.3	=	0.00134	mg / person / day

			External exposure		Abs. factor		Systemic exposure	
Inhalation	mix/load	I_M =	0.0107	x	100%	=	0.0107	mg / person / day
	spray	I_A =	0.0013	x	100%	=	0.0013	mg / person / day
Dermal	mix/load	D_M =	2.6800	x	0.5%	=	0.0134	mg / person / day
	spray	D_A =	2.7336	x	4.0%	=	0.1093	mg / person / day

Total exposure (assuming person weighing 70 kg):	=	**0.135**	**mg / person / day**
Total exposure (mg/kg bw/day):	=	**0.002**	**mg/kg bw/day**
Total exposure (% AOEL):	=	**1.9%**	

Appendix 7.2-3: Pyraclostrobin: UK POEM estimations for tractor mounted boom sprayer application without PPE

Application method	**Tractor-mounted/trailed boom sprayer: hydraulic nozzles**		
Product	BAS 516 07 F	Active ingredient	**Pyraclostrobin**
Formulation type	**WG or SG**	a.i. concentration	**67** mg/g
Dermal absorption from product	0.2 %	Dermal absorption from spray	5 %
PPE during mix/loading	None	PPE during application	None
Dose	**0.25** kg product/ha	Work rate/day	**30** ha
Application volume	**200** litres spray/ha	Duration of spraying	**6** h

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg a.i.	5.72	mg/kg a.i.
Hand contamination/day	2.87	mg/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to a.i.	**2.87**	**mg/day**

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg a.i.	0.0358	mg/kg a.i.
Inhalation exposure/day	0.018	mg/day
RPE	None	
Transmission through RPE	100	%
Inhalation exposure to a.i.	**0.018**	**mg/day**

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	200	litres spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	5%	15%	
Dermal exposure	6.5	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	ml/day		
Concentration of a.i. in spray solution	0.08	mg/ml		
Dermal exposure to a.i.	**3.48**	**mg/day**		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure to spray	0.01	ml/h
Duration of exposure	6	h
Concentration of a.i. in spray	0.08	mg/ml
Inhalation exposure to a.i.	0.005	mg/day
Percent absorbed	100	%
Absorbed dose	**0.005**	**mg/day**

ABSORBED DOSE

	Mix/load		Application	
Dermal exposure to a.i.	2.87	mg/day	3.48	mg/day
Percent absorbed	0.2	%	5	%
Absorbed dose (dermal route)	0.006	mg/day	0.174	mg/day
Inhalation exposure to a.i.	0.018	mg/day	0.005	mg/day
Absorbed dose	**0.02**	**mg/day**	**0.18**	**mg/day**

PREDICTED EXPOSURE

Total absorbed dose	0.20	mg/day
Operator body weight	60	kg
Operator exposure	0.003	mg/kg bw/day
Total exposure (% AOEL)	**23%**	

Appendix 7.2-4: Boscalid: UK POEM estimations for tractor mounted boom sprayer application without PPE

Application method	**Tractor-mounted/trailed boom sprayer: hydraulic nozzles**		
Product	BAS 516 07 F	Active ingredient	**Boscalid**
Formulation type	**WG or SG**	a.i. concentration	**267** mg/g
Dermal absorption from product	0.5 %	Dermal absorption from spray	4 %
PPE during mix/loading	None	PPE during application	None
Dose	**0.25** kg product/ha	Work rate/day	**30** ha
Application volume	**200** litres spray/ha	Duration of spraying	**6** h

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg a.i.	5.72	mg/kg a.i.
Hand contamination/day	11.4543	mg/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to a.i.	**11.4543**	**mg/day**

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg a.i.	0.0358	mg/kg a.i.
Inhalation exposure/day	0.072	mg/day
RPE	None	
Transmission through RPE	100	%
Inhalation exposure to a.i.	**0.072**	**mg/day**

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	200	litres spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	5%	15%	
Dermal exposure	6.5	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	ml/day		
Concentration of a.i. in spray solution	0.33375	mg/ml		
Dermal exposure to a.i.	**13.8673125**	**mg/day**		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure to spray	0.01	ml/h
Duration of exposure	6	h
Concentration of a.i. in spray	0.33375	mg/ml
Inhalation exposure to a.i.	0.020025	mg/day
Percent absorbed	100	%
Absorbed dose	**0.020025**	**mg/day**

ABSORBED DOSE

	Mixing/loading		Spray application	
Dermal exposure to a.i.	11.4543	mg/day	13.8673125	mg/day
Percent absorbed	0.5	%	4	%
Absorbed dose (dermal route)	0.057	mg/day	0.555	mg/day
Inhalation exposure to a.i.	0.072	mg/day	0.020025	mg/day
Absorbed dose	**0.13**	**mg/day**	**0.57**	**mg/day**

PREDICTED EXPOSURE

Total absorbed dose	0.70	mg/day
Operator body weight	60	kg
Operator exposure	0.0117	mg/kg bw/day
Total exposure (% AOEL)	**12%**	

Appendix 7.2-5: Pyraclostrobin: BBA model estimations for tractor mounted boom sprayer application with PPE during mixing/loading and application

Product:	BAS 516 07 F		**Formulation type:**	WG	
Active substance:	pyraclostrobin		**Concentration:**	67	g/kg
Active ingredient:	0.015	mg/kg bw/day	**Assessment factor:**	100	
Maximum Rate:	0.017	kg a.i. per ha	**Area treated per day:**	20	ha
Amount of a.i. handled / day:	0.3	kg a.i. per day	**Dermal absorption (M/L):**	0.2%	
Application technique	tractor-mounted boom sprayers with hydraulic nozzles, field crop		**Dermal absorption (Spray):**	5.0%	
Personal protective equipment:	gloves during mixing/loading and gloves and coverall and sturdy footwear during application				

$D_{M(H)}$ =	2	mg / person x kg a.i.	x	0.3	x 1%	=	0.007	mg / person / day
$D_{A(H)}$ =	0.38	mg / person x kg a.i.	x	0.3	x 1%	=	0.001	mg / person / day
$D_{A(B)}$ =	1.6	mg / person x kg a.i.	x	0.3	x 5%	=	0.027	mg / person / day
$D_{A(C)}$ =	0.06	mg / person x kg a.i.	x	0.3		=	0.020	mg / person / day
I_M =	0.008	mg / person x kg a.i.	x	0.3		=	0.003	mg / person / day
I_A =	0.001	mg / person x kg a.i.	x	0.3		=	0.000	mg / person / day

			External exposure		Abs. factor		Systemic exposure	
Inhalation	mix/load	I_M =	0.0027	x	100%	=	0.0027	mg / person / day
	spray	I_A =	0.0003	x	100%	=	0.0003	mg / person / day
Dermal	mix/load	D_M =	0.0068	x	0.2%	=	0.0000	mg / person / day
	spray	D_A =	0.0489	x	5.0%	=	0.0024	mg / person / day

Total exposure (assuming person weighing 70 kg):	=	**0.0055**	**mg / person / day**
Total exposure (mg/kg bw/day):	=	**0.0001**	**mg/kg bw/day**
Total exposure (% AOEL):	=	**0 5%**	

Appendix 7.2-6: Boscalid: BBA model estimations for tractor mounted boom sprayer application with PPE during mixing/loading and application

Product:	BAS 516 07 F		**Formulation type:**	WG	
Active ingredient:	Boscalid		**Concentration:**	267.0	g/kg
AOELsys:	0.100	mg/kg bw/day	**Assessment factor:**	100	
Maximum Rate:	0.067	kg a.i. per ha	**Area treated per day:**	20	ha
Amount of a.i. handled / day:	1.34	kg a.i. per day	**Dermal absorption (M/L):**	0.5%	
Application technique	tractor-mounted boom sprayers with hydraulic nozzles, field crop		**Dermal absorption (Spray):**	4.0%	
Personal protective equipment:	gloves during mixing/loading and gloves and coverall and sturdy footwear during application				

$D_{M(H)}$ =	2	mg / person x kg a.i.	x	1.3	x	1%	=	0.027 mg / person / day
$D_{A(H)}$ =	0.38	mg / person x kg a.i.	x	1.3	x	1%	=	0.005 mg / person / day
$D_{A(B)}$ =	1.6	mg / person x kg a.i.	x	1.3	x	5%	=	0.107 mg / person / day
$D_{A(C)}$ =	0.06	mg / person x kg a.i.	x	1.3			=	0.080 mg / person / day
I_M =	0.008	mg / person x kg a.i.	x	1.3			=	0.0107 mg / person / day
I_A =	0.001	mg / person x kg a.i.	x	1.3			=	0.001 mg / person / day

			External exposure		Abs. factor		Systemic exposure	
Inhalation	mix/load	I_M =	0.0107	x	100%	=	0.0107	mg / person / day
	spray	I_A =	0.0013	x	100%	=	0.0013	mg / person / day
Dermal	mix/load	D_M =	0.0268	x	0.5%	=	0.0001	mg / person / day
	spray	D_A =	0 1927	x	4.0%	=	0.0077	mg / person / day

Total exposure (assuming person weighing 70 kg):	=	**0.01990**	**mg / person / day**
Total exposure (mg/kg bw/day):	=	**0.00028**	**mg/kg bw/day**
Total exposure (% AOEL):	=	**0.3%**	

Appendix 7.2-7: Pyraclostrobin: UK POEM estimations for tractor mounted boom sprayer application with PPE during mixing/loading and application

Application method	**Tractor-mounted/trailed boom sprayer: hydraulic nozzles**		
Product	BAS 516 07 F	Active ingredient	**Pyraclostrobin**
Formulation type	**WG or SG**	a.i. concentration	**67** mg/g
Dermal absorption from product	0.2 %	Dermal absorption from spray	5 %
PPE during mix/loading	Gloves	PPE during application	Gloves
Dose	**0.25** kg product/ha	Work rate/day	**30** ha
Application volume	**200** litres spray/ha	Duration of spraying	**6** h

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg a.i.	5.72	mg/kg a.i.
Hand contamination/day	2.87	mg/day
Protective clothing	Gloves	
Transmission to skin	1	%
Dermal exposure to a.i.	**0.0287**	**mg/day**

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg a.i.	0.0358	mg/kg a.i.
Inhalation exposure/day	0.018	mg/day
RPE	None	
Transmission through RPE	100	%
Inhalation exposure to a.i.	**0.018**	**mg/day**

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	200	litres spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	Gloves	Permeable	Permeable	
Penetration	10%	5%	15%	
Dermal exposure	0.65	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	6.45	ml/day		
Concentration of a.i. in spray solution	0.08	mg/ml		
Dermal exposure to a.i.	**0.54**	**mg/day**		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure to spray	0.01	ml/h
Duration of exposure	6	h
Concentration of a.i. in spray	0.08	mg/ml
Inhalation exposure to a.i.	0.005	mg/day
Percent absorbed	100	%
Absorbed dose	**0.005**	**mg/day**

ABSORBED DOSE

	Mix/load		Application	
Dermal exposure to a.i.	0.03	mg/day	0.54	mg/day
Percent absorbed	0.2	%	5	%
Absorbed dose (dermal route)	0.000	mg/day	0.027	mg/day
Inhalation exposure to a.i.	0.018	mg/day	0.005	mg/day
Absorbed dose	**0.02**	**mg/day**	**0.03**	**mg/day**

PREDICTED EXPOSURE

Total absorbed dose	0.05	mg/day
Operator body weight	60	kg
Operator exposure	0.001	mg/kg bw/day
Total exposure (% AOEL)	**6%**	

Appendix 7.2-8: Boscalid: UK POEM estimations for tractor mounted boom sprayer application with PPE during mixing/loading and application

Application method	**Tractor-mounted/trailed boom sprayer: hydraulic nozzles**		
Product	BAS 516 07 F	Active ingredient	**Boscalid**
Formulation type	**WG or SG**	a.i. concentration	**267** mg/g
Dermal absorption from product	0.5 %	Dermal absorption from spray	4 %
PPE during mix/loading	gloves	PPE during application	gloves
Dose	**0.25** kg product/ha	Work rate/day	**30** ha
Application volume	**200** litres spray/ha	Duration of spraying	**6** h

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg a.i.	5.72	mg/kg a.i.
Hand contamination/day	11.4543	mg/day
Protective clothing	gloves	
Transmission to skin	1	%
Dermal exposure to a.i.	**0.114543**	**mg/day**

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg a.i.	0.0358	mg/kg a.i.
Inhalation exposure/day	0.072	mg/day
RPE	none	
Transmission through RPE	100	%
Inhalation exposure to a.i.	**0.072**	**mg/day**

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	200	litres spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	gloves	Permeable	Permeable	
Penetration	10%	5%	15%	
Dermal exposure	0.65	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	6.45	ml/day		
Concentration of a.i. in spray solution	0.33375	mg/ml		
Dermal exposure to a.i.	**2.1526875**	**mg/day**		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure to spray	0.01	ml/h
Duration of exposure	6	h
Concentration of a.i. in spray	0.33375	mg/ml
Inhalation exposure to a.i.	0.020025	mg/day
Percent absorbed	100	%
Absorbed dose	**0.020025**	**mg/day**

ABSORBED DOSE

	Mix/load		Application	
Dermal exposure to a.i.	0.114543	mg/day	2.1526875	mg/day
Percent absorbed	0.5	%	4	%
Absorbed dose (dermal route)	0.001	mg/day	0.086	mg/day
Inhalation exposure to a.i.	0.072	mg/day	0.020025	mg/day
Absorbed dose	**0.07**	**mg/day**	**0.11**	**mg/day**

PREDICTED EXPOSURE

Total absorbed dose	0.18	mg/day
Operator body weight	60	kg
Operator exposure	0.0030	mg/kg bw/day
Total exposure (% AOEL)	**3%**	

Appendix 7.2-9: Estimated exposure and risk assessment for bystanders exposed to pyraclostrobin during the product application of BAS 516 07 F

Product:	BAS 516 07 F	**Active ingredient:**	pyraclostrobin
Crop:	field crop	**AOELsys:**	0.015 mg/kg bw/day
Max. application rate (AR):	0.017 kg/ha	**Dermal absorption spray (DA):**	5.0%
Max. application rate (ar):	1.7 mg a.i./m²		
		Inhalation absorption (IA):	100%
Area treated (A):	20 ha		
Drift deposition at 10-m distance (D):		2.77 % of application rate	
Exposure duration operator during spraying (TO):		6 h	
Exposure duration bystander (TB):		5 min	
Exposure duration factor (TF = TB / TO):		0.014	

		Adults	**Children**
Exposed body surface area (BSA):		1 m²	0.21 m²
Specific inhalation exposure operator (IA*):		0.001 mg/kg a.i.	0.00057 mg/kg a.i
Respiration rate (R):		1.74 m³/h	1 m³/h
Body weight:		60 kg	16.15 kg
		mg / person / day	mg / person / day
External exposure of bystanders via the dermal route			
ar x D x BSA	=	0.0471	0.01
External exposure of bystanders via the inhalative route			
IA* x AR x A x TF	=	0.000005	0.000003
		mg / kg bw / day	mg / kg bw / day
Systemic exposure via the dermal route (SDE_B)			
(ar x D x BSA x DA) / BW	=	0.00004	0.000031
Systemic exposure via the inhalation route (SIE_B)			
(IA* x AR x A x TF x IA) / BW	=	0.0000001	0.0000002
Total systemic exposure (SE_R)		**Adults**	**Children**
		mg / kg bw / day	mg / kg bw / day
= SDE_B + SIE_B	=	0.00004	0.000031
% AOEL	=	**0.26**	**0.21**

According to the bystander exposure model proposed by Martin et al. 2008

Appendix 7.2-10: Estimated exposure and risk assessment for bystanders exposed to boscalid during the product application of BAS 516 07 F

Product:	BAS 516 07 F	**Active ingredient:**	boscalid
Crop:	field crop	**AOELsys:**	0.10 mg/kg bw/day
Max. application rate (AR):	0.067 kg/ha	**Dermal absorption spray (DA):**	4.0%
Max. application rate (ar):	6.7 mg a.i./m²	**Inhalation absorption (IA):**	100%
Area treated (A):	20 ha		
Drift deposition at 10-m distance (D):	2.77 % of application rate		
Exposure duration operator during spraying (TO):	6 h		
Exposure duration bystander (TB):	5 min		
Exposure duration factor (TF = TB / TO):	0.014		

		Adults	**Children**
Exposed body surface area (BSA):		1 m²	0.21 m²
Specific inhalation exposure operator (IA*):		0.001 mg/kg a.i.	0.00057 mg/kg a.i
Respiration rate (R):		1.74 m³/h	1 m³/h
Body weight:		60 kg	16.15 kg
		mg / person / day	mg / person / day
External exposure of bystanders via the dermal route			
ar x D x BSA	=	0.1856	0.0390
External exposure of bystanders via the inhalative route			
IA* x AR x A x TF	=	0.000018	0.000011
		mg / kg bw / day	mg / kg bw / day
Systemic exposure via the dermal route (SDE_B)			
(ar x D x BSA x DA) / BW	=	0.000124	0.00010
Systemic exposure via the inhalation route (SIE_B)			
(IA* x AR x A x TF x IA) / BW	=	0.0000003	0.000001
Total systemic exposure (SE_R)		**Adults**	**Children**
		mg / kg bw / day	mg / kg bw / day
= SDE_B + SIE_B	=	0.0001243	0.000101
% AOEL	=	**0.12%**	**0.10%**

According to the bystander exposure model proposed by Martin et al. 2008

Appendix 7.2-11: Estimated exposure and risk assessment for adult and child residents exposed to pyraclostrobin during the product application of BAS 516 07 F

Product:	BAS 516 07 F		**Active ingredient:**	pyraclostrobin	
Crop:	field crop		**AOELsys:**	0.015 mg/kg bw/day	
Applications per season:	4				
Max. application rate (x2):	0.034 kg a.i./ha		**Oral absorption (OA):**	50%	
Max. application rate (Ar):	0.0003 mg a.i./cm²		**Dermal absorption spray (DA):**	5.0%	
Drift deposition at 10-m distance (D):			**Inhalation absorption (IA):**	100%	
2.38% of application rate (82nd percentile)			**Vapour pressure:**	2.60E-08 Pa	
Dislodgeable foliar residue (DFR):	20%		**Volatility of pyraclostrobin:**	non-volatile	
Turf Transferable Residues (TTR):	5%		**Airborne vapour conc. (ACv):**	0 mg/m³	
			Adults	**Children**	
Duration of exposure					
- dermal (H):			2 h	2 h	
- inhalation:			24 h	24 h	
- mouthing (H):			----	2 h	
Transfer Coefficient (TC):			7300 cm²/h	2600 cm²/h	
Body weight (BW):			60 kg bw	16.15 kg bw	
Inhalation rate (IR):			16.57 m³/day	8.31 m³/day	
Saliva extraction factor (SE):			----	50%	
Hand surface area (SA):			----	20 cm²	
Hand-to-mouth frequency (Freq):			----	20 events/h	
Ingestion rate for mouthing of grass/day (IgR):			----	25 cm²	
			mg / person / day	mg / person / day	
External exposure of residents via the dermal route					
Ar x D x TTR x TC x H		=	0.006	0.0021	
External exposure of residents via the inhalative route					
ACv x IR (a.i. is considered non-volatile)		=	0.0000	0.00000	
Exposure via hand to mouth route					
Ar x D x TTR x SE x SA x Freq x H		=	----	0.00016	
Exposure via object to mouth route					
Ar x D x DFR x IgR		=	----	0.00004	
			mg / kg bw / day	mg / kg bw / day	
Systemic exposure via the dermal route (SDE_R)					
(Ar x D x TTR x TC x H x DA) / BW		=	0.000005	0.000007	
Systemic exposure via the inhalation route (SIE_R)					
(ACv x IR x IA) / BW		=	0.00000	0.00000	
Systemic exposure via hand-to-mouth (SOE_H)					
(Ar x D x TTR x SE x SA x Freq x H x OA) / BW		=	----	0.0000051	
Systemic exposure via object-to-mouth (SOE_O)					
(Ar x D x DFR x IgR x OA) / BW		=	----	0.0000013	
Total systemic exposure (SE_R)			**Adults**	**Children**	
			mg / kg bw / day	mg / kg bw / day	
= SDE_R + SIE_R		=	0.000005	----	
= SDE_R + SIE_R + SOE_H + SOE_O		=	----	0.000013	
% AOEL		=	0.033%	0.09%	

According to the bystander exposure model proposed by Martin et al. 2008.

Appendix 7.2-12: Estimated exposure and risk assessment for adult and child residents exposed to boscalid during the product application of BAS 516 07 F

Product:	BAS 516 07 F	**Active ingredient:**	boscalid
Crop:	field crop	**AOELsys:**	0.10mg/kg bw/day
Applications per season:	4		
Max. application rate (x2):	0.134 kg a.i./ha	**Oral absorption (OA):**	44%
Max. application rate (Ar):	0.0013 mg a.i./cm²	**Dermal absorption spray (DA):**	4.0%
Drift deposition at 10-m distance (D):		**Inhalation absorption (IA):**	100%
2.38% of application rate(82nd percentile)		**Vapour pressure:**	7.20E-07 Pa
Dislodgeable foliar residue (DFR):	20%	**Volatility of boscalid:**	non-volatile
Turf Transferable Residues (TTR):	5%	**Airborne vapour conc. (ACv):**	0 mg/m³

		Adults	**Children**
Duration of exposure			
- dermal (H):		2 h	2 h
- inhalation:		24 h	24 h
- mouthing (H):		----	2 h
Transfer Coefficient (TC):		7300 cm²/h	2600 cm²/h
Body weight (BW):		60 kg bw	16 kg bw
Inhalation rate (IR):		16.6 m³/day	8.3 m³/day
Saliva extraction factor (SE):		----	50%
Hand surface area (SA):		----	20 cm²
Hand-to-mouth frequency (Freq):		----	20 events/h
Ingestion rate for mouthing of grass/day (IgR):		----	25 cm²
		mg / person / day	mg / person / day
External exposure of residents via the dermal route			
Ar x D x TTR x TC x H	=	0.023	0.0084
External exposure of residents via the inhalative route			
ACv x IR (a.i. is considered non-volatile)	=	0.0000	0.00000
Exposure via hand to mouth route			
Ar x D x TTR x SE x SA x Freq x H	=	----	0.00064
Exposure via object to mouth route			
Ar x D x DFR x IgR	=	----	0.00016
		mg / kg bw / day	mg / kg bw / day
Systemic exposure via the dermal route (SDE_R)			
(Ar x D x TTR x TC x H x DA) / BW	=	0.000016	0.000021
Systemic exposure via the inhalation route (SIE_R)			
(ACv x IR x IA) / BW	=	0.00000	0.00000
Systemic exposure via hand-to-mouth (SOE_H)			
(Ar x D x TTR x SE x SA x Freq x H x OA) / BW	=	----	0.0000175
Systemic exposure via object-to-mouth (SOE_O)			
(Ar x D x DFR x IgR x OA) / BW	=	----	0.0000044
Total systemic exposure (SE_R)		**Adults**	**Children**
		mg / kg bw / day	mg / kg bw / day
= SDE_R + SIE_R	=	0.000016	----
= SDE_R + SIE_R + SOE_H + SOE_O	=	----	0.00004
% AOEL	=	0.02%	0.04%

According to the bystander exposure model proposed by Martin et al. 2008

Appendix 7.2-13: Estimated exposure and risk assessment for an unprotected worker exposed to pyraclostrobin from the product BAS 516 07 F (standard assessment)

Product:	BAS 516 07 F	**Active ingredient:**		Pyraclostrobin	
Maximum application rate (MR):	0.067 kg a.i. per ha	**AOELsys:**		0.015	mg/kg bw/day
Crop:	Potatoes	**Assessment factor:**		100	
Growth stage BBCH:	*51-89*	**Dermal absorption (DF):**		5%	
Activity:	Scouting and crop inspection	**Working duration (A):**		2	hours/day
		Worker bodyweight:		60	kg
Dislodgeable Foliar Residue (DFR):	0.003 mg/cm²	**Transfer coefficient (TC):**		1,500	cm²/hour
Protective clothing:	**None**	**Transmission to skin (TR):**		100%	
Dermal exposure (mg/person/d):		MR x A x DFR x TC x TR	=	0.603	mg/person/day
Systemic exposure (mg/person/d):		MR x A x DFR x TC x TR x DF	=	0.0302	mg/person/day
Total systemic exposure (assuming person of 60 kg):				**0.0005**	**mg/kg bw/day**
Total exposure (% AOEL):				**3.3%**	

Based on the Europoem - Re-entry exposure model final draft adopted by more specific US EPA agricultural transfer coefficients.

Appendix 7.2-14: Estimated exposure and risk assessment for an unprotected worker exposed to boscalid from the product BAS 516 07 F (standard assessment)

Product:	BAS 516 07 F	**Active ingredient:**		Boscalid	
Maximum application rate (MR):	0.267 kg a.i. per ha	**AOELsys:**		0.015	mg/kg bw/day
Crop:	potatoes	**Assessment factor:**		100	
Growth stage BBCH:	*51-89*	**Dermal absorption (DF):**		4%	
Activity:	Scouting and crop inspection	**Working duration (A):**		2	hours/day
		Worker bodyweight:		60	kg
Dislodgeable Foliar Residue (DFR):	0.003 mg/cm²	**Transfer coefficient (TC):**		1,500	cm²/hour
Protective clothing:	**None**	**Transmission to skin (TR):**		100%	
Dermal exposure (mg/person/d):		MR x A x DFR x TC x TR	=	2.403	mg/person/day
Systemic exposure (mg/person/d):		MR x A x DFR x TC x TR x DF	=	0.09612	mg/person/day
Total systemic exposure (assuming person of 60 kg):				**0.0016**	**mg/kg bw/day**
Total exposure (% AOEL):				**1.6%**	

Based on the Europoem - Re-entry exposure model final draft adopted by more specific US EPA agricultural transfer coefficients.

CP 7.3 Dermal absorption

Report: CP 7.3/1
Hassler S., 2014(a)
Radiolabeled BAS 510 F in BAS 516 07 F - In vitro study to investigate the dermal penetration through human skin
2014/1001401

Guidelines: OECD Guideline for testing of chemicals No. 428 (Skin absorption: In vitro method (2004)), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.45

GLP: yes
(certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

In an in-vitro experiment, the dermal penetration of boscalid (BAS 510 F) formulated as BAS 516 07 F through human skin was determined. For this a high dose (formulation 'concentrate' – 1:2 aqueous slurry feasible for application; nominal dose 945 µg bocalid per cm²), a mid dose (1:150 aqueous dilution; 17 µg boscalid per cm²) and a low dose (1:1200 aqueous dilution; 2.1 µg boscalid per cm²) was applied to human dermatomed skin at 10 µL/cm². The skin membranes were set up in flow through diffusion cells. The exposure of the skin to the test material lasted 8 hours; thereafter the skin was thoroughly washed. Further penetration of the remaining test item after removal was measured for additional 16 hours. The receptor fluid (water/ethanol 9:1 (v/v) for low and middle doses, and water/ethanol 7:3 (v/v) for the high dose level) was collected in hourly intervals between 0-8 hours and thereafter in 2 hour intervals until the end of the experiments.

In summary, boscalid, applied as BAS 516 07 F to human skin membrane, penetrated at a very low extent at all concentrations tested. Summing up the absorbed dose with that associated to the skin membrane, the following dermal absorption estimates are proposed for boscalid: 0.23 ± 0.30% for the formulation concentrate as well as 1.05 ± 1.18% and 3.00 ± 0.91% for the mid and low dose, respectively. The dermal penetration estimates to be used for risk assessment were therefore calculated to be 0.5% and 4% for the formulation concentrate and the aqueous spray dilutions, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

	a) ^{14}C-BAS 510 F (pyridin-3-C14)
	b) BAS 516 CO F (BAS 516 07 F blank formulation, without BAS 510 F)
	c) unlabeled BAS 510 F
Description:	a) solution in acetonitril
	b) solid, brownish
	c) solid, white
Lot/Batch #:	a) 640-2201
	b) FD-131202-0032
	c) N37
Purity:	a) radiochemical purity: 99.7%; specific activity: 5.34 MBq/mg
	b) BAS 516 07 F, but without BAS 510 F
	c) 94.4%
Stability of test compound:	a) The storage stability of the test substance was confirmed by HPLC analysis.
	b) The storage stability of the test substance in the formulation was confirmed by HPLC analysis.
	c) Stable: Expiry date 30.06.2018

2. Vehicle: water

3. Human skin preparations:

Source:	Institute for Pathology, Kantonspital Basel, Switzerland
Preparation:	Dermatomed (split thickness) human skin membranes with a thickness of about 400 µm were prepared.
Storage of skin samples:	Human skin samples were stored at -20°C wrapped until preparation of the split thickness skin membranes and the skin membranes were stored refrigerated (+5°C) until use.

4. Reagents:

Receptor fluid:	Water/ethanol (7:3 (v/v); boscalid solubility 68 mg/L) for the high dose formulation
	Water/ethanol (9:1 (v/v); boscalid solubility 44 mg/L) for the low and mid dose
Extraction media:	none
Washing solution:	mild shower gel solution: Nivea douche fitness, Beiersdorf, Germany (1% in tap water)

5. Preparation of the dosing solutions

High dose A3 (formulation 'concentrate', 1:2 aqueous slurry):
The formulation 'concentrate' was diluted in the lowest possible amount of water (1 part formulation + 2 parts water) feasible for dermal application with a pipette. An amount of 266 mg unlabeled BAS 510 F was weighed in a flask and a volume (260 µL) of the stock solution containing 1.0 mg [^{14}C]-BAS 510 F was added and further diluted with 4.74 mL of dichloromethane. This procedure led to a final specific activity of 20 kBq/mg. An amount (733 mg) of BAS 516 CO F blank formulation was added. The organic solvent was removed using a rotary evaporator. The dry formulation was then suspended in 2 ml water. The final formulation had a concentration of 107.5 mg [^{14}C] BAS 510 F/mL. The concentration of radioactivity was 2151 kBq/mL (measured). A density of 1.14 g/mL was determined in the final formulation.

Mid dose A2 (1:150 aqueous dilution):
An amount of 16.7 mg unlabeled BAS 510 F was weighed in a flask and a volume (2600 µL) of the stock solution containing 10.1 mg [^{14}C]-BAS 510 F was added and further diluted with 2.4 mL of dichloromethane. This procedure led to a final specific activity of 2017 kBq/mg. An amount of 73.3 mg of BAS 516 CO F blank formulation was added. The organic solvent was removed using a rotary evaporator. This formulation was further diluted with 15.0 mL water according to a ratio of 1:150. The final preparation had a concentration of radioactivity of 3451 kBq/mL corresponding to 1.71 mg [^{14}C]-BAS 510 F/mL.

Low dose A1 (1:1200 aqueous dilution):
A volume of the middle dose formulation A2, i.e. 1.5 mL containing about 0.01 g BAS 516 07 F, was further diluted with 10.5 mL water to a total volume of 12 mL in order to achieve a dilution ratio of 1:1200. The final formulation had a concentration of radioactivity of 462 kBq/mL corresponding to 0.23 mg [^{14}C]-BAS 510 F/mL.

Analyses:
Liquid scintillation counting and/or HPLC verified the homogeneity and accuracy of the test substance preparations. Taking and analyzing samples at time of application confirmed the stability of the test-substance in the preparation.

The conditions of the HPLC analysis are described in the table below:

Column:	Nucleosil 120-5 C18 (incl. pre-column RP18) 5µm, 250 mm x 4 mm (l x i.d.)
Mobile Phase: Eluent A:	Water/acetonitrile/sulfuric acid (500/500/0.5 v/v/v)
Flow:	1 mL/min
Gradient: 0-40 min	100% A (isocratic)
Wavelength:	254 nm
Detection:	UV-detector (Merck-Hitachi Model L-4000), Radiomatic 500TR radioactivity flow monitor

B. TEST PERFORMANCE

1. Dates of experimental work: 13-Jan-2014 - 20-Jan-2014

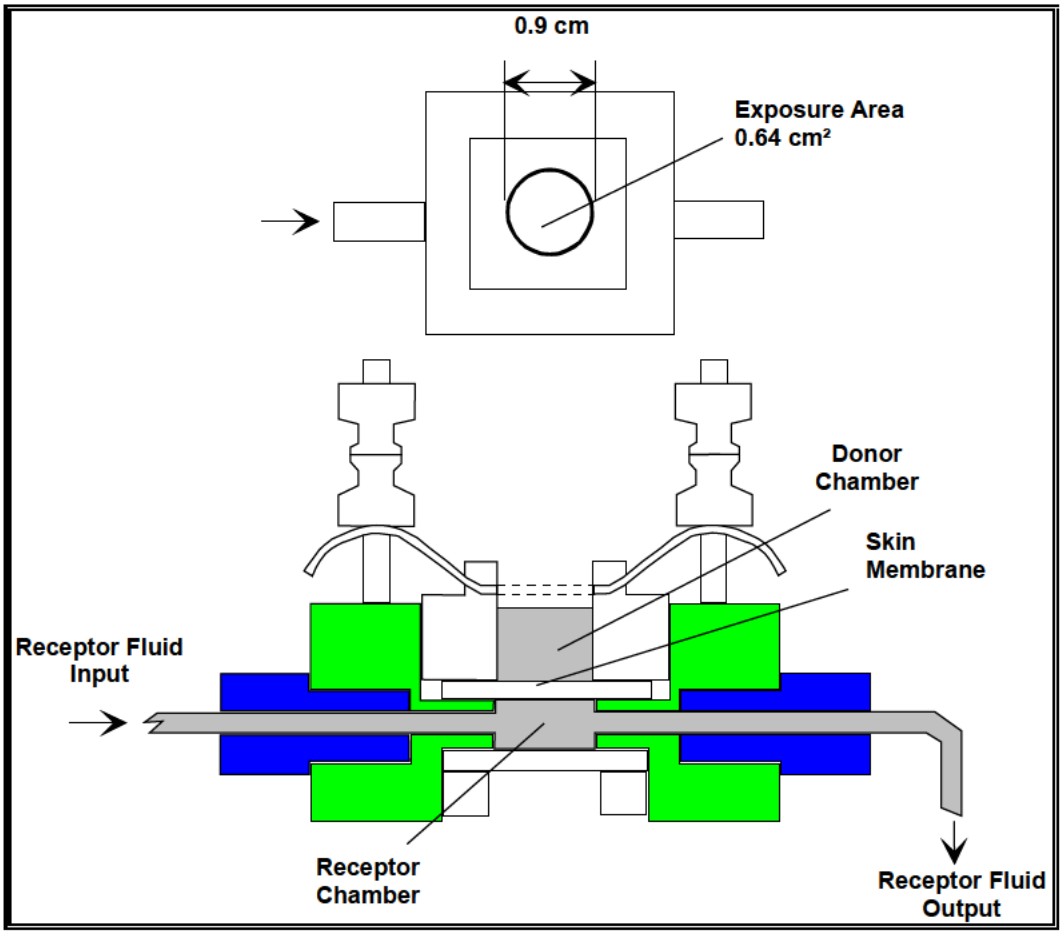
2. Study design:

The penetration of BAS 510 F through human dermatomed skin was determined using an automated flow through cell system (see Figure 7.3-1). Seven flow-through diffusion cells were placed in one aluminium manifold connected to a water bath to maintain the temperature of the skin membranes at 32 ± 1°C. Each diffusion cell consisted of a donor and receptor chamber. The area of skin membrane exposed to the donor chamber was 0.64 cm^2. The receptor chambers were connected to a multi-channel peristaltic pump. The pump speed was adjusted to about 3 mL/h. During the given time intervals, the effluent from the cell was collected directly into vials on a fraction collector.

Pieces of the skin membranes (approximately 1.8 x 1.8 cm) were cut and mounted in the diffusion cells between the donor and receptor chamber, 7 cells per formulation each. The cells were placed in the manifolds and connected to the peristaltic pump. For an equilibration period of 0.5 – 1 hour, saline (0.9% NaCl w/v) was pumped through the receptor chamber at a flow rate of about 3 mL/h.

The integrity of the skin membranes was checked by applying 50 µl tritium water (about 200000 dpm) to the skin membrane surface. The donor chamber was covered with adhesive tape (occluded conditions). The cumulative penetration was determined over 6 hours by collecting hourly fractions. The permeability coefficient (K_p) of each skin membrane was calculated for the 3 - 6 hours interval. Human membranes with $K_p > 3.0 \cdot 10^{-3}$ cm/h were excluded from the subsequent experiment.
After 6 hours, adhesive tape was removed from the donor cell chamber and the cells left open overnight with the saline flowing through the receptor chamber.

Figure 7.3-1: Schematic depiction of a flow-through diffusion cell



Those cells with skin membranes of acceptable K_p values in the integrity test were arranged on the manifold. Skin from a total of 5 donors was used and skin from 4 different donors were used per dose group. An aliquot of 6 µl of the application solution was applied to the surface of the skin membranes. The donor chambers were covered by a permeable tape (non-occluded conditions). The receptor fluid water/ethanol (9:1 v/v for the 1:150 and 1:1200 dilution and 7:3 v/v for formulation 'concentrate'), was delivered at a flow rate of about 3 mL/h. The perfusates were collected at ambient temperature in time intervals as follows:

0 - 8 hours:	1 hour intervals	(8 intervals)
8 - 24 hours:	2 hours intervals	(8 intervals)

At the end of the exposing period, i.e. 8 hours after start of exposure, the surface of the skin membrane was thoroughly rinsed (3 times / 0.5 mL) with a mild shower gel solution (1% in tap water), then the skin membrane was gently brushed with cotton swabs wetted with the mild shower gel solution (2 times). The skin membrane was finally rinsed once with 0.5 mL water and dried with one cotton swab. The skin membrane rinse and the used cotton swabs were combined to one specimen and analyzed for radioactivity (skin membrane wash 8 h). The donor chamber was covered again with a permeable tape (non-occluded conditions) until end of experiment (24 hours).

Twenty-four hours after application the perfusate sampling was terminated. The skin membrane surface was washed again as described after the exposure period. The skin membrane rinse and the used cotton swabs were combined to one specimen and analysed for radioactivity (skin membrane wash 24 h). The skin membranes were removed from the diffusion cell and consecutively stripped with adhesive tape until the stratum corneum/epidermis was removed from the skin membrane, i.e. 15 tape strips were needed. The first five stripping tapes were analysed individually (Tape Strips I-V) and the stripping tape 6 to 10 and 11 to 15 were combined to a specimen pool of five tape strips each, i.e. Tape Strips VI and VII.

Aliquots of each specimen were measured for radioactivity after solubilization with tissue solubilizer (Solvable). The skin membranes remaining after stripping were digested in tissue solubilizer (Solvable) and the radioactivity was determined by LSC.
The donor cell was finally washed with 15 ml ethanol/water (1:1) and the radioactivity in the donor cell wash was determined by LSC.

II. RESULTS AND DISCUSSION

A. STABILITY, HOMOGENEITY AND CONTENT

The stability, homogeneity and content of boscalid in the application medium were confirmed by analysis. The radiochemical purity was in the range of 96.7 to 97.7%.

B. INTEGRITY TEST OF SKIN SAMPLES

All skin samples passed the integrity test. The mean permeability coefficients for the formulation ‘concentrate’ and the 1:150 and 1:1200 aqueous dilutions were 1.77 ± 0.61, 1.63 ± 0.41 and 1.87 ± 0.38, respectively.

C. APPLIED AND RECOVERY OF DOSE

A 6µL aliquot of the application solution was applied manually to each skin membrane preparation. The doses applied and the respective concentrations of the application solution are given in Table 7.3-1.

Table 7.3-1: Applied doses

Dose group	Actual nominal dose [µg/cm²]	dpm/cell	Concentration [mg/cm³]
A1 Low dose	2.1	166145	0.23
A2 Mid dose	16.0	1242332	1.71
A3 High dose	1008	774281	107.5

The mean recoveries were 100.1, 102.3 and 104.0% for the high, mid and low dose levels, respectively (see Table 7.3-2). As group mean total recovery for all dose groups were > 95% - in agreement with the EFSA Guidance on Dermal Absorption - no adjustment of the dermal penetration values were necessary. The individual recovery for the high, mid and low dose was in the range of 94.2 – 108.2%, 96.7 – 105.9% and 96.8 – 106.2%, respectively. At the low dose, cell #3 was excluded from the calculations of means because of a too high recovery (128.9%).

In the high (formulation 'concentrate') and mid dose groups (1:150 aqueous dilution) absorption was not essentially completed. After 12 hours 64.8% and 61% of the total penetrated radioactivity, respectively, was recovered in the receptor media (see Table 7.3-3). Accordingly, only the first two tape strips were not added to the absorption estimate. In the low dose group absorption was essentially complete. After 12 hours 80% of the total penetrated radioactivity, respectively, was recovered in the receptor media (see Table 7.3-3). Accordingly, none of the tape strips were added to the absorption estimate.

Nearly the entire dose, i.e. 99.9% was recovered from the skin washing, donor chamber and tape strips (1st pool) at the high dose. In case of the cells treated with the mid and low dose, this value was 101.2 and 100.5%, respectively. The amount associated with the skin preparations was 0.08, 0.21 and 0.24% for the high, mid and low dose, respectively.

The absorbed dose, i.e. the sum of perfusates, amounted to < 0.05, 0.62 and 2.76% at the high, mid and low doses, respectively.

The total amount of boscalid recovered in the cumulative penetration after 24 hours was 0.494 µg/cm², 0.099 µg/cm² and 0.059 µg/cm² for the high, mid and low dose, respectively. Since the skin membrane area was 0.64 cm² following amounts of BAS 510 F were found after 24 h: 0.316 µg, 0.063 µg and 0.038 µg for the high, mid and low dose, respectively. When compared to the maximum solubilisation capacity in the total receptor medium volume of 72 mL (3 mL/h for 24 h) of 4896 µg and 3168 µg, the solubility in the receptor media was about 15494, 50286 and 83368-fold higher than actually needed for the high, mid and low dose, respectively. These results show that the maximum concentration of the test substance in the receptor chamber does not exceed 10% of the saturation concentration as recommended by EFSA.

Table 7.3-2: In-vitro dermal penetration of boscalid formulated as BAS 516 07 F through human skin - Recovery data

Dose group	High dose		Mid dose		Low dose	
	A3		A2 (1:150)		A1 (1:1200)	
Concentration [mg/mL]	107.5		1.71		0.23	
Applied dose [µg/cm²]	1008		16.0		2.1	
Number of cells used/Valid cells	7/7		7/7		6/7	
	Recovery [%]		Recovery [%]		Recovery [%]	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Unabsorbed dose						
Skin washing after 8 hours	99.0	5.23	89.2	7.41	91.9	9.62
Skin washing after 24 hours	0.46	0.38	5.05	2.63	4.43	2.98
Donor chamber	0.12	0.19	0.37	0.66	0.15	0.16
Dose associated to skin[a]						
Tape strip (1st pool, strips 1 -2)	0.28	0.27	6.62	3.89	3.96	3.71
Tape strips (2nd pool; strips 3 - 15)	0.10	0.14	0.22	0.32	0.54	0.71
Skin preparation	0.08	0.14	0.21	0.38	0.24	0.16
Absorbed dose						
Perfusate 0-8 h	0.02	0.02	0.25	0.20	1.66	0.48
Perfusate 8-12 h	< 0.01	< 0.01	0.13	0.12	0.55	0.20
Perfusate 12-24 h	0.02	0.02	0.24	0.30	0.55	0.28
Total recovery[#]	**100.13**	**4.77**	**102.29**	**2.87**	**104.02**	**3.64**
Absorption essentially complete at end of study (>75% absorption within half the study duration)	**No**		**No**		**Yes**	
Absorption estimates when absorption not essentially completed (= absorbed dose + dose associated to skin - tape strips 1 and 2)[#]	**0.23**	**0.30**	**1.05**	**1.18**	**n.a.**	**n.a.**
Absorption estimates when absorption essentially completed (= absorbed dose + dose associated to skin - all tapes)[#]	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**3.00**	**0.91**
Absorption estimate normalized[b]	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**
Absorption estimates used for risk assessment[c]	**0.5**		**2**		**4**	

[#] Values may not calculate exactly due to rounding of figures

[a] Grouping is different than in the report: In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2012;10(4):2665) the radioactivity in the second tape-strip pool (3rd to 6th tape strip) is considered potentially absorbable if less than 75% of the absorption occurred in the first half of the study (see Table 7.3-3). Finally, the skin preparation is also considered potentially absorbable.

[b] Cells with insufficient recovery (<95%) were corrected by normalization of absorption estimate to 100% recovery.

[c] In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2012;10(4):2665) one standard deviation was added to the mean % dermal penetration in cases where the standard deviation was ≥ 25% of the mean value. This value was then rounded to the required number of significant figures.

n.a.: not applicable

D. ABSORPTION KINETICS

The mean values of the kinetic parameters determined from the linear region of the cumulative absorbed dose curve are presented in Table 7.3-3. Within the exposure period of 8 hours very low amounts of the applied radioactivity penetrated through the human skin membrane into the receptor fluid at all dose levels, i.e. 1.66% of the low dose, 0.25% of the middle dose, and 0.02% of the high dose. For the high dose level most values measured were close to or below the Limit of Quantitation (LOQ), therefore the calculated cumulative values should be considered as worst case and the corresponding standard deviation of the mean values was high. The absorption rate was calculated to be 0.0092 and 0.0070 µg/cm²·h for the low and middle dose levels, respectively. The absorption rate for the high dose level could not be calculated since all values were below the LOQ level.

Table 7.3-3: Cumulative in-vitro dermal penetration of boscalid formulated as BAS 516 07 F through human skin - Penetration kinetics

Dose group	**High dose A3 (1:2)**		**Mid dose A2 (1:150)**		**Low dose A1 (1:1200)**	
Concentration [mg/cm³]	107.5		1.71		0.23	
Applied dose [µg/cm²]	1008		16.0		2.1	
Number of used/valid cells	7/7		7/7		6/7	
	Mean cumulative absorption		Mean cumulative absorption		Mean cumulative absorption	
	[µg/cm²]	[%]	[µg/cm²]	[%]	[µg/cm²]	[%]
Sample time [h]						
1	0.036*	< 0.01	0.001	< 0.01	0.001	0.06
2	0.065*	< 0.01	0.008	0.05	0.012	0.57
4	0.094*	< 0.01	0.022	0.14	0.025	1.16
6	0.146*	0.01	0.032	0.20	0.031	1.46
8	0.220*	0.02*	0.040	0.25	0.036	1.66
12	0.320*	0.03*	0.060	0.38	0.047	2.21
24	0.494*	0.05*	0.099	0.62	0.059	2.76
Absorption rate [µg/cm²·h]	n.a.		0.0070		0.0092	
% absorbed within 12 hours#	64.8		61.0		80.2	

values may not calculate exactly due to rounding of figures; values are based on unrounded figures
* corresponding values of the time interval were < LOQ
LOQ limit of quantification according to Currie
n.a. not applicable

III. CONCLUSION

The in-vitro dermal absorption of ^{14}C-BAS 510 F formulated as BAS 516 07 F through human skin is very low. The absorption estimates (sum of the absorbed dose and the remaining dose associated to the skin membrane) for boscalid were 0.23 ± 0.30% for the formulation 'concentrate' (1:2 aqueous slurry) and 1.05 ± 1.18% and 3.00 ± 0.91% of the 1:150 and 1:1200 spray dilutions, respectively. According to the EFSA Guidance on Dermal Penetration this translates into absorption estimates to be used for risk assessment of 0.5 and 4.0% for the formulations concentrate and aqueous dilutions, respectively.

Report:	CP 7.3/2 Hassler S., 2014b Radiolabeled BAS 500 F in BAS 516 07 F - In vitro study to investigate the dermal penetration through human skin 2014/1001402
Guidelines:	OECD Guideline for testing of chemicals No. 428 (Skin absorption: In vitro method (2004)), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.45
GLP:	yes (certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

In an in-vitro experiment, the dermal penetration of pyraclostrobin (BAS 500 F) formulated as BAS 516 07 F through human skin was determined. For this a high dose (formulation 'concentrate' – 1:2 aqueous slurry feasible for application; nominal dose 231 µg pyraclostrobin per cm²), a mid dose (1:150 aqueous dilution; 3.8 µg pyraclostrobin per cm²) and a low dose (1:600 aqueous dilution; 0.92 µg pyraclostrobin per cm²) was applied to human dermatomed skin at a volume of 10 µL/cm². The skin membranes were set up in flow through diffusion cells. The exposure of the skin to the test material lasted 8 hours; thereafter the skin was thoroughly washed. Further penetration of the remaining test item after this wash was measured for additional 16 hours. During the experimental period the receptor fluid (water/ethanol 9:1 (v/v) for low and middle doses, and water/ethanol 8:2 (v/v) for the high dose level) was collected in hourly intervals between 0-8 hours and thereafter in 2 hours intervals until the end of the experiments.

In summary, pyraclostrobin, applied as BAS 516 07 F to human skin membrane, penetrated at a very low extent at all concentrations tested. Summing up the absorbed dose with that associated to the skin membrane, dermal absorption estimates for pyraclostrobin of 0.12 ± 0.07% for the formulation concentrate and 2.37 ± 2.78% and 1.65 ± 1.79% for the mid and low dose, respectively, are proposed. The dermal penetration estimates to be used for risk assessment were therefore calculated to be 0.2 and 5% for the formulation concentrate and the aqueous spray dilutions, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

a) ^{14}C- pyraclostrobin (tolyl-ring-U-C14)
b) BAS 516 CN F (BAS 516 07 F blank formulation without pyraclostrobin, but containing all other ingredients including bocalid)
c) unlabeled pyraclostrobin

Description:
a) Solution in toluene
b) solid, brown
c) solid / melt, red to brown

Lot/Batch #:
a) 566-5101
b) FD-131202-0018
c) COD-001236

Purity:
a) radiochemical purity: 99.9%; specific activity: 6.6 MBq/mg
b) BAS 516 07 F without pyraclostrobin
c) 99.02%

Stability of test compound:
a) The storage stability of the test substance was confirmed by HPLC analysis prior to and after termination of the study
b) no data
c) Stable: Expiry date 01.10.2015

2. Vehicle: water

3. Human skin preparations:

Source: Institute for pathology, Kantonspital Basel, Switzerland

Preparation: Dermatomed (split thickness) human skin membranes with a thickness of about 400 µm were prepared.

Storage of skin samples: Human skin samples were stored at -20°C wrapped until preparation of the split thickness skin membranes and the skin membranes were stored refrigerated (+5°C) until use.

4. Reagents:

Receptor fluid: Water/ethanol (8:2 (v/v); pyraclostrobin solubility 8.2 mg/L) for the high dose formulation
Water/ethanol (9:1 (v/v); pyraclostrobin solubility 0.88 mg/L) for the low and mid dose

Extraction media: none

Washing solution: mild shower gel solution: Nivea douche fitness, Beiersdorf, Germany (1% in tap water)

5. Preparation of the dosing solutions

High dose A3 (formulation 'concentrate', 1:2 aqueous slurry):
The formulation 'concentrate' was diluted in the lowest possible amount of water (1 part formulation + 2 parts water) feasible for dermal application with a pipette. An amount of 66.2 mg unlabeled pyraclostrobin was weighed in a flask and a volume (300 µL) of the stock solution containing 1.1 mg [^{14}C]- pyraclostrobin was added and further diluted with 4.7 mL of dichloromethane. This procedure led to a final specific activity of approximately 112 kBq/mg. An amount (932.8 mg) of BAS 516 CN F blank formulation was added. The organic solvent was removed using a rotary evaporator. The dry formulation was then suspended in 2 ml water. The final formulation was measured prior to administration by LSC and had a concentration of 24 mg [^{14}C]-pyraclostrobin/mL. The concentration of radioactivity was 2698 kBq/mL (measured). A density of 1.16 g/mL was determined in the final formulation.

Mid dose A2 (aqueous 1:150 dilution):
A volume (1950 µL) of the stock solution containing approximately 6.2 mg [^{14}C]-pyraclostrobin was placed in a flask and 93.3 mg of the blank formulation BAS 516 CN F was added. The solvent of the stock solution was removed under a stream of nitrogen. Once dried, this formulation was further diluted with 15 mL water according to a ratio of 1:150. The final formulation was measured prior to administration by LSC and had a concentration of radioactivity of 2728 kBq/mL corresponding to 0.41 mg [^{14}C]-pyraclostrobin/mL.

Low dose A1 (aqueous 1:600 dilution):
A volume of the middle dose formulation A2, i.e. 1.5 mL containing about 0.01 g BAS 516 07 F, was further diluted with 4.5 mL water to a total volume of 6 mL in order to achieve a dilution ratio of 1:600. The final formulation was measured prior to administration by LSC and had a concentration of radioactivity of 698 kBq/mL corresponding to 0.11 mg [^{14}C]- pyraclostrobin/mL.

Analyses:
Liquid scintillation counting and/or HPLC verified the homogeneity and accuracy of the test substance preparations. Taking and analyzing samples at time of application confirmed the stability of the test-substance in the preparation.

The conditions of the HPLC analysis are described in the table below:

Column:	Nucleosil 120-5 C18 (incl. pre-column RP18) 5µm, 250 mm x 4 mm (l x i.d.)
Mobile Phase: Eluent A:	Water/acetonitrile/tetrahydrofuran/acetic acid (450/500/50/1)
Flow:	1 mL/min
Gradient: 0-40 min	100 % A (isocratic)
Wavelength:	275 nm
Detection:	UV-detector (Merck-Hitachi Model L-4000), Radiomatic 500TR radioactivity flow monitor

B. TEST PERFORMANCE

1. Dates of experimental work: 06-Jan-2014 - 20-Jan-2014

2. Study design:

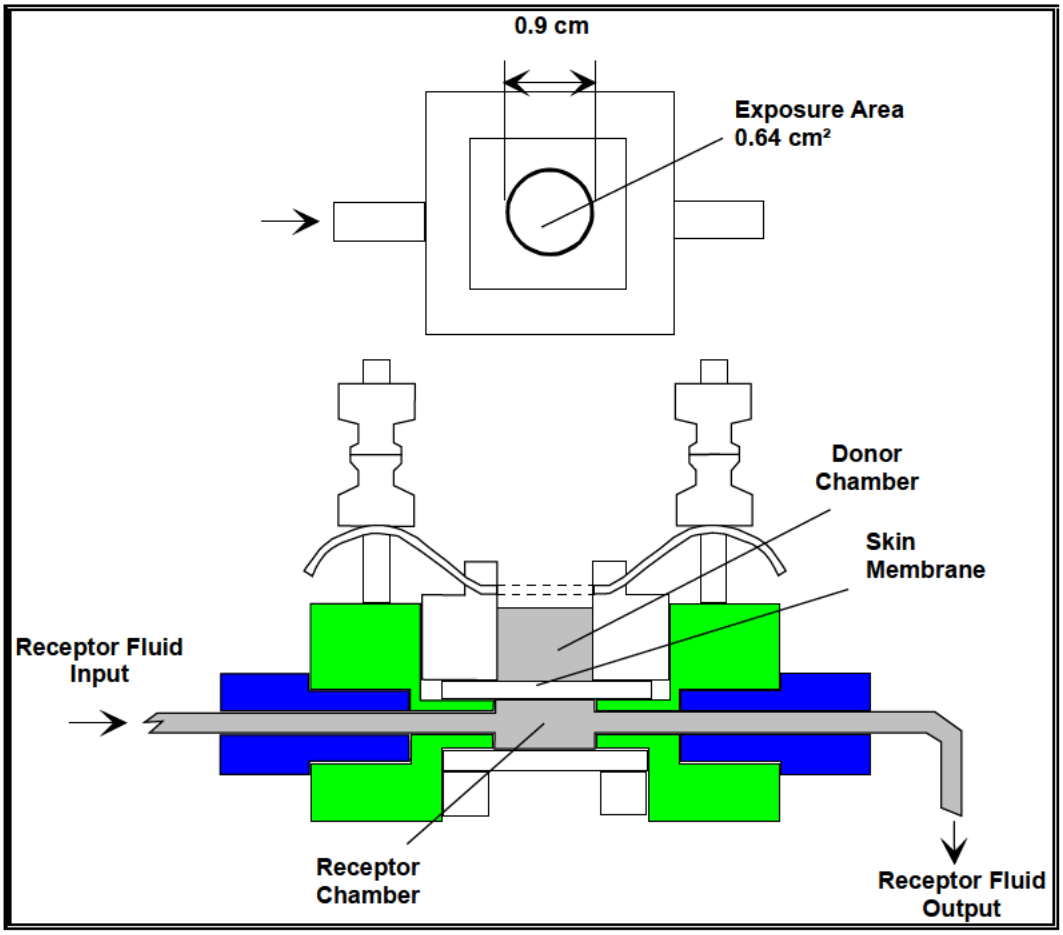
The penetration of pyraclostrobin through human dermatomed skin was determined using an automated flow through cell system (see Figure 7.3-2). Seven flow-through diffusion cells were placed in one aluminium manifold connected to a water bath to maintain the temperature of the skin membranes at 31 ± 1°C. Each diffusion cell consisted of a donor and receptor chamber. The area of skin membrane exposed to the donor chamber was 0.64 cm^2. The receptor chambers were connected to a multi-channel peristaltic pump. The pump speed was adjusted to about 3 mL/h. During the given time intervals, the effluent from the cell was collected directly into vials on a fraction collector.

Pieces of the skin membranes (approximately 1.8 x 1.8 cm) were cut and mounted in the diffusion cells between the donor and receptor chamber, 7 cells per formulation each. The cells were placed in the manifolds and connected to the peristaltic pump. For an equilibration period of 0.5 – 1 hour, saline (0.9% NaCl w/v) was pumped through the receptor chamber at a flow rate of about 3 mL/h.

The integrity of the skin membranes was checked by applying 50 µl tritium water (about 200000 dpm) to the skin membrane surface. The donor chamber was covered with adhesive tape (occluded conditions). The cumulative penetration was determined over 6 hours by collecting hourly fractions. The permeability coefficient (K_p) of each skin membrane was calculated for the 3 - 6 hours interval. Human membranes with $K_p > 3.0 \cdot 10^{-3}$ cm/h were excluded from the subsequent experiment.

After 6 hours, adhesive tape was removed from the donor cell chamber and the cells left open overnight with the saline flowing through the receptor chamber.

Figure 7.3-2: Schematic depiction of a flow-through diffusion cell



Those cells with skin membranes of acceptable K_p values in the integrity test were arranged on the manifold. Skin from a total of 5 donors was used and skin from 4 different donors were used per dose group. Aliquots of 6 µl of the application solution was applied to the surface of the skin membranes. The donor chambers were covered by a permeable tape (non-occluded conditions). The receptor fluid water/ethanol (9:1 v/v for the 1:150 and 1:600 dilutions and 8:2 v/v for the formulation 'concentrate'), was delivered at a flow rate of ca. 3 mL/h. The perfusates were collected at ambient temperature in time intervals as follows:

0 - 8 hours:	1 hour intervals	(8 intervals)
8 - 24 hours:	2 hours intervals	(8 intervals)

At the end of the exposing period, i.e. 8 hours after start of exposure, the surface of the skin membrane was thoroughly rinsed (3 times / 0.5 mL) with a mild shower gel solution (1% in tap water), then the skin membrane was gently brushed with cotton swabs wetted with the mild shower gel solution (2 times). The skin membrane was finally rinsed once with 0.5 mL water and dried with one cotton swab. The skin membrane rinse and the used cotton swabs were combined to one specimen and analyzed for radioactivity (skin membrane wash 8 h). The donor chamber was covered again with a permeable tape (non-occluded conditions) until end of experiment (24 hours).

Twenty-four hours after application the perfusate sampling was terminated. The skin membrane surface was washed again as described after the exposure period. The skin membrane rinse and the used cotton swabs were combined to one specimen and analysed for radioactivity (skin membrane wash 24 h). The skin membranes were removed from the diffusion cell and consecutively stripped with adhesive tape until the stratum corneum/epidermis was removed from the skin membrane, i.e. 15 tape strips were needed. The first five stripping tapes were analysed individually (Tape Strips I-V) and the stripping tape 6 to 10 and 11 to 15 were combined to a specimen pool of five tape strips each, i.e. Tape Strips VI and VII.

Aliquots of each specimen were measured for radioactivity after solubilization with tissue solubilizer (Solvable). The skin membranes remaining after stripping were digested in tissue solubilizer (Solvable) and the radioactivity was determined by LSC.
The donor cell was finally washed with 15 ml ethanol/water (1:1) and the radioactivity in the donor cell wash was determined by LSC.

II. RESULTS AND DISCUSSION

A. STABILITY, HOMOGENEITY AND CONTENT

The stability, homogeneity and content of pyraclostrobin in the application medium were confirmed by analysis. The radiochemical purity was in the range of 98.3 to 99.2%.

B. INTEGRITY TEST OF SKIN SAMPLES

All skin samples passed the integrity test. The mean permeability coefficients for the formulation ‘concentrate’ and the 1:150 and 1:600 aqueous dilutions were 1.84 ± 0.60, 1.95 ± 0.71 and 1.61 ± 0.41, respectively.

C. APPLIED DOSE AND RECOVERY OF DOSE

The measurement of the control doses of formulation A1 (low dose) revealed an amount of radioactivity accounting for 198211 dpm/6 µL (± 4.3%). This concentration was more than 20% lower as measured immediately after preparation of the formulation, i.e. 251152 dpm/6 µL (± 4.7%). Furthermore, the balance results of formulation A1 revealed a mean recovery of 118.25%. Both observations give strong indication for a higher dose level as compared to the measured control doses. All penetration values (% of dose) were therefore normalized to 100% recovery and the applied dose for formulation A1 was adapted regarding to the mean recovery of 118.25%. The applied doses per dose group are given in Table 7.3-4.

Table 7.3-4: Applied doses

Dose group	Actual nominal dose [µg/cm²]	dpm/cell	Concentration [mg/cm³]
A1 Low dose	0.8 0.92*	198211 234386*	0.083 0.099*
A2 Mid dose	3.8	970073	0.408
A3 High dose	231	991739	24.6

* Values adapted to 118.25% recovery

The mean recoveries were 97.9, 102.8 and 100.0% (normalized) for the high, mid and low dose levels, respectively (see Table 7.3-5). As group mean total recovery for all dose groups were > 95% - in agreement with the EFSA Guidance on Dermal Absorption - no adjustment of the dermal penetration values were necessary. The individual recovery for the high, mid and low dose was in the range of 92.4 – 101.5%, 99.1 – 107.9% and 107.8 – 133.7% (before adaption to 100%), respectively.

In all dose groups absorption was not essentially completed. After 12 hours 59.7, 54.2 or 58.2% of the total penetrated radioactivity was recovered in the receptor media at the high, mid and low dose, respectively (see Table 7.3-6). Accordingly, only the first two tape strips were not added to the absorption estimate.

Nearly the entire dose, i.e. 97.78% was recovered from the skin washing, donor chamber and the 1st and 2nd tape strip at the high dose. In case of the cells treated with the mid and low dose, the values were 100.4 and 98.35%, respectively. The amount associated with the skin preparations was 0.08, 1.69 and 0.98% for the high, mid and low dose, respectively.

The absorbed dose, i.e. the sum of perfusates, amounted to < 0.01, 0.68 and 0.67% at the high, mid and low doses, respectively. The total absorption, which corresponds to the amount recovered from skin including the 3rd to 15th tape strip and the absorbed dose, thus, sum up to 0.11, 2.37 and 1.65% for the high, mid and low dose, respectively.

The total amount of pyraclostrobin recovered in the cumulative penetration after 24 hours was 0.055 µg/cm², 0.026 µg/cm² and 0.006 µg/cm² for the high, mid and low dose, respectively. Since the skin membrane area was 0.64 cm² following amounts of pyraclostrobin were found after 24 h: 0.0352 µg, 0.0166 µg and 0.0038 µg for the high, mid and low dose, respectively. When compared to the maximum solubilisation capacity in the total receptor medium volume of 72 mL (3 mL/h for 24 h) of 590.4 µg and 63.4 µg, the solubility in the receptor media was about 16773, 3817 and 16674-fold higher than actually needed for the high, mid and low dose, respectively. These results show that the maximum concentration of the test substance in the receptor chamber does not exceed 10% of the saturation concentration as recommended by EFSA.

Table 7.3-5: In-vitro dermal penetration of pyraclostrobin formulated as BAS 516 07 F through human skin - Recovery data

Dose group	High dose		Mid dose		Low dose	
	A3		A2 (1:150)		A1 (1:600)	
Concentration [mg/mL]	24.6		0.408		0.099[1]	
Applied dose [µg/cm²]	231		3.8		0.92[1]	
Number of cells used/Valid cells	7/7		7/7		7/7	
	Recovery [%]		Recovery [%]		Recovery[2] [%]	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Unabsorbed dose						
Skin washing after 8 hours	96.33	3.64	80.09	9.82	77.12	11.08
Skin washing after 24 hours	1.02	0.78	9.44	3.07	13.37	7.96
Donor chamber	0.12	0.10	0.43	0.50	0.55	0.50
Dose associated to skin[a]						
Tape strip (1st pool, strips 1 -2)	0.31	0.27	10.44	5.08	7.31	5.75
Tape strips (2nd pool; strips 3 - 15)	0.06	0.08	1.27	2.63	0.63	0.83
Skin preparation	0.02	0.01	0.42	0.36	0.35	0.35
Absorbed dose						
Perfusate 0-8 h	< 0.01	< 0.01	0.24	0.13	0.27	0.25
Perfusate 8-12 h	< 0.01	< 0.01	0.13	0.09	0.12	0.13
Perfusate 12-24 h	< 0.01	< 0.01	0.31	0.28	0.28	0.29
Total recovery[#]	**97.9**	**3.2**	**102.8**	**3.0**	**100.00**	**-**
Absorption essentially complete at end of study (>75% absorption within half the study duration)	**No**		**No**		**No**	
Absorption estimates when absorption not essentially completed (= absorbed dose + dose associated to skin - tape strips 1 and 2)[#]	**0.12**	**0.07**	**2.37**	**2.78**	**1.65**	**1.79**
Absorption estimates when absorption essentially completed (= absorbed dose + dose associated to skin - all tapes)[#]	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**
Absorption estimate normalized[b#]	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**
Absorption estimates used for risk assessment[c]	**0.2**		**5**		**3**	

1 Applied dose and concentration of group A1 were adapted to 118.25% recovery
2 Recovery data for the low dose were normalized
Values may not calculate exactly due to rounding of figures
a Grouping is different than in the report: In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2012;10(4):2665) the radioactivity in the second tape-strip pool (3rd to 6th tape strip) is considered potentially absorbable if less than 75% of the absorption occurred in the first half of the study (see Table 7.3-6). Finally, the skin preparation is also considered potentially absorbable.
b Cells with insufficient recovery (<95%) were corrected by normalization of absorption estimate to 100% recovery.
c In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2012;10(4):2665) one standard deviation was added to the mean % dermal penetration in cases where the standard deviation was ≥ 25% of the mean value. This value was then rounded to the required number of significant figures.
n.a. not applicable

D. ABSORPTION KINETICS

The mean values of the kinetic parameters determined from the linear region of the cumulative absorbed dose curve are presented in Table 7.3-6. Within the exposure period of 8 hours very low amounts of the applied radioactivity penetrated through the human skin membrane into the receptor fluid at all dose levels, i.e. 0.27% of the low dose, 0.24% of the middle dose, and < 0.01% of the high dose. For all dose levels most values measured were close to or below the LOQ (limit of quantification) level, therefore the calculated cumulative values should be considered as worst case. The absorption rate was calculated to be 0.0004 and 0.0015 µg/cm²·h for the low and middle dose levels, respectively. The absorption rate for the high dose level could not be calculated since all values were below the LOQ level.

Table 7.3-6: Cumulative in-vitro dermal penetration of pyraclostrobin formulated as BAS 516 07 F through human skin - Penetration kinetics

Dose group	**High dose A3 (1:2)**		**Mid dose A2 (1:150)**		**Low dose A1 (1:600)**	
Concentration [mg/cm³]	24.6		0.408		0.099[1]	
Mean dose [µg/cm²]	231		3.8		0.92[1]	
Number of used/valid cells	7/7		7/7		7/7	
	Mean cumulative absorption		Mean cumulative absorption		Mean cumulative absorption	
	[µg/cm²]	[%]	[µg/cm²]	[%]	[µg/cm²]	[%]
Sample time [h]						
1	0.005*	< 0.01	< 0.001	0.02	< 0.001*	0.02
2	0.009*	< 0.01	0.003	0.07	< 0.001	0.07
4	0.011*	< 0.01	0.005	0.13	0.001	0.14
6	0.015*	< 0.01	0.007	0.18	0.002*	0.22
8	0.022*	< 0.01	0.009	0.24	0.002*	0.27
12	0.033*	0.01	0.014	0.37	0.004	0.39
24	0.055*	0.02	0.026	0.68	0.006	0.67
Absorption rate [µg/cm²·h]	n.a.		0.0015		0.0004	
% absorbed within 12 hours[#]	59.7		54.2		58.2	

[#] Values may not calculate exactly due to rounding of figures; values are based on unrounded figures
[1] Applied dose and concentration of Group A1 were adapted to 118.25 % recovery
* Corresponding values of the time interval were < LOQ
LOQ Limit of quantification according to Currie
n.a. not applicable

III. CONCLUSION

The in-vitro dermal absorption of ^{14}C-pyraclostrobin formulated as BAS 516 07 F through human skin is very low. The absorption estimates (sum of the absorbed dose and the remaining dose associated to the skin membrane) for pyraclostrobin were 0.12 ± 0.07% for the formulation 'concentrate' and 2.37 ± 2.78% and 1.65 ± 1.79% of the 1:150 and 1:600 spray dilutions, respectively. The dermal penetration estimates to be used for risk assessment were therefore calculated to be 0.2% and 5% for the formulation concentrate and the aqueous spray dilutions, respectively.

CP 7.4 Available toxicological data relating to co-formulants

Confidential information - data provided in Document J.

BASF
The Chemical Company

BAS 516 07 F

DOCUMENT M-CP, Section 8

RESIDUES IN OR ON TREATED PRODUCTS, FOOD OR FEED

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Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

CP 8 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

Supplementary data and information on residues in or on treated products, food and feed are discussed in M-CA 6.

The Chemical Company

BAS 516 07 F

DOCUMENT M-CP, Section 9

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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Table of Contents

CP 9 FATE AND BEHAVIOUR IN THE ENVIRONMENT

BAS 516 07 F is a new representative formulation, which has not been evaluated during previous Annex I inclusion processes.

CP 9.1 Fate and behaviour in soil

No studies were performed with BAS 516 07 F. The fate and behavior in soil is sufficiently addressed by information given in M-CA 7.1.

CP 9.1.1 Rate of degradation in soil

No studies were performed with BAS 516 07 F. The rate of degradation in soil is sufficiently addressed by information given in M-CA 7.1.2.

CP 9.1.1.1 Laboratory studies

No laboratory studies were performed with BAS 516 07 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.1.2.1.

CP 9.1.1.2 Field studies

No field studies were performed with BAS 516 07 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.1.2.2.

CP 9.1.1.2.1 Soil dissipation studies

No soil dissipation studies were performed with BAS 516 07 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.1.2.2.1.

CP 9.1.1.2.2 Soil accumulation studies

No soil accumulation studies were performed with BAS 516 07 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.1.2.2.2.

CP 9.1.2 Mobility in the soil

No studies were performed with BAS 516 07 F. The mobility in soil is sufficiently addressed by information given in M-CA 7.1.4.

CP 9.1.2.1 Laboratory studies

No laboratory studies were performed with BAS 516 07 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.1.4.1.

CP 9.1.2.2 Lysimeter studies

Due to the negligible leaching risk of pyraclostrobin and its metabolites, lysimeter studies are not considered necessary.

CP 9.1.2.3 Field leaching studies

Due to the negligible leaching risk of pyraclostrobin and its metabolites, field leaching studies are not considered necessary.

CP 9.1.3 Estimation of concentrations in soil

Predicted environmental concentrations in soil (PEC_S)

Report: CP 9.1.3/1
Kallweit W., 2014a
Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes
2014/1000685

Guidelines: FOCUS Groundwater (2000) Sanco/321/2000, FOCUS groundwater (2009): SANCO/13144/2010, FOCUS Groundwater (2012) Generic guidance for FOCUS groundwater scenarios v 2.1, FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011, FOCUS Surface Water Report SANCO/4802/2001 rev. 2, FOCUS Surface Water (2012) Generic guidance for FOCUS surface water scenarios v 1.1, FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 139 pp., FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 169 pp.

GLP: no

Executive Summary

PEC_{soil} were calculated for a fourfold application of pyraclostrobin and its aerobic soil metabolites BF 500-6 and BF 500-7 to potatoes at the nominal application rate and interval. Anaerobic soil metabolites (BF 500-3, BF 500-4 and BF 500-5) are not considered relevant for the assessment in soil, since soil organisms are not viable under these conditions. In addition, the anaerobic soil metabolites are immediately further degraded when aerobic conditions are re-established. Conservative crop interception values were selected in accordance with the guidance of the FOCUS groundwater scenarios workgroup (2012). A crop interception value of 80% was chosen for all four applications to potatoes.

A soil bulk density of 1.5 g cm^{-3} and a soil layer depth of 5 cm were assumed for the calculations.

Maximum PEC_{soil} for pyraclostrobin and its soil metabolites were calculated. No accumulation is triggered for pyraclostrobin (DT_{90} of trigger endpoints < 365 days). However, $PEC_{soil,plateau}$ and $PEC_{soil,accu}$ of the metabolites were also taken into account due to multi-year application of pyraclostrobin to potatoes.

Table 9.1.3-1: PEC_{soil} concentrations of pyraclostrobin and its soil metabolites BF 500-6 and BF 500-7 after application to potatoes

Compound	$PEC_{soil,plateau}$ [mg kg^{-1}] in 0 - 20 cm depth	$PEC_{soil,max}$ [mg kg^{-1}] in 0 - 5 cm depth	$PEC_{soil,accu}$ [mg kg^{-1}] (= $PEC_{soil,plateau}$ + $PEC_{soil,max}$) in 0 - 5 cm depth
Pyraclostrobin	-	0.015	-
BF 500-6	0.004	0.004	0.008
BF 500-7	0.002	0.003	0.005

I. MATERIAL AND METHODS

Application scenario

Calculations were carried out for a worst-case application scenario of pyraclostrobin applied to potatoes. Conservative crop interception values were chosen as recommended by the FOCUS groundwater scenarios workgroup [*FOCUS (2012): Generic guidance for Tier 1 FOCUS Ground Water Assessments, v2.1, 64 pp.*]. The aerobic soil metabolites BF 500-6 and BF 500-7 were additionally considered in the assessment. Anaerobic soil metabolites (BF 500-3, BF 500-4 and BF 500-5) are not considered relevant for the assessment in soil, since soil organisms are not viable under these conditions. In addition, the anaerobic soil metabolites are immediately further degraded when aerobic conditions are re-established.

Table 9.1.3-2 summarizes the worst-case application scenario of pyraclostrobin applied to potatoes. The minimum application interval for the respective crop was considered as conservative scenario.

Table 9.1.3-2: Worst-case application scenario of pyraclostrobin applied to potatoes considered for the PEC_{soil} calculations

Crop	Potatoes
Growth stage at first application [BBCH]	51
Application rate [g a.s. ha^{-1}]	17
No. of applications [-]	4
Interval [d]	10
Interception [%]	80 / 80 / 80 / 80
Amount reaching the soil surface [g a.s. ha^{-1}]	3.4 / 3.4 / 3.4 / 3.4
Total yearly soil load [g a.s. ha^{-1}]	13.6

Environmental fate parameters

Degradation of pyraclostrobin in soil

The aerobic soil degradation of pyraclostrobin under laboratory conditions was investigated in five studies with altogether 10 soils for 120 to 360 days at a temperature of 20°C and a soil moisture of 40 to 53% of maximum water holding capacity (MWHC) (see M-CA 7.1.2.1). The degradation kinetics of these studies were re-evaluated in a separate study [*CA 7.1.2.1.1/3, Eickler B. – BASF DocID 2014/1093424*]. An overview of the non-normalized DT_{50} values is given in the table at the end of M-CA 7.1.2.1.2.

The dissipation behavior of pyraclostrobin was investigated in two different field dissipation studies conducted in Europe between 1997 and 1999 with altogether six trials located in Germany (n=3), Spain (n=2) and Sweden (n=1) (see section 7.1.2.2). The degradation kinetics were evaluated in a separate study [*CA 7.1.2.2.1/1, Eickler B. – BASF DocID 2014/1093423*]. An overview of the DT_{50} values is given in the table at the end of study *CA 7.1.2.2.1/4*.

In addition, four terrestrial field dissipation trials were conducted in Europe between 2011 and 2012 [*CA 7.1.2.2.1/2, Bayer H., Marwitz A. - BASF DocID 2013/1348661*]. These trials were located in Denmark, Germany, Italy and France, and the plots were covered with sand to exclude surface processes according to the requirements of the EFSA guidance for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ (2010) [*EFSA (2010): Guidance for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ values of plant protection products in soil. EFSA Journal 2010;8(12):1936, 67 pp.*]. Due to the specific design of the studies, the obtained degradation half-lives in the soil matrix are not appropriate for derivation of trigger endpoints and were therefore not considered for PEC_{soil} calculation.

For the calculations of PEC_{soil} the worst-case DT_{50} from the field studies of 55.8 days (SFO) was used.

Formation and degradation of BF 500-6 and BF 500-7 in soil

Two metabolites of pyraclostrobin, BF 500-6 and BF 500-7, were found in laboratory aerobic soil metabolism and degradation studies with maximum occurrences of 12 to 31% and of 1 to 14% of the applied radioactivity (AR), respectively.

In the older pyraclostrobin field dissipation trials [*old EU Dossier, A II M 7.1.1.2.2/1, Kellner O., Zangmeister W. – BASF DocID 1999/11301; old EU Dossier, A II M 7.1.1.2.2/2, Kellner O., Zangmeister W. – BASF DocID 1999/11292*] BF 500-6 was found only rarely at a single trial site, and BF 500-7 was not detected (limit of quantification 0.01 mg kg^{-1}). In the new field dissipation study [*CA 7.1.2.2.1/2, Bayer H., Marwitz A. – DocID 2013/1348661*], the metabolites BF 500-6 and BF 500-7 were detected at maximum occurrences of 28 and 19% of applied parent amount, respectively.

For PEC_{soil} calculations, the maximum occurrences of 31% observed in the laboratory and 19% observed in the field study were used for BF 500-6 and BF 500-7, respectively.

The $DegT_{50}$ values of the metabolites calculated from laboratory studies with the parent or with the metabolites ranged from 95.9 to 971.2 days and from 81.5 to >1000 days for BF 500-6 and BF 500-7, respectively. As the $DegT_{50}$ values of BF 500-6 and BF 500-7 were close to the worst-case default value of 1000 days this default value was used for PEC_{soil} calculations.

Calculation methods

Maximum, actual and time-weighted average concentrations in soil ($PEC_{soil,max}$, $PEC_{soil,act}$, $PEC_{soil,twa}$) were calculated for the parent substance and its soil metabolites. The calculations were carried out based on the approach given in the guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 of November 2011, 436 pp.*]. Additionally, the accumulation behaviour of the metabolites BF 500-6 and BF 500-7 in soil was assessed assuming application over many years.

Calculation of maximum concentrations in soil ($PEC_{soil,max}$)

The metabolites BF 500-6 and BF 500-7 are formed by a dimeric reaction, i.e. one metabolite molecule is formed out of two parent molecules. Hence, for the calculation of the respective f_{mol} the molar mass of the parent has to be considered twice (i.e. $f_{mol} = M_{met} / (2 \cdot M_{par})$). The resulting f_{mol} are 0.788 and 0.768 for the metabolites BF 500-6 and BF 500-7, respectively.

The maximum PEC values in soil ($PEC_{soil,max}$) were calculated considering the respective application rate, a soil bulk density of 1.5 g cm^{-3} and a thickness of the soil layer of 5 cm (Equation 9.1.3-1).

Equation 9.1.3-1 Calculation of the maximum PEC_{soil}

$$PEC_{soil,max,n} = \sum_{i=1}^{n} \left[\left(\frac{A_i \cdot (1 - f_{int,i})}{100 \cdot d \cdot bd_{soil}} \cdot f_{max,soil} \cdot f_{mol} \right) \cdot e^{-k \cdot (t_n - t_i)} \right]$$

with:	$PEC_{soil,max}$	maximum concentration in soil after n applications	[mg kg^{-1}]
	n	number of applications	[-]
	A_i	application rate at i-th application	[g a.s. ha^{-1}]
	$f_{int,i}$	fraction intercepted by plant cover at i-th application	[-]
	d	depth of soil layer (5 cm)	[cm]
	bd_{soil}	soil bulk density (1.5 g cm^{-3})	[g cm^{-3}]
	k	degradation rate (= ln (2) / DT_{50})	[d^{-1}]
	t_i	time of i-th application	[d]
	t_n	time of n-th (last) application	[d]
	$f_{max,soil}$	maximum observed formation in soil	[-]
	f_{mol}	correction factor for molar mass difference	[-]

Actual and time-weighted average concentrations ($PEC_{soil,act}$ and $PEC_{soil,twa}$)

The actual concentration in soil ($PEC_{soil,act}$) was calculated for different time points t = 1, 2, 4, 7, 14, 21, 28, 50 and 100 days after the occurrence of the maximum concentration according to Equation 9.1.3-2. First order kinetics (SFO) is assumed, since this type of kinetics was observed in laboratory studies and field trials.

Equation 9.1.3-2 Calculation of the actual PEC_{soil}

$$PEC_{soil,act} = PEC_{soil,max} \cdot e^{-k \cdot t}$$

with:	$PEC_{soil,act}$	actual concentration in soil	[mg kg^{-1}]
	$PEC_{soil,max}$	maximum concentration in soil	[mg kg^{-1}]
	k	degradation rate (= ln (2) / DT_{50})	[d^{-1}]
	t	time after last application	[d]

The maximum time-weighted average concentrations ($PEC_{soil,twa}$) for exposure periods of 1, 2, 4, 7, 14, 21, 28, 50 and 100 days were derived from the time series of PEC values ranging from day of the first application up to 200 DAT (days after first treatment) using a moving time-frame Excel spreadsheet. For a given exposure period t, the spreadsheet calculates the respective set of time-weighted average concentrations from the time series first (moving time-frame) and scans for the highest value in the set afterwards, see Equation 9.1.3-3.

Equation 9.1.3-3 Calculation of the time-weighted average PEC_{soil}

$$PEC_{soil,twa}(\Delta t) = \max\left[\frac{1}{m}\sum_{t=t_j}^{t_j+\Delta t} PEC_{soil}(t)\right] \qquad \text{for } j = 0,\ldots,200$$

with:	$PEC_{soil,twa}$	worst-case time-weighted average concentration in soil for time interval Δt	[mg kg^{-1}]
	PEC_{soil}	concentration in soil at time t	[mg kg^{-1}]
	t	time	[d]
	t_j	start time point for integration	[d]
	Δt	time interval	[d]
	j	running variable for time step	[-]
	m	number of time steps in time interval t	[-]

Maximum PEC_{soil} due to multi-year application ($PEC_{soil,accu}$)

The potential of accumulation in soil was assessed for metabolites BF 500-6 and BF 500-7. For this purpose, the plateau concentration in soil at steady state ($PEC_{soil,plateau}$) and the overall accumulation PEC in soil ($PEC_{soil,accu}$) after application of pyraclostrobin over many years were determined following Equation 9.1.3-4 and Equation 9.1.3-5. The ploughing depth was set to 20 cm to represent the depth of soil cultivation in arable crops. This conservative standard approach with regard to field crops covers also the mixing depth of 30 cm for vegetables which could be considered for potatoes.

Equation 9.1.3-4 Calculation of the plateau PEC_{soil}

$$PEC_{soil,plateau} = \frac{PEC_{soil,max,d}}{1 - e^{-kt}} \cdot e^{-k \cdot (t-i)}$$

with:	$PEC_{soil,plateau}$	plateau concentration at steady state	[mg kg^{-1}]
	$PEC_{soil,max,d}$	maximum soil concentration following last application considering a certain mixing depth	[mg kg^{-1}]
	t	interval between application seasons (365 days)	[d]
	i	interval between first application and last application in the cropping season	[d]
	k	degradation rate (= ln(2) / DT_{50})	[d^{-1}]

The overall accumulation PEC in soil ($PEC_{soil,accu}$) represents the highest potential soil concentration considering the multi-year accumulation load as background concentration ($PEC_{soil,plateau}$) plus the peak concentration ($PEC_{soil,max}$) after application in the top soil layer. For this purpose, the $PEC_{soil,plateau}$ and the $PEC_{soil,max}$ in the top soil layer of 5 cm were added (see Equation 9.1.3-5).

Equation 9.1.3-5 Calculation of the overall accumulation PEC_{soil}

$$PEC_{soil,accu} = PEC_{soil,plateau} + PEC_{soil,max}$$

with:	$PEC_{soil,accu}$	maximum concentration in soil for the accumulation risk assessment	[mg kg^{-1}]
	$PEC_{soil,plateau}$	concentration at steady state (plateau concentration) related to the plough layer depth of 20 cm	[mg kg^{-1}]
	$PEC_{soil,max}$	maximum concentration that gives respect to the soil load after one application period related to a soil layer depth of 5 cm	[mg kg^{-1}]

II. RESULTS AND DISCUSSION

The maximum, actual and time weighted average PEC_{soil} values of pyraclostrobin and its metabolites for a soil layer depth of 5 cm are shown in Table 9.1.3-3.

Table 9.1.3-3: PEC_{soil} of pyraclostrobin, BF 500-6 and BF 500-7 after application to potatoes

	Time[a] [d]	PEC_{soil} [mg kg^{-1}]					
		Pyraclostrobin		BF 500-6		BF 500-7	
		Act	TWA	Act	TWA	Act	TWA
Global maximum	0	0.015	-	0.004	-	0.003	-
Short-term	1	0.015	0.015	0.004	0.004	0.003	0.003
	2	0.015	0.015	0.004	0.004	0.003	0.003
	4	0.014	0.015	0.004	0.004	0.003	0.003
Long-term	7	0.014	0.015	0.004	0.004	0.003	0.003
	14	0.013	0.014	0.004	0.004	0.003	0.003
	21	0.012	0.013	0.004	0.004	0.003	0.003
	28	0.011	0.013	0.004	0.004	0.003	0.003
	50	0.008	0.012	0.004	0.004	0.003	0.003
	100	0.004	0.010	0.004	0.004	0.002	0.003

[a] Time: days after maximum concentration ($PEC_{soil,act}$) or time interval ($PEC_{soil,twa}$)

$PEC_{soil,accu}$ of the metabolites BF 500-6 and BF 500-7 after application for a period of many years are given in Table 9.1.3-4 and Table 9.1.3-5, respectively.

Table 9.1.3-4: $PEC_{soil,plateau}$ and $PEC_{soil,accu}$ of BF 500-6 following multi-year use in potatoes

Crop	$PEC_{soil,plateau}$ [mg kg^{-1}] in 0 - 20 cm depth	$PEC_{soil,max}$ [mg kg^{-1}] in 0 - 5 cm depth	$PEC_{soil,accu}$ [mg kg^{-1}] (= $PEC_{soil,plateau}$ + $PEC_{soil,max}$) in 0 - 5 cm depth
Potatoes	0.004	0.004	0.008

Table 9.1.3-5: $PEC_{soil,plateau}$ and $PEC_{soil,accu}$ of BF 500-7 following multi-year use in potatoes

Crop	$PEC_{soil,plateau}$ [mg kg^{-1}] in 0 - 20 cm depth	$PEC_{soil,max}$ [mg kg^{-1}] in 0 - 5 cm depth	$PEC_{soil,accu}$ [mg kg^{-1}] (= $PEC_{soil,plateau}$ + $PEC_{soil,max}$) in 0 - 5 cm depth
Potatoes	0.002	0.003	0.005

III. CONCLUSION

Initial, short-term and long-term actual and time-weighted average PEC_{soil} were calculated for pyraclostrobin, active substance in the formulated product BAS 516 07 F, and its aerobic soil metabolites BF 500-6 and BF 500-7.

The predicted concentrations in soil are appropriate to be used for the subsequent risk assessment for soil organisms.

Report:	CP 9.1.3/2 Kallweit W., 2014b Predicted environmental concentrations of BAS 510 F - Boscalid in soil, groundwater, surface water and sediment following application to potatoes in Europe 2014/1001461
Guidelines:	SANCO/321/2000, FOCUS Groundwater (2012) Generic guidance for FOCUS groundwater scenarios v 2.1, FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011, SANCO/4802/2001 rev. 2 (FOCUS surface water scenarios) May 2003, FOCUS Surface Water (2012) SANCO/4802/2001 v1.1, FOCUS groundwater (2009): SANCO/13144/2009
GLP:	no

Executive Summary

Predicted environmental concentrations in soil (PEC_{soil}) were calculated for a fourfold application of boscalid to potatoes at the nominal application rate and interval. A conservative crop interception value of 80% for all applications was selected in accordance with the guidance of the FOCUS groundwater scenarios workgroup. This represents a worst-case assumption regarding the soil load of boscalid.

A soil bulk density of 1.5 g cm^{-3} and a soil layer depth of 5 cm were assumed for the calculations. Additionally, the accumulation behavior of boscalid in soil was assessed with the FOCUS standard approach and with two approaches based on accumulation studies with boscalid in the field.

The maximum PEC_{soil}, the actual PEC_{soil} after 100 days, the time-weighted average PEC_{soil} for a period of 100 days and the maximum $PEC_{soil,accu}$ values are summarized in the following table.

Table 9.1.3-6: PEC_{soil} values of boscalid following application to potatoes

$PEC_{soil,max}$ [mg kg^{-1}]	$PEC_{soil,act}$ 100 days after global max. [mg kg^{-1}]	$PEC_{soil,twa}$ 100 days interval [mg kg^{-1}]	Maximum $PEC_{soil,accu}$ [mg kg^{-1}] Classical approach	Maximum* $PEC_{soil,accu}$ [mg kg^{-1}] Based on boscalid accumulation study
0.069	0.057	0.063	0.080	0.245

* results of approach 2 (based on accumulation behavior observed in field studies)

I. MATERIAL AND METHODS

Application scenario

Calculations were carried out for a worst-case application scenario of boscalid applied to potatoes as given in Table 9.1.3-7.

Table 9.1.3-7: Worst-case application scenario of boscalid applied to potatoes considered for the PEC_{soil} calculations

Potatoes	
Max. no. of applications [-]	4
Crop growth stage at first application [BBCH]	51
Minimum application interval [d]	10
Application rate [g a.s. ha^{-1}]	67
Interception [%]	80 / 80 / 80 / 80
Amount reaching the soil surface [g a.s. ha^{-1}]	13.4 / 13.4 / 13.4 / 13.4
Total yearly soil load [g a.s. ha^{-1}]	53.6

Environmental fate parameters

Degradation of boscalid in soil

The degradation behavior of boscalid was investigated in five field soils [*Kellner O., Keller W. – BASF DocID 2000/1000123; Bayer H., Grote C. – BASF DocID 2000/10013295*]. Standardization of the field data to 20°C [*Platz K. – BASF DocID 2000/1017044; Platz K. – BASF DocID 2012/1288168*] was only possible for three out of the five studies due to scattering of data and a high uncertainty of degradation rates estimated from the Spanish sites Huelva and Sevilla. The standardized worst-case field half-life (Q_{10} = 2.2) from the three studies was 212 days and the respective geometric mean (Q_{10} = 2.2) was 130 days.

PEC_{soil} were calculated using the worst-case field half-life at 15°C, which was calculated from the respective value of 212 days at 20°C with the Arrhenius Equation. The Q_{10} value was set to 2.58 instead of 2.2 following the opinion of EFSA [*EFSA (2007) "Opinion on a request from EFSA related to the default Q_{10} value used to describe the temperature effect on transformation rates of pesticides in soil. Scientific Opinion of the Panel on Plant Protection Products and their Residues (PPR-Panel). Question No EFSA-Q-2007-048. The EFSA Journal (2007) 622, 1-32*]. The combination of low Q_{10} of 2.2 for normalization of field DT_{50} to 20°C [*BASF DocID 2000/1017044; BASF DocID 2012/1288168*] and high Q_{10} of 2.58 for recalculating DT_{50} to 15°C leads to worse-case degradation conditions for temperatures below 20°C. PEC_{soil} calculations can therefore be considered conservative regarding the use of the Q_{10} value.

The resulting worst-case field DT_{50} of 340.5 days was used for calculating maximum, actual and time-weighted average PEC_{soil}.

Calculation methods

Calculation of maximum concentrations in soil ($PEC_{soil,max}$)

The maximum PEC values in soil ($PEC_{soil,max}$) were calculated considering the respective application rate, a soil bulk density of 1.5 g cm^{-3} and a thickness of the soil layer of 5 cm (Equation 9.1.3-6). This formula has been derived from Equation 9.1.3-1 based on the constant dosage and interception rate of boscalid for the application to potatoes.

Equation 9.1.3-6 Calculation of the maximum PEC_{soil}

$$PEC_{soil,max} = \frac{A \cdot (1 - f_{int})}{100 \cdot d \cdot bd} \cdot \frac{(1 - e^{-n \cdot k \cdot i})}{(1 - e^{-k \cdot i})}$$

with:	$PEC_{soil,max}$	global maximum concentration in soil after multiple applications	[mg kg^{-1}]
	A	application rate of the active substance boscalid	[g a.s. ha^{-1}]
	f_{int}	fraction intercepted by the plant cover	[-]
	d	depth of soil layer (5 cm)	[cm]
	bd	soil bulk density (1.5 g cm^{-3})	[g cm^{-3}]
	k	degradation rate (= ln(2) / DT_{50})	[d^{-1}]
	i	minimum interval between applications	[d]
	n	maximum number of applications	[-]

Actual and time-weighted average concentrations ($PEC_{soil,act}$ and $PEC_{soil,twa}$)

Calculations of actual and time-weighted average concentrations in soil ($PEC_{soil,act}$, $PEC_{soil,twa}$) were calculated for boscalid using the same methods as for pyraclostrobin [described in *CP 9.1.3/1*].

Maximum PEC_{soil} due to multi-year application ($PEC_{soil,accu}$)

The accumulation behavior of the substance boscalid in soil was assessed with the FOCUS standard approach and with two approaches based on accumulation studies with boscalid in the field.

$PEC_{soil,accu}$ based on standard approach according to FOCUS

The plateau concentration in soil at steady state ($PEC_{soil,plateau}$) and the overall accumulation PEC in soil ($PEC_{soil,accu}$) after application over many years were determined for boscalid using the same equations as for pyraclostrobin [described in *CP 9.1.3/1*]. The maximum concentration in soil is derived using Equation 9.1.3-5 considering a 30 cm mixing depth for potatoes.

$PEC_{soil,accu}$ based on accumulation behavior observed in field studies

$PEC_{soil,accu}$ was calculated in two ways that consider the accumulation behavior of boscalid as observed during accumulation studies in grapevine [*Kellner et al. – BASF DocID 2004/1003851*] and vegetable crops [*Penning et al. – BASF DocID 2009/1070939*]. The first approach was based on measured total boscalid residues in soil. The residues were used to derive an accumulation factor, which was then applied to calculate $PEC_{soil,accu}$ in the soil layer of interest (top 5 cm) from the yearly application rate of the use under assessment. The second approach was based on measured concentrations in the top 10 cm soil layer of the accumulation studies that are used to predict concentrations in the soil layer of interest (top 5 cm).

The maximum $PEC_{soil,accu}$ values obtained with these two approaches are considered a conservative and adequate estimate to be used in soil risk assessment.

Approach 1

$PEC_{soil,accu}$ was calculated as the sum of the maximum PEC_{soil} resulting from the annual application pattern ($PEC_{soil,max}$) and the plateau PEC_{soil} ($PEC_{soil,plateau}$) reflecting the background level after multi-year use before the beginning of the annual application period (Equation 9.1.3-7).

Equation 9.1.3-7: Calculation of $PEC_{soil,accu}$ (approach 1)

$$PEC_{soil,accu} = PEC_{soil,max} + PEC_{soil,plateau}$$

with:	$PEC_{soil,accu}$	PEC_{soil} after multi-year application at the end of the annual application period in a soil layer of 5 cm	[mg kg^{-1}]
	$PEC_{soil,max}$	maximum PEC_{soil} due to single-year application in a soil layer of 5 cm	[mg kg^{-1}]
	$PEC_{soil,plateau}$	PEC_{soil} after multi-year application before the beginning of the annual application period	[mg kg^{-1}]

The $PEC_{soil,max}$ for the use under assessment was calculated with the approach described in the relevant section. The respective $PEC_{soil,plateau}$ was calculated considering the accumulation factor (f_{accu}) that was concluded from the ratio of modeled residue plateau to yearly application rate (see Table 9.1.3-8). $PEC_{soil,plateau}$ is the yearly application rate in the GAP multiplied by f_{accu} and then related to a typical soil cultivation layer of defined depth and bulk density, where the residues are distributed evenly due to ploughing (Equation 9.1.3-8). The depth of the soil cultivation layer is 0.1 m for permanent cultures and 0.3 m for vegetables considered for potatoes.

Equation 9.1.3-8: Calculation of $PEC_{soil,plateau}$ (approach 1)

$$PEC_{soil,plateau} = \frac{f_{accu} \cdot A_{GAP} \cdot 1000}{depth \cdot 10000 \cdot bd}$$

with:	$PEC_{soil,plateau}$	PEC_{soil} after multi-year application before the beginning of the annual application period	[mg kg^{-1}]
	f_{accu}	accumulation factor concluded from relevant accumulation study (see Table 9.1.3-8)	[-]
	A_{GAP}	Yearly application rate of use under assessment	[g ha^{-1}]
	depth	depth of soil cultivation layer	[m]
	bd	bulk density of soil cultivation layer (1500 kg m^{-3})	[kg m^{-3}]

Table 9.1.3-8: Accumulated fraction (f_{accu}) of boscalid from field studies in grapevine and vegetables

Accumulation study	Modeled minimum residue plateau [kg ha^{-1}]	Yearly application rate [kg ha^{-1}]	Ratio of residue plateau to application rate (f_{accu}) [-]
Grapevine	2.0	2.10	0.95
Vegetables	1.5	1.27[a]	1.18

[a] Average rate of triennial crop rotation scheme (1st year: 2.1 kg ha^{-1}, 2nd year: 1.7 kg ha^{-1}, 3rd year: 0 kg ha^{-1})

In the present study, $PEC_{soil,accu}$ (approach 1) was calculated using the results of the vegetables accumulation study for potatoes.

Approach 2

$PEC_{soil,accu}$ was calculated based on the maximum PEC_{soil} in the soil layer of interest (top 5 cm) from the relevant accumulation study ($PEC_{soil,study,max}$). The approach is considered to be an advanced version of the estimation approach proposed by the Rapporteur Member State in the 2nd addendum to the Draft Assessment Report.

The $PEC_{soil,accu}$ for the use under assessment was calculated by rescaling the $PEC_{soil,study,max}$ from the relevant accumulation study with the ratio of the yearly application rate in the GAP and the study as described in Equation 9.1.3-9.

Equation 9.1.3-9: Calculation of $PEC_{soil,accu}$ (approach 2)

$$PEC_{soil,accu} = PEC_{soil,study,max} \cdot \frac{A_{GAP}}{A_{study}}$$

with:	$PEC_{soil,accu}$	PEC_{soil} after multi-year application at the end of the annual application period	[mg kg^{-1}]
	$PEC_{soil,study,max}$	maximum PEC_{soil} in soil layer of interest (top 5 cm) in relevant accumulation study at the end of the annual application period	[mg kg^{-1}]
	A_{GAP}	Yearly application rate of use under assessment	[g ha^{-1}]
	A_{study}	Yearly application rate of relevant accumulation study related to $PEC_{soil,study,max}$	[g ha^{-1}]

$PEC_{soil,study,max}$ for the top 5 cm was not measured in the accumulation studies, but can be calculated from the reported concentrations in the upper 10 cm soil layer. Table 9.1.3-9 summarizes $PEC_{soil,study,max}$ values of the two accumulation studies that were calculated for different soil layer depths.

Table 9.1.3-9: Maximum PEC_{soil} of boscalid in two accumulation studies after the annual application period with regard to different top soil layer depths

Accumulation study	A_{study} [g ha^{-1}]	$PEC_{soil,study,max}$ of boscalid [mg kg^{-1}]		
		1 cm	2.5 cm	5 cm
Grapevine	2100	7.024	3.076	2.094
Vegetables	1700[a]	7.214	2.954	1.553

[a] $PEC_{soil,study,max}$ values resulted from those years in the crop rotation scheme of the study, when 1700 g ha^{-1} boscalid were applied

In the present study, $PEC_{soil,accu}$ (approach 2) was calculated using the results of the vegetables accumulation study for potatoes.

II. RESULTS AND DISCUSSION

Maximum, actual and time-weighted average PEC_{soil}

The PEC_{soil} values of boscalid after spray application to potatoes are shown in Table 9.1.3-10.

Table 9.1.3-10: PEC_{soil} values of boscalid following application to potatoes

	Time[a] [d]	Single application		Multiple application	
		$PEC_{soil,act}$ [mg kg^{-1}]	$PEC_{soil,twa}$ [mg kg^{-1}]	$PEC_{soil,act}$ [mg kg^{-1}]	$PEC_{soil,twa}$ [mg kg^{-1}]
Global max.	0	0.018	-	0.069	-
Short-term	1	0.018	0.018	0.069	0.069
	2	0.018	0.018	0.069	0.069
	4	0.018	0.018	0.069	0.069
Long-term	7	0.018	0.018	0.068	0.069
	14	0.017	0.018	0.067	0.068
	21	0.017	0.017	0.066	0.068
	28	0.017	0.017	0.065	0.067
	50	0.016	0.017	0.063	0.066
	100	0.015	0.016	0.057	0.063

[a] Time: days after maximum concentration ($PEC_{soil,act}$) or time interval ($PEC_{soil,twa}$)

Maximum PEC_{soil} due to multi-year application ($PEC_{soil,accu}$)

$PEC_{soil,accu}$ calculated with the standard approach according to FOCUS is given in Table 9.1.3-11.

Table 9.1.3-11: $PEC_{soil,plateau}$ and $PEC_{soil,accu}$ of boscalid following multi-year application to potatoes (standard approach)

Crop	Cultivation soil depth [cm]	$PEC_{soil,plateau}$ [mg kg^{-1}]	$PEC_{soil,max}$ in 5 cm depth [mg kg^{-1}]	$PEC_{soil,accu}$ (= $PEC_{soil,plateau}$ + $PEC_{soil,max}$) in 5 cm depth [mg kg^{-1}]
Potatoes	30	0.011	0.069	0.080

$PEC_{soil,accu}$ in a soil layer of 0 - 5 cm calculated with approach 1 together with the relevant values of $PEC_{soil,plateau}$ and $PEC_{soil,max}$ are given in Table 9.1.3-12.

Table 9.1.3-12: $PEC_{soil,accu}$ of boscalid following multi-year application to potatoes (approach 1)

Crop	Cultivation soil depth [cm]	$PEC_{soil,plateau}$ [mg kg^{-1}]	$PEC_{soil,max}$ in 5 cm depth [mg kg^{-1}]	$PEC_{soil,accu}$ (= $PEC_{soil,plateau}$ + $PEC_{soil,max}$) in 5 cm depth [mg kg^{-1}]
Potatoes	30	0.070	0.069	0.140[a]

[a] exact value from Excel data with higher numerical precision

$PEC_{soil,accu}$ in a soil layer of 0 - 5 cm calculated with approach 2 together with the relevant $PEC_{soil,study,max}$ and the ratio of total yearly application rates in the GAP and the study are given in Table 9.1.3-13.

Table 9.1.3-13: $PEC_{soil,accu}$ of boscalid following multi-year application to potatoes (approach 2)

Crop	$PEC_{soil,study,max}$ in 5 cm depth [mg kg^{-1}]	A_{GAP}/A_{Study} [-]	$PEC_{soil,accu}$ ($PEC_{soil,study,max}$ x A_{GAP}/A_{Study}) in 5 cm depth [mg kg^{-1}]
Potatoes	1.553	0.158	0.245[a]

[a] exact value from Excel data with higher numerical precision

A comparison of the $PEC_{soil,accu}$ values obtained with the FOCUS standard approach and the two approaches based on field accumulation studies shows that calculation with approach 2 results in the highest $PEC_{soil,accu}$ values.

III. CONCLUSION

Initial, short-term and long-term actual and time-weighted average PEC_{soil} were calculated for boscalid, active substance in the formulated product BAS 516 07 F.

The predicted concentrations in soil are appropriate to be used for the subsequent risk assessment for soil organisms.

CP 9.2 Fate and behaviour in water and sediment

CP 9.2.1 Aerobic mineralisation in surface water

No studies were performed with BAS 516 07 F. The aerobic mineralisation in surface water is sufficiently addressed by information given in M-CA 7.2.2.2.

CP 9.2.2 Water/sediment study

No water/sediment studies were performed with BAS 516 07 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.2.2.3.

CP 9.2.3 Irradiated water/sediment study

No irradiated water/sediment studies were performed with BAS 516 07 F. Data for pyraclostrobin and its metabolites are covered by information given in M-CA 7.2.2.4.

CP 9.2.4 Estimation of concentrations in groundwater

CP 9.2.4.1 Calculation of concentrations in groundwater

Predicted environmental concentrations in groundwater (PEC_{GW})

Report: CP 9.2.4/1
Kallweit W., 2014a
Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes
2014/1000685

Guidelines: FOCUS Groundwater (2000) Sanco/321/2000, FOCUS groundwater (2009): SANCO/13144/2010, FOCUS Groundwater (2012) Generic guidance for FOCUS groundwater scenarios v 2.1, FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011, FOCUS Surface Water Report SANCO/4802/2001 rev. 2, FOCUS Surface Water (2012) Generic guidance for FOCUS surface water scenarios v 1.1, FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 139 pp., FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 169 pp.

GLP: no
(certified by <none>)

Executive Summary

Predicted environmental concentrations in groundwater (PEC_{gw}) of pyraclostrobin and its aerobic soil metabolites BF 500-6 and BF 500-7, as well as its anaerobic soil metabolites BF 500-3, BF 500-4 and BF 500-5 following application of BAS 516 07 F to potatoes were calculated in accordance with the guidance of the FOCUS groundwater scenarios work group (2000, 2009, 2012). Although these anaerobic soil metabolites are degraded again quickly under aerobic conditions, they were considered (according to Regulation 1107/2009) for an assessment in groundwater.

Tier 1 PEC_{gw} of pyraclostrobin and its soil metabolites were calculated with the models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3 for all available FOCUS scenarios of potatoes (fourfold application of 17 g a.s. ha^{-1}, BBCH 51, 10 day interval, 80% interception for all applications) with an annual application pattern (continuous cropping over a period of 26 years).

The maximum PEC_{gw} for pyraclostrobin and its soil metabolites were < 0.001 µg L^{-1} and thus clearly below the 0.1 µg L^{-1} threshold value.

I. MATERIAL AND METHODS

The leaching assessment for pyraclostrobin and its metabolites was conducted at Tier 1 of the tiered assessment scheme proposed by the FOCUS groundwater higher tier working group. The aerobic soil metabolites BF 500-6 and BF 500-7 were considered in the assessment. In addition the anaerobic soil metabolites BF 500-3, BF 500-4 and BF 500-5 were considered even though they are degraded again quickly under aerobic conditions.

The simulations were carried out using FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3 in combination with the FOCUS standard scenarios.

Applied procedures methods and scenarios follow the recommendations of the following guidelines:

- *FOCUS (2009): Assessing Potential for Movement of Active Substances and their Metabolites to Groundwater in the EU Final Report of the Groundwater Workgroup of FOCUS, amending FOCUS (2000), EC Document Reference Sanco/13144/2010, 604 pp.*
- *FOCUS (2000): "FOCUS groundwater scenarios in the EU plant protection product review process" Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000, 197 pp.*
- *FOCUS (2012): Generic guidance for Tier 1 FOCUS Ground Water Assessments, v2.1, 64 pp.*].

Application scenarios

PEC_{gw} values of pyraclostrobin were calculated for the same worst-case application scenario as for the PEC_{soil} assessment (M-CP 9.1.3). PEC_{gw} calculations were performed for all FOCUS groundwater scenarios that are parameterized for potatoes.

Application dates were selected following FOCUS recommendations. The worst-case application scenario and application dates for all relevant FOCUS scenarios are shown in Table 9.2.4.1-1.

Table 9.2.4.1-1: Worst-case application scenario for PEC_{gw} calculations of pyraclostrobin applied to potatoes

Crop	Potatoes			
Growth stage at first application [BBCH]	51			
No. of applications [-]	4			
Interval [days]	10			
Application rate [g a.s. ha^{-1}]	17			
Interception [%]	80 / 80 / 80 / 80			
Amount reaching the soil surface [g a.s. ha^{-1}]	3.4 / 3.4 / 3.4 / 3.4			
Total yearly soil load [g a.s. ha^{-1}]	13.6			
Scenario	**Application dates**			
	1st application	**2nd application**	**3rd application**	**4th application**
Châteaudun	30th May	9th June	19th June	29th June
Hamburg	9th June	19th June	29th June	9th July
Jokioinen	5th July	15th July	25th July	4th August
Kremsmünster	9th June	19th June	29th June	9th July
Okehampton	30th May	9th June	19th June	29th June
Piacenza	20th May	30th May	9th June	19th June
Porto	14th April	24th April	4th May	14th May
Sevilla	2nd March	12th March	22nd March	1st April
Thiva	31st March	10th April	20th April	30th April

Environmental fate parameters

Degradation parameters of the parent

A total of 18 degradation endpoints for environmental fate modeling from different laboratory and field trials were reported (see M-CP 9.1.3) in four studies [*CA 7.1.2.1.1/3, Eickler B. – BASF DocID 2014/1093424; CA 7.1.2.1.1/1, Hassink J., Kuhnke G. – BASF DocID 2013/1337273; CA 7.1.2.2.1/1, Eickler B. – BASF DocID 2014/1093423; CA 7.1.2.2.1/4, Pape L. – BASF DocID 2014/1105764*]. As the endpoint in *Hassink J., Kuhnke G.* [*BASF DocID 2013/1337273*] was calculated for actual study conditions, it was further normalized to reference conditions (20°C, pF2). In all other studies normalized degradation endpoints were reported. In study *CA 7.1.2.2.1/1* [*Eickler B. – BASF DocID 2014/1093423*] the two Spanish field trials (ALO/01/98 and ALO/02/98) were considered to be not appropriate for derivation of modeling endpoints as the extreme climatic conditions during the trials were not representative for the EU.

An overview of individual DT_{50} values considered to select endpoints for PEC_{gw} calculations is given in Table 7.1.2.1.1-26 and/or Table 7.1.2.1.1-27 (laboratory studies) and in Table 7.1.2.2.1-24 (field studies).

For derivation of an appropriate degradation endpoint for PEC_{gw} modeling the EFSA endpoint selector was used [*EFSA $DegT_{50}$ and Sorption Endpoint Selector. Download from: http://www.efsa.europa.eu/en/130725a/docs/130725aax1.xls, date of access: 15.04.2014*]. Accordingly, the bias-corrected geometric mean $DegT_{50}$ value of 28.3 days based on the results of the field studies was used as modeling input.

Sorption of pyraclostrobin to soil

The sorption behavior of pyraclostrobin was investigated in six soils by batch-equilibrium experiments [*old EU Dossier, A II M 7.1.2/1, Ziegler G. - BASF DocID 1998/10650*]. An overview of individual $K_{f,oc}$ and 1/n values considered to select endpoints for PEC_{gw} calculations is given in M-CA 7.1.3.1.1. For the PEC_{gw} calculations of pyraclostrobin, an arithmetic mean $K_{f,oc}$ of 9304 mL g^{-1} (equivalent to a $K_{f,om}$ value of 5397 mL g^{-1}) and an arithmetic mean 1/n of 0.95 were used.

Formation and degradation of the metabolites in soil

BF 500-3
In laboratory experiments under aerobic conditions and in field dissipation trials with pyraclostrobin, the metabolite BF 500-3 was either not detected or formed in amounts far below 5% of the amount of applied parent (see M-CA 7.1.1). Under anaerobic conditions it was formed in high amounts by a very fast de-methoxylation of pyraclostrobin (see M-CA 7.1.1). This reaction is the first step also in the aerobic soil degradation process. However, further reaction to successive metabolites and bound residues is too fast to detect this short-lived transient intermediate.

To account for the rapid formation and degradation of BF 500-3 under aerobic conditions, a conservative default DT_{50} of 1 day and a formation fraction of 1 from pyraclostrobin were considered for PEC_{gw} calculations.

BF 500-4
The metabolite BF 500-4 was never found in soil under aerobic conditions. Under anaerobic conditions it was observed with up to 11.1% TAR *[old EU Dossier, A II M 7.1.1.2.1/5, Kellner O. - BASF DocID 1999/10079; old EU Dossier, A II M 7.1.1.2.1/6, Kellner O. - BASF DocID 1999/11103*]. The aerobic degradation of BF 500-4 under laboratory conditions was investigated in three soils [*CA 7.1.2.1.2/3, Ebert D., Dalkmann P. – BASF DocID 2013/1294779*]. The corresponding DT_{50} values were normalized to reference conditions (20°C, pF2), see Table 7.1.2.1.2-24 at the end of study *CA 7.1.2.1.2/3*. The geometric mean of the normalized DT_{50} values of 2.82 days was used as input parameter for BF 500-4 for PEC_{gw} calculations.

In the kinetic re-evaluation of the anaerobic soil degradation studies of pyraclostrobin formation fractions of BF 500-4 from BF 500-3 of 1 and 0.692 were reported [*CA 7.1.2.1.3/1, Pape L. – BASF DocID 2014/1000701*]. Therefore, a formation fraction of 1 was used as worst-case assumption for PEC_{gw} calculations.

BF 500-5
Under aerobic conditions, the metabolite BF 500-5 occurred only in one soil with 2.8% TAR. Under anaerobic conditions it was found with up to 7.7% TAR [*Kellner O. - BASF DocID 1999/11103*]. The aerobic degradation of BF 500-5 under laboratory conditions was investigated in three soils [*CA 7.1.2.1.2/4, Schoof S., Possienke M. – BASF DocID 2013/1294780*]. The corresponding DT_{50} were normalized to reference conditions (20°C, pF2), see Table 7.1.2.1.2-34 at the end of study *CA 7.1.2.1.2/4*. As all DT_{50} values were below 1 day a default DT_{50} value of 1 day was used for PEC_{gw} calculations as worst-case assumption.

In the kinetic re-evaluation of the anaerobic soil degradation studies of pyraclostrobin a formation fraction of BF 500-5 from BF 500-4 of 0.409 was reported and was considered for PEC_{gw} calculations [*CA 7.1.2.1.3/1, Pape L. – BASF DocID 2014/1000701*].

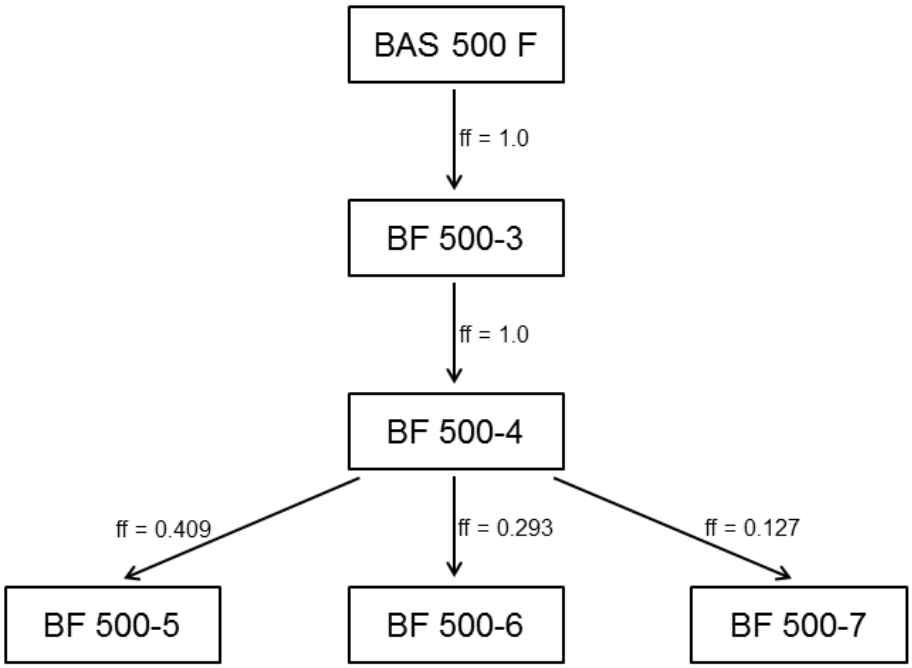
BF 500-6 and BF 500-7
The metabolites BF 500-6 and BF 500-7 were found in aerobic soil degradation studies as well as in field dissipation studies with pyraclostrobin. Degradation endpoints for modeling are available from kinetic evaluations of aerobic laboratory degradation studies with pyraclostrobin and aerobic laboratory degradation studies with the metabolites (see table at the end of study *CA 7.1.2.1.2/4*) as well as with the new field dissipation study with pyraclostrobin (see table at the end of study *CA 7.1.2.2.1/4*) [*Hassink J., Kuhnke G. – BASF DocID 2013/1337273; Eickler B. – BASF DocID 2014/1093424; Pape L. – BASF DocID 2014/1105764; CA 7.1.2.1.2/1, Tornisielo A., Sacchi R.R. – BASF DocID 2011/1142307; CA 7.1.2.1.2/2, Tornisielo A., Sacchi R.R. – BASF DocID 2011/1142308*].

Where necessary, the reported DT_{50} values were normalized to reference conditions (20°C, pF2), see tables at the end of study *CA 7.1.2.1.2/1* (BF 500-6) and at the end of study *CA 7.1.2.1.2/2* (BF 500-7). For derivation of appropriate degradation endpoints for PEC_{gw} modeling the EFSA endpoint selector was used. Accordingly, the bias-corrected geometric mean of pooled laboratory and field DT_{50} values of 321.0 days for BF 500-6 and 312.0 days for BF 500-7 was used for the PEC_{gw} calculations.

Formation fractions of BF 500-6 and BF 500-7 were reported in the kinetic evaluations of laboratory and field studies with pyraclostrobin either directly from the parent or from BF 500-3 (see table at the end of study *CA 7.1.2.1.2/4* and table at the end of study *CA 7.1.2.2.1/4*).

In the aerobic degradation study with BF 500-4 the metabolites occurred as degradates of BF 500-4 [*CA 7.1.2.1.2/3, Ebert D., Dalkmann P. – BASF DocID 2013/1294779*]. Consequently, this pathway was also assumed for PEC_{gw} calculations. For the preceeding metabolites BF 500-3 and 500-4 formation fractions of 1 were considered, respectively (see above). Thus, for BF 500-6 and BF 500-7 the reported formation fractions from pyraclostrobin or from BF 500-3 can be directly considered. As such the arithmetic mean values derived from pooled field and laboratory data (following the EFSA approach for degradation rates) of 0.293 (BF 500-6) and 0.127 (BF 500-7) were used.

The complete transformation scheme considered for the PEC_{gw} calculations is shown in Figure 9.2.4.1-1.



ff formation fraction

Figure 9.2.4.1-1: Transformation scheme of pyraclostrobin in soil used for PEC_{gw} simulations

Sorption of the metabolites to soil

An overview of individual K_{oc} values for the metabolites of pyraclostrobin considered to select endpoints for PEC_{gw} calculations is given in M-CA 7.1.3.1.2 and summarized in the tables below. For BF 500-3 and BF 500-4 arithmetic mean K_{oc} of 9315 mL g^{-1} (n = 6) and 9819 mL g^{-1} (n = 5) as well as a worst-case default 1/n of 1 were used for PEC_{gw} calculations, respectively. For BF 500-5, an arithmetic mean $K_{f,oc}$ of 705 mL g^{-1} (n = 5) and the corresponding arithmetic mean 1/n of 0.85 were considered, while for BF 500-6 and BF 500-7, the median of 11 K_{oc} values of 107301 mL g^{-1} and 149900 mL g^{-1} and a default 1/n of 1 were used.

Input parameters for pyraclostrobin and its metabolites

A summary of the substance parameters used for the Tier 1 PEC_{gw} calculations is given in Table 9.2.4.1-2 to Table 9.2.4.1-7.

Table 9.2.4.1-2: Overview of input parameters for pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	Value	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	387.8	
Water solubility	[mg L^{-1}]	1.9 (20°C)	
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	2.6 x 10^{-8} (20°C)	
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	28.3 (20°C, pF2)	Bias-corrected geometric mean of field studies (n=8)
Degradation rate to metabolite (PELMO)	[d^{-1}]	0.0245 (to BF 500-3)	=ln2/DT_{50}*formation fraction (ff = 1)
Q_{10} value (PELMO)	[-]	2.58	EFSA opinion
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	5397	Arithmetic mean (n=6)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	9304	
Freundlich exponent 1/n	[-]	0.95	
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

Table 9.2.4.1-3: Overview of input parameters for metabolite BF 500-3 of pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	BF 500-3	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	357.8	
Water solubility	[mg L^{-1}]	0.03 (20°C)	
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	1 x 10^{-10} (20°C)	Worst-case assumption
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	1	Worst-case assumption
Transformation rate (PELMO)	[d^{-1}]	0.6931 (to BF 500-4)	=ln2/DT_{50}*formation fraction (ff = 1)
Formation fraction (PEARL)	[-]	1 (from parent)	Worst-case assumption
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	5403	Arithmetic mean (n=6)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	9315	
Freundlich exponent 1/n	[-]	1	Worst-case assumption
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

Table 9.2.4.1-4: Overview of input parameters for metabolite BF 500-4 of pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	BF 500-4	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	299.8	
Water solubility	[mg L^{-1}]	1000	Worst-case assumption
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	1 x 10^{-10} (20°C)	Worst-case assumption
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	2.82	Geometric mean from laboratory study (n=3)
Transformation rate (PELMO)	[d^{-1}]	0.1008 (to BF 500-5) 0.0720 (to BF 500-6) 0.0312 (to BF-500-7) 0.0418 (to SINK)	=ln2/DT_{50}*formation fraction (ff = 0.409) (ff = 0.293) (ff = 0.127)
Formation fraction (PEARL)	[-]	1 (from BF 500-3)	Worst-case assumption
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	5695	Arithmetic mean (n=5)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	9819	
Freundlich exponent 1/n	[-]	1	Worst-case assumption
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

Table 9.2.4.1-5: Overview of input parameters for metabolite BF 500-5 of pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	BF 500-5	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	194.6	
Water solubility	[mg L^{-1}]	21.8 (20°C)	
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	1 x 10^{-10} (20°C)	Worst-case assumption
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	1	Worst-case assumption
Transformation rate (PELMO)	[d^{-1}]	0.6931 (to SINK)	$=\ln 2/DT_{50}$
Formation fraction (PEARL)	[-]	0.409 (from BF 500-4)	
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	409	Arithmetic mean (n=5)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	705	
Freundlich exponent 1/n	[-]	0.85	
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

Table 9.2.4.1-6: Overview of input parameters for metabolite BF 500-6 of pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	BF 500-6	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	305.8	0.5 of molar mass as metabolite is a dimer
Water solubility	[mg L^{-1}]	0.003 (20°C)	
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	1 x 10^{-10} (20°C)	Worst-case assumption
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	321.0	Bias-corrected geometric mean of lab- and field-DT_{50}
Transformation rate (PELMO)	[d^{-1}]	0.0019 (to SINK)	=ln2/DT_{50}
Formation fraction (PEARL)	[-]	0.293 (from BF 500-4)	
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	62240	Median (n=11)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	107301	
Freundlich exponent 1/n	[-]	1	Worst-case assumption
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

Table 9.2.4.1-7: Overview of input parameters for metabolite BF 500-7 of pyraclostrobin for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	BF 500-7	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	297.8	0.5 of molar mass as metabolite is a dimer
Water solubility	[mg L^{-1}]	0.005 (20°C)	
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure	[Pa]	1 x 10^{-10} (20°C)	Worst-case assumption
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water	[m^2 d^{-1}]	4.3 x 10^{-5} (20°C)	
Diffusion coefficient in gas	[m^2 d^{-1}]	0.43 (20°C) (PEARL)	
	[cm^2 s^{-1}]	0.05 (PELMO)	
DEGRADATION PARAMETERS			
Half-life at reference conditions	[d]	312.0	Bias-corrected geometric mean of lab- and field-DT_{50}
Transformation rate (PELMO)	[d^{-1}]	0.0022 (to SINK)	=ln2/DT_{50}
Formation fraction (PEARL)	[-]	0.127 (from BF 500-4)	
Arrhenius activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Exponent of moisture correction function	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
$K_{f,om}$ value (PEARL)	[mL g^{-1}]	86949	Median (n=11)
$K_{f,oc}$ value (PELMO)	[mL g^{-1}]	149900	
Freundlich exponent 1/n	[-]	1	Worst-case assumption
pH dependency	[-]	pH independent	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0	

II. RESULTS AND DISCUSSION

The 80th percentiles of the predicted annual leachate concentrations in groundwater after application to potatoes were below 0.001 µg L^{-1} for pyraclostrobin and its metabolites BF 500-3, BF 500-4, BF 500-5, BF 500-6 and BF 500-7 for all scenarios. Consequently, the leaching of unacceptable amounts of pyraclostrobin after application to potatoes is highly unlikely.

III. CONCLUSION

Predicted environmental concentrations in groundwater (PEC_{gw}) of pyraclostrobin and its metabolite BF 500-3, BF 500-4, BF 500-5, BF 500-6 and BF 500-7 were calculated following application of BAS 516 07 F to potatoes. A tiered approach was considered to address the risk for groundwater following the guidelines of FOCUS groundwater. No risk was identified for the parent compound, nor for its soil metabolites at Tier 1 (PEC_{gw} < 0.001 µg L^{-1}).

Report: CP 9.2.4/2
Kallweit W., 2014b
Predicted environmental concentrations of BAS 510 F - Boscalid in soil, groundwater, surface water and sediment following application to potatoes in Europe
2014/1001461

Guidelines: SANCO/321/2000, FOCUS Groundwater (2012) Generic guidance for FOCUS groundwater scenarios v 2.1, FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011, SANCO/4802/2001 rev. 2 (FOCUS surface water scenarios) May 2003, FOCUS Surface Water (2012) SANCO/4802/2001 v1.1, FOCUS groundwater (2009): SANCO/13144/2009

GLP: no

Executive Summary

PEC_{gw} values were calculated according to the guidance of the FOCUS groundwater scenarios workgroup using the models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3. All scenarios available for the $FOCUS_{gw}$ crop potatoes were considered for the leaching assessment. Continuous cropping over a period of 26 years was assumed and annual application of the active substance was taken into account. Calculations were based on the worst-case scenario considered for calculating PEC_{soil}.

The 80th percentiles of the predicted annual average leachate concentrations of boscalid were below 0.001 µg L^{-1} in all tested scenarios. Therefore, the leaching of unacceptable amounts of boscalid is highly unlikely.

I. MATERIAL AND METHODS

The leaching assessment for boscalid was conducted at Tier 1 of the tiered assessment scheme proposed by the FOCUS groundwater higher tier working group. The simulations were carried out using FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3 in combination with the FOCUS standard scenarios.

Application scenario

PEC_{gw} values of boscalid were calculated for the same worst-case application scenario as for the PEC_{soil} assessment (M-CP 9.1.3). All FOCUS groundwater scenarios that are parameterized for the $FOCUS_{gw}$ crop potatoes were considered.

Application dates were selected following FOCUS recommendations and are equal to the ones for pyraclostrobin. A summary of the application scenario considered for the PEC_{gw} calculations is given in Table 9.2.4.1-8.

Table 9.2.4.1-8: Worst-case application scenario of boscalid applied to potatoes considered for the PEC_{gw} calculations

Crop	Potatoes
$FOCUS_{gw}$ crop	Potatoes
Max. no. of applications [-]	4
Crop growth stage at first application [BBCH]	51
Minimum application interval [d]	10
Application rate[g a.s. ha^{-1}]	67
Interception [%]	80 / 80 / 80 / 80
Amount reaching the soil surface [g a.s. ha^{-1}]	13.4 / 13.4 / 13.4 / 13.4
Total yearly soil load [g a.s. ha^{-1}]	53.6

Environmental fate parameters

Degradation of boscalid in soil

The degradation behavior of boscalid in soil was described using the geometric mean DT_{50} of 130.0 days that was calculated from field DT_{50} normalized to 20°C (Q_{10} = 2.2) (see M-CP 9.3.1 for details). PEC_{gw} were calculated using a Q_{10} value of 2.58 as recommended by EFSA [*EFSA (2007) "Opinion on a request from EFSA related to the default Q_{10} value used to describe the temperature effect on transformation rates of pesticides in soil. Scientific Opinion of the Panel on Plant Protection Products and their Residues (PPR-Panel). Question No EFSA-Q-2007-048. The EFSA Journal (2007) 622, 1-32*]. The combination of Q_{10} = 2.2 for normalization and Q_{10} = 2.58 for simulation leads to worse-case degradation conditions for temperatures below 20°C, which are predominant in the FOCUS scenarios. The leaching assessment can therefore be considered conservative regarding the use of the Q_{10} value.

Sorption of boscalid to soil

The sorption behavior of boscalid was investigated in batch-equilibrium sorption studies for a variety of soils [*Seher, A. – BASF DocID 1998/10513*]. The $K_{f,oc}$-values ranged from 507 to 1110 mL g^{-1} with corresponding Freundlich exponents 1/n between 0.839 and 0.887. The respective arithmetic mean $K_{f,oc}$ of 771 mL g^{-1} (corresponding to a $K_{f,om}$ value of 447 mL g^{-1}) and Freundlich exponent of 0.864 were used for the PEC_{gw} calculations.

Summary of input parameters

The substance specific input parameters such as DT_{50}, $K_{f,oc}$, molecular mass, vapor pressure and aqueous solubility as well as further parameters, which are set to standard assumptions, are given in Table 9.2.4.1-9.

Table 9.2.4.1-9: Overview of input parameters for boscalid for the leaching simulation models FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3

Input parameter	Unit	Value	Remarks
PHYSICO-CHEMICAL PARAMETERS			
Molecular weight	[g mol^{-1}]	343.2	Phys.-chem. properties
Water solubility (20°C)	[mg L^{-1}]	4.6	Phys.-chem. properties
Molar enthalpy of dissolution	[kJ mol^{-1}]	27	FOCUS recommendation
Saturated vapor pressure (20°C)	[Pa]	1×10^{-10}	Default value
Molar enthalpy of vaporization	[kJ mol^{-1}]	95	FOCUS recommendation
Diffusion coefficient in water (20°C) (PEARL)	[m^2 d^{-1}]	4.3×10^{-5}	FOCUS recommendation
Diffusion coefficient in gas (20°C)	[m^2 d^{-1}] [cm^2 s^{-1}]	0.43 (PEARL) 0.05 (PELMO)	FOCUS recommendation
DEGRADATION PARAMETERS			
Half-life at reference temperature (20°C)	[d]	130.0	Geometric mean of field studies (n = 3, standardized)
Transformation rate to sink (PELMO)	[d^{-1}]	0.005332	Calculated (ln(2) / DT_{50})
Molar activation energy (PEARL)	[kJ mol^{-1}]	65.4	EFSA opinion
Q_{10} value (PELMO)	[-]	2.58	
Exponent of moisture correction function (PEARL)	[-]	0.7	FOCUS recommendation
SORPTION PARAMETERS			
Soil adsorption option	[-]	pH-independent	-
$K_{f,oc}$ value	[mL g^{-1}]	771	Arithmetic mean (n=6)
$K_{f,om}$ value	[mL g^{-1}]	447	
Freundlich exponent 1/n	[-]	0.864	
CROP RELATED PARAMETERS			
TSCF (crop uptake)	[-]	0.5	FOCUS recommendation

II. RESULTS AND DISCUSSION

The simulated concentrations of boscalid in groundwater following fourfold application of 67 g a.s. ha^{-1} to potatoes were < 0.001 µg L^{-1} in all tested scenarios using both models. Consequently, the leaching of unacceptable amounts of boscalid after application to potatoes is highly unlikely.

III. CONCLUSION

Predicted environmental concentrations in groundwater (PEC_{gw}) of boscalid were calculated following application of BAS 516 07 F to potatoes. PEC values in groundwater were derived in accordance with the guidance of the FOCUS groundwater scenarios work group. The 80th percentile average annual leachate concentrations of boscalid were below 0.001 µg L^{-1} in all tested scenarios. The leaching of unacceptable amounts of boscalid after application to potatoes is highly unlikely.

CP 9.2.4.2 Additional field tests

No additional field tests were performed with BAS 516 07 F. Data for the active substance and its metabolites are covered by information given in M-CA 7.

CP 9.2.5 Estimation of concentrations in surface water and sediment

Predicted environmental concentrations in surface water (PEC_{SW}) and sediment (PEC_{SED})

Report: CP 9.2.5/1
Kallweit W., 2014a
Predicted environmental concentrations of BAS 500 F - Pyraclostrobin and its metabolites in soil, groundwater, surface water and sediment following application to cereals, maize and potatoes
2014/1000685

Guidelines: FOCUS Groundwater (2000) Sanco/321/2000, FOCUS groundwater (2009): SANCO/13144/2010, FOCUS Groundwater (2012) Generic guidance for FOCUS groundwater scenarios v 2.1, FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011, FOCUS Surface Water Report SANCO/4802/2001 rev. 2, FOCUS Surface Water (2012) Generic guidance for FOCUS surface water scenarios v 1.1, FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 139 pp., FOCUS (2007): Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations SANCO/10422/2005 v2.0. 169 pp.

GLP: no

Executive Summary

Predicted environmental concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) were calculated for pyraclostrobin after single and fourfold spray application of 17 g a.s. ha^{-1} to potatoes considering the entry pathways spray drift, drainage and runoff.

The metabolites of pyraclostrobin which were observed in major amounts in water (BF 500-5, BF 500-11, BF 500-13, BF 500-14) and in sediment (BF 500-3, BF 500-6, BF 500-7) were also considered for the assessment in the respective environmental compartments, taking into account their formation after entry of the parent substance.

Additionally, for the metabolites BF 500-6 and BF 500-7 occurring in major amounts in soil under aerobic conditions the entry pathways runoff and drainage of the metabolites into surface water were taken into account. Since both metabolites have very low water solubilities (2 - 3 µg L^{-1}) and extremely high sorption values (K_{oc}>10^5 mL g^{-1}), they will reach surface water bodies via runoff or drainage in a soil particle-bound form. They then sink to the sediment surface, i.e. they are extremely unlikely to occur freely dissolved in molecular form in the water phase. Consequently BF 500-6 and BF 500-7 were only considered for PEC calculations in the sediment compartment.

For the metabolites BF 500-3 and BF 500-5 runoff and drainage of the metabolites are not relevant as entry pathways into surface water as the metabolites did not occur in relevant amounts in soil under aerobic conditions. The metabolite BF 500-4 was not found either in water/sediment studies or in soil under aerobic conditions and was therefore not considered in the assessment.

The calculations were performed according to the FOCUS surface water guidance documents [*FOCUS (2001, 2012)*].

Calculations for pyraclostrobin were carried out at Step 1 to Step 3. A tiered approach was considered for the parent substance, with endpoints derived from a dark water/sediment study being used at Tier 1 and endpoints from an irradiated water/sediment study being used at Tier 2. PEC values for the metabolites were calculated for FOCUS Step 1 and 2 level. Input parameters for the metabolites were derived from the study in which they occurred, i.e. in dark or irradiated water/sediment systems.

The software packages STEPS1-2 in FOCUS version 2.1, FOCUS-PRZM version 1.5.6, FOCUS-MACRO version 4.4.2 and FOCUS-TOXSWA version 3.3.1 for Step 3 were used for the calculations. At Step 2 of the assessment, the regions ‘North Europe’ and ‘South Europe’ were taken into account. At Step 3, all FOCUS scenarios available for potatoes were considered.

A summary of the maximum PEC_{sw} of pyraclostrobin and its metabolites calculated for each step of the assessment is given in the tables below.

Pyraclostrobin

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-1: Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 17 g a.s. ha^{-1} to potatoes (Tier 1)

FOCUS level	SURFACE WATER			SEDIMENT
	Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	2.316	-[a]	-[a]	157.319
Step 2	0.164	-[a]	-[a]	14.142
Step 3	0.088 D3 d + D6 d, single	0.065 D6 d, single	0.043 D6 d, multiple	1.550 R1 s, multiple

s = stream, d = ditch
single = single application, multiple = multiple application
[a] only maximum values for Step 1 and 2 are reported

Tier 2 (parameters from irradiated water/sediment study)

Table 9.2.5-2: Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 17 g a.s. ha^{-1} to potatoes (Tier 2)

FOCUS level	SURFACE WATER			SEDIMENT
	Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	2.316	-[a]	-[a]	157.319
Step 2	0.156	-[a]	-[a]	13.003
Step 3	0.088 D3 d, single	0.062 D6 d, single	0.040 D6 d, multiple	1.327 R1 s, multiple

s = stream, d = ditch
single = single application, multiple = multiple application
[a] only maximum values for Step 1 and 2 are reported

Metabolites of pyraclostrobin

Metabolites observed in dark water/sediment study: BF 500-3, BF 500-5, BF 500-6, BF 500-7

Parameters from dark water/sediment study

Table 9.2.5-3: Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 17 g a.s. ha^{-1} to potatoes

Potatoes				
Compound	SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]		SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]	
	Step 1	Step 2	Step 1	Step 2
BF 500-3	not relevant [1]		2.724	1.805
BF 500-6	not relevant [2]		41.471	4.912
BF 500-7			24.885	2.989
BF 500-5	0.035	0.016	not relevant [3]	

[1] BF 500-3 did not occur in soil or water phase in significant amounts
[2] BF 500-6 and BF 500-7 will not occur free in water phase due to low water solubility and high sorption values
[3] BF 500-5 did not occur in soil or sediment phase in significant amounts

Metabolites observed in irradiated water/sediment study: BF 500-3, BF 500-11, BF 500-13, BF 500-14

Parameters from irradiated water/sediment study

Table 9.2.5-4: Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 17 g a.s. ha^{-1} to potatoes (irradiated water/sediment study)

Potatoes				
Compound	SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]		SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]	
	Step 1	Step 2	Step 1	Step 2
BF 500-3	not relevant [1]		0.885	0.511
BF 500-11	0.055	0.024	not relevant [2]	
BF 500-13	0.072	0.041		
BF 500-14	0.078	0.031		

[1] BF 500-3 did not occur in soil or water phase in significant amounts
[2] BF 500-11, BF 500-13 and BF 500-14 did not occur in soil or sediment phase in significant amounts

I. MATERIAL AND METHODS

Predicted environmental concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) were calculated for pyraclostrobin after single and fourfold spray application of 17 g a.s. ha^{-1} to potatoes considering the entry pathways spray drift, drainage and runoff.

The metabolites of pyraclostrobin, which were observed in major amounts in water (BF 500-5, BF 500-11, BF 500-13, BF 500-14) and in sediment (BF 500-3, BF 500-6, BF 500-7), were also considered for the assessment in the respective environmental compartments, taking into account their formation after entry of the parent substance. Additionally, for the metabolites occurring in major amounts in soil under aerobic conditions (BF 500-6, BF 500-7) the entry pathways runoff and drainage of the metabolites into surface water were taken into account.

Since both metabolites have very low water solubilities (2 - 3 µg L^{-1}) and extremely high sorption values (K_{oc}>10^5 mL g^{-1}), they will reach surface water bodies via runoff or drainage in a soil particle-bound form. They then sink to the sediment surface, i.e. they are extremely unlikely to occur freely dissolved in molecular form in the water phase. Consequently BF 500-6 and BF 500-7 were only considered for PEC calculations in the sediment compartment.

For the metabolites BF 500-3 and BF 500-5 runoff and drainage of the metabolites are not relevant as entry pathways into surface water as the metabolites did not occur in relevant amounts in soil under aerobic conditions. Metabolite BF 500-4 was neither found in water/sediment studies nor in soil under aerobic conditions and was therefore not considered in the assessment.

The calculations were performed according to the FOCUS surface water guidance documents [*FOCUS (2001): FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC Report of the FOCUS Working Group on Surface Water Scenarios. EC Document Reference SANCO/4802/2001-rev.2. final. 238 pp; FOCUS (2012): "FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC" Report of the FOCUS Working Group on Surface Water Scenarios. EC Document Reference SANCO/4802/2001 version 1.2. 357 pp.*].

Application scenarios

The worst-case application scenarios of pyraclostrobin considered for PEC_{sw} and PEC_{sed} calculations are given in Table 9.2.5-29.

Table 9.2.5-5: Application scenarios of pyraclostrobin applied to potatoes considered for PEC_{sw} and PEC_{sed} calculations

$FOCUS_{sw}$ crop	Potatoes
Growth stage [BBCH]	51
Application rate [g a.s. ha^{-1}]	17
Max. no. of applications [-]	4
Interval [d]	10
Total yearly application rate [g a.s. ha^{-1}]	80 / 80 / 80 / 80

Environmental fate parameters

Fate and behavior in soil

Degradation and sorption of pyraclostrobin

The degradation behavior of pyraclostrobin in soil has been investigated under both laboratory and field conditions. An overview of individual DT_{50} considered to select endpoints for PEC calculations is given in the tables at the end of study *CA 7.1.2.1.1/3* and study *CA 7.1.2.2.1/4*. The sorption behavior of pyraclostrobin is described in M-CP 9.2.4 (section on PEC_{gw}).

The normalized bias-corrected geometric mean half-life of 28.3 days (at 20°C and pF2) from the eight relevant field trials, an arithmetic mean $K_{f,oc}$ of 9304 mL g^{-1} and the corresponding 1/n of 0.95 were used for the PEC_{sw} calculations.

Degradation and sorption of the metabolites of pyraclostrobin

The degradation behavior of the metabolites of pyraclostrobin in soil has been investigated under laboratory (BF 500-3, BF 500-5, BF 500-6, BF 500-7) and field conditions (BF 500-6, BF 500-7). An overview of individual DT_{50} considered to select endpoints for PEC calculations is given in the tables at the end of study *CA 7.1.2.1.2/4* and study *CA 7.1.2.2.1/4*. The sorption behavior of the metabolites of pyraclostrobin is described in M-CP 9.2.4 (section on PEC_{gw}).

As the metabolites BF 500-3 and BF 500-5 did not occur in relevant amounts in soil under aerobic conditions, a maximum occurrence in soil of 0.01% and a DT_{50} of 1 day were assumed as worst-case default input parameters. The arithmetic mean K_{oc} values of 9315 mL g^{-1} and 705 mL g^{-1} for BF 500-3 and BF 500-5, respectively, were considered for the calculations.

For the metabolite BF 500-6 a maximum occurrence in soil of 31% TAR observed in the laboratory, a bias-corrected geometric mean DT_{50} of 321.0 days and a median K_{oc} value of 107301 mL g^{-1} were used as modeling input.

For the metabolite BF 500-7 a maximum occurrence in soil of 19% TAR observed in field degradation studies, a bias-corrected geometric mean DT_{50} of 312.0 days and a median K_{oc} value of 149900 mL g^{-1} were used as modeling input.

The metabolites BF 500-11, BF 500-13 and BF 500-14 occur only in water. As there are no data on soil degradation and sorption available, a maximum occurrence in soil of 0.01%, a DT_{50} of 1 day and a K_{oc} of 1 x 10^{-10} mL g^{-1} were assumed as worst-case default input parameters.

Fate and behavior in aquatic systems

Pyraclostrobin

Degradation and partitioning of pyraclostrobin in aquatic systems have been investigated in a range of studies with different test designs and an increasing level of environmental relevance: dark and irradiated water/sediment systems and outdoor mesocosm [*old EU Dossier, A II M 7.2.1.3.2/1, Staudenmaier H. - BASF DocID 1999/11241*; *old EU Dossier, A II M 7.2.1.3.2/2, Ebert D. - BASF DocID 1999/11791*; *CA 7.2.2.4/2, Ebert D. – BASF DocID 2011/1101715; old EU Dossier, A III M 10.2.2/1, Dohmen G. P. - BASF DocID 2000/1000011*]. In order to reflect the different test designs in the model calculations a tiered approach was considered.

At **Tier 1** the assessment was based on the results of the water/sediment study conducted under dark laboratory conditions [*Staudenmaier H. - BASF DocID 1999/11241*]. The degradation kinetics were re-evaluated in a separate study [*CA 7.2.2.3/1, Wiedemann G. – BASF DocID 2012/1165029*]. At Level P-I, for System A whole system DT_{50} values of 23.3 and 26.8 days were reported for chlorophenyl- and tolyl-labeled pyraclostrobin, respectively, while for System B no reliable endpoints could be derived. The kinetic evaluation at Level P-II did not result in reliable endpoints. According to FOCUS kinetics a case-by-case decision should be made if reliable endpoints (P-I, System B) cannot be derived with the recommended standard procedures. For System B a $DegT_{90}$ in the whole system of approximately 30 days can be estimated visually, which is considerably shorter than the endpoint calculated for System A. Hence, the results of the kinetic evaluation of System A represent the worst-case of the two w/s systems investigated. Consequently, only the results of the kinetic evaluation of System A are to be considered as worst-case for further usage. In accordance with FOCUS degradation kinetics [*FOCUS (2006)*] the geometric mean DT_{50} in the total system of 25.0 days was considered for the total system at Step 1. Due to the high sorption tendency of pyraclostrobin the total system DT_{50} value of 25.0 days was used at Step 2 and 3 for the sediment phase, while the conservative default value of 1000 days was used for the water phase.

At **Tier 2** the assessment was based on the results of the water/sediment-studies conducted under irradiated laboratory conditions [*Ebert D. - BASF DocID 1999/11791*; *Ebert D. – BASF DocID 2011/1101715*]. While in *Ebert D.* [*BASF DocID 2011/1101715*] degradation endpoints could be derived only at Level P-I, the kinetic re-evaluation of *Ebert D.* [*BASF DocID 1999/11791*] resulted in appropriate degradation endpoints at Level P-I and Level P-II [*CA 7.2.2.4/1, Miles B. - BASF DocID 2012/1021122*]. The reported endpoints (dissipation half-life in water of 4.47 days) are considered as a realistic worst-case scenario compared to the outdoor mesocosm study, where a dissipation half-life in water of 3.5 days was reported. Consequently, the respective Level P-II degradation half-lives of 7.50 days in water and 6.48 days in sediment were used as input for modeling at Step 2 and 3 while for Step 1 calculations a $DegT_{50}$ in the total system calculated at Level P-I of 7.2 days was considered in accordance with FOCUS kinetics [*FOCUS (2006)*].

Metabolites of pyraclostrobin

In the dark water/sediment study the metabolites BF 500-3, BF 500-6 and BF 500-7 occurred as major metabolites [*Staudenmaier H. - BASF DocID 1999/11241*]. Additionally, BF 500-3, BF 500-11, BF 500-13 and BF 500-14 occurred in relevant amounts in the irradiated water/sediment study [*Ebert D. - BASF DocID 1999/11791*], and BF 500-5 was detected as major metabolite in the study on aerobic mineralization in surface water [*CA 7.2.2.2/1, Ebert D., Possienke M. – BASF DocID 2013/1002741*]. An overview of the DT_{50} values for the metabolites of pyraclostrobin in water/sediment systems is shown at the end of M-CA 7.2.2.

BF 500-3 was observed at amounts >5% TAR in the sediment compartment of the water/sediment studies under dark as well as under irradiated conditions, whereas in the water compartment no residues above 5% TAR were detected [*Staudenmaier H. - BASF DocID 1999/11241; Ebert D. - BASF DocID 1999/11791; Ebert D. – BASF DocID 2011/1101715*]. Therefore, PEC_{sed} calculations were conducted considering the results from the dark and the irradiated studies separately. Regarding the calculations representing the dark conditions, the maximum occurrence in the total system (mean of two labels) of 67.7% TAR [*Staudenmaier H. - BASF DocID 1999/11241*] and a conservative default DT_{50} of 1000 days were used, while for irradiated conditions the maximum occurrence in the total system of 21.9% TAR and a DT_{50} in the total system of 92.5 days from the kinetic re-evaluation were considered [*Ebert D. - BASF DocID 1999/11791; Miles B. - BASF DocID 2012/1021122*].

BF 500-5 was observed as major metabolite in the water phase of the study on aerobic mineralization in surface water, whereas it did not occur in relevant amounts in the sediment phase [*Ebert D., Possienke M. – BASF DocID 2013/1002741*]. Therefore, PEC_{sw} calculations were conducted considering the maximum occurrence of 10.9% TAR observed in the pelagic test and a conservative default DT_{50} of 1000 days.

BF 500-6 and BF 500-7 were observed at amounts >5% TAR in the sediment compartment of the water/sediment study under dark conditions, whereas in the water compartment no residues above 5% TAR were detected [*Staudenmaier H. - BASF DocID 1999/11241*]. Therefore, PEC_{sed} calculations were conducted considering the maximum occurrences of 6.5% TAR and 6.3% TAR (mean of two labels) for BF 500-6 and BF 500-7, respectively, and a conservative default DT_{50} of 1000 days.

BF 500-11, BF 500-13 and BF 500-14 were observed as major metabolites under irradiated conditions in the water compartment, whereas they did not occur in relevant amounts in the sediment compartment [*Ebert D. - BASF DocID 1999/11791*]. Therefore, PEC_{sw} calculations were conducted considering the sum of the maximum occurrences in water and sediment of 12.0% TAR, 17.8% TAR and 12.1% TAR for BF 500-11, BF 500-13 and BF 500-14, respectively. For BF 500-11 and BF 500-14 the DT_{50} values in the total system of 22.6 days and 17.3 days from the kinetic re-evaluation were considered, respectively [*Miles B. - BASF DocID 2012/1021122*]. For BF 500-13 a reliable half-life could not be estimated from the data of the irradiated water/sediment study because the maximum occurrence of the metabolite was at the last sampling date [*Ebert D. - BASF DocID 1999/11791*]. However, results of the aqueous photolysis study show a significant degradation of BF 500-13 in water with a calculated half-life of 30.7 days (rounded value) under continuous irradiation [*old EU Dossier, A II M 7.2.1.2/2, Scharf J. - BASF DocID 1999/11286*]. In order to represent a realistic radiation scenario (12 h radiation followed by 12 h darkness) the reported half-life was multiplied with a factor of 2 resulting in a DT_{50} of 61.3 days, which was used as input parameter for PEC_{sed} calculations.

Surface water assessment according to FOCUS

The calculations were performed in a stepwise approach according to the recommendations of the FOCUS working group on surface water scenarios (2001, 2012).

For all calculations, the following model versions were used: STEPS1-2 in FOCUS version 2.1, FOCUS-PRZM version 1.5.6, FOCUS-MACRO version 4.4.2 and FOCUS-TOXSWA version 3.3.1. The modeling runs were set up with SWASH version 3.1.

Setup of FOCUS surface water runs

Pyraclostrobin

FOCUS Step 1 to Step 3 calculations were carried out for PEC_{sw} and PEC_{sed} for pyraclostrobin considering the entry routes spray drift, drainage and runoff for spray application to potatoes.

STEP 1 and Step 2 scenario settings

At Step 1 and 2, appropriate application periods and parameters for crop interception according to the actual GAP for potatoes were considered as shown in Table 9.2.5-6. The regions 'North Europe' and 'South Europe' as implemented in FOCUS STEPS1-2 were considered.

Table 9.2.5-6: Steps 1-2 FOCUS scenarios for the selected FOCUS crops

FOCUS$_{sw}$ crop	Interception class	Application period	Region
Potatoes	Full canopy	Mar – May	North and South Europe

Step 3 scenario settings

At Step 3, all FOCUS scenarios parameterized for potatoes (D3, D4, D6, R1 - R3) were selected for the simulations. In the SWASH shell the application method was set to ‘ground spray’. The chemical application method (CAM) was set to option '2' (application to foliage). The length of the application window that is required for the Pesticide Application Tool (PAT) to determine actual application dates was chosen to cover the whole application window as specified in the GAP. Considering the minimum application interval of 10 days, a minimum length of the application window of 60 days is required for multiple application to potatoes. The actual dates for field application differ according to the crop development in the different European regions and from year to year.

The start date of the application windows was set to coincide with the earliest growth stage defined for application to potatoes in the GAP (BBCH 51). To cover the whole application period according to GAP, the first application was assumed to take place 30 days after emergence and the last application was assumed 3 days prior to harvest (derived from the pre-harvest interval (PHI) provided in the GAP).

The detailed application timing used for the simulations is shown in Table 9.2.5-7.

Table 9.2.5-7: Application timing for pyraclostrobin in potatoes in the relevant scenarios (Step 3)

Scenario	Water body	Application window[a]	Application dates according to PAT[b]
Potatoes			
D3 - Vredepeel	ditch	9th June - 12th September	14th June / 26th June / 8th July / 24th July
D4 - Skousbo	pond	21st June - 20th September	21st June / 4th July / 27th August / 10th September
D4 - Skousbo	stream	21st June - 20th September	21st June / 4th July / 27th August / 10th September
D6 – Thiva (1st)	ditch	10th May - 12th July	17th May / 4th June / 23rd June / 5th July
D6 – Thiva (2nd)	ditch	4th September - 22nd November	4th September / 14th September / 27th September / 7th October
R1 - Weiherbach	pond	4th June - 5th September	9th June / 29th June / 11th July / 28th July
R1 - Weiherbach	stream	4th June - 5th September	9th June / 29th June / 11th July / 28th July
R2 - Porto	stream	13th April - 12th June	22nd April / 7th May / 20th May / 3rd June
R3 - Bologna	stream	10th May - 29th August	18th May / 1st June / 11th June / 21st June

[a] calculations for single and multiple applications were conducted in separate runs
[b] for calculation of single application the first date was considered

Metabolites of pyraclostrobin

For the metabolites of pyraclostrobin, PEC values were calculated at FOCUS Step 1 and 2. The results of the calculations are reported for those compartments where the respective metabolites had been detected as major metabolites. The simulations were carried out according to the approach and application scenarios for the parent compound.

Overview of the FOCUS levels used for the simulations

Table 9.2.5-8 gives an overview of the FOCUS levels, at which the PEC values of the respective compounds were simulated.

Table 9.2.5-8: Summary of the selection of the FOCUS levels for each compound

Compound	PEC_{sw} calculations conducted with FOCUS Step	PEC_{sed} calculations conducted with FOCUS Step	Reported PEC_{sw}	Reported PEC_{sed}
Pyraclostrobin	1, 2, 3, 4	1, 2, 3	max, act, twa	max
BF 500-3	-[1]	1, 2	-[1]	max
BF 500-5	1, 2	-[2]	max	-[2]
BF 500-6	-[3]	1, 2	-[3]	max
BF 500-7	-[3]	1, 2	-[3]	max
BF 500-11	1, 2	-[4]	max	-[4]
BF 500-13	1, 2	-[4]	max	-[4]
BF 500-14	1, 2	-[4]	max	-[4]

[1] BF 500-3 did not occur in soil or water phase in significant amounts
[2] BF 500-5 did not occur in soil or sediment phase in significant amounts
[3] BF 500-6 and BF 500-7 will not occur free in water phase due to low water solubility and high sorption values
[4] BF 500-11, BF 500-13 and BF 500-14 did not occur in soil or sediment phase in significant amounts

Pyraclostrobin

For a summary of the environmental fate parameters of pyraclostrobin used for modeling at Step 1 to Step 3 of the assessment, see Table 9.2.5-9. The input parameters for the metabolites are summarized in Table 9.2.5-10 to Table 9.2.5-16. Some default assumptions were made as the measured data could not cover all required model input, e.g. solubility in water, maximum occurrence in soil, DT_{50} in soil and K_{oc} of the aquatic metabolites BF 500-11, BF 500-13 and BF 500-14.

Table 9.2.5-9: Summary of FOCUS input parameters for pyraclostrobin

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	
Molecular weight [g mol^{-1}]	387.8	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	1.9	Phys.-chem. properties
Vapor pressure at 20°C [Pa]	2.6×10^{-8}	Phys.-chem. properties
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	28.3	Bias-corrected geometric mean of field study
Temperature correction function Reference temperature [°C] MACRO temp. exponent [K^{-1}] PRZM Q_{10} [-]	 20 0.095 2.58	EFSA opinion
Moisture correction function Reference moisture [-] PRZM / MACRO moisture exponent [-]	 pF 2 0.7	FOCUS recommendation
SORPTION TO SOIL		
$K_{f,oc}$ [mL g^{-1}]	9304	Arithmetic mean (n=6)
1/n [-]	0.95	Arithmetic mean (n=6)
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1) - Tier 1 - Tier 2	 25.0 7.2	 System $DegT_{50}$ level P-I (dark study) System $DegT_{50}$ level P-I (irradiated study)
DT_{50} water [d] (Step 2, Step 3) - Tier 1 - Tier 2	 1000 7.5	 Conservative assumption Water $DegT_{50}$ level P-II (irradiated study)
DT_{50} sediment [d] (Step 2, Step 3) - Tier 1 - Tier 2	 25.0 6.5	 System $DegT_{50}$ level P-I (dark study) Sediment $DegT_{50}$ level P-II (irradiated study)
DT_{50} crop [d] (Step 3)	10	FOCUS recommendation
Temperature correction function Reference temperature [°C] TOXSWA: activation energy [J mol^{-1}]	 20 65400	EFSA opinion
MANAGEMENT RELATED PARAMETERS		
Crop uptake factor [-]	0	
Wash off coefficient PRZM: [cm^{-1}] MACRO: [mm^{-1}]	 0.5 0.05	FOCUS recommendation

Table 9.2.5-10: Summary of FOCUS input parameters for BF 500-3

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	357.8	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	0.03	Phys.-chem. properties
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	1	Worst-case default value
Max. observed occurrence in soil [%]	0.01	Worst-case default value
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	9315	Arithmetic mean (n=6)
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1) - parameters from dark study - parameters from irradiated study	 1000 92.5	 Worst-case default value System DT_{50} level M-I (irradiated study)
DT_{50} water [d] (Step 2) - parameters from dark study - parameters from irradiated study	 1000 92.5	 Worst-case default value System DT_{50} level M-I (irradiated study)
DT_{50} sediment [d] (Step 2) - parameters from dark study - parameters from irradiated study	 1000 92.5	 Worst-case default value System DT_{50} level M-I (irradiated study)
Sum of max. observed occurrence in water/sediment [%] - parameters from dark study - parameters from irradiated study	 67.7 21.9	 Dark water/sediment study Irradiated water/sediment study

Table 9.2.5-11: Summary of FOCUS input parameters for BF 500-5

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	194.6	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	21.8	
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	1	Worst-case default value
Max. observed occurrence in soil [%]	0.01	Worst-case default value
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	705	Arithmetic mean (n=5)
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	1000	Worst-case default value
DT_{50} water [d] (Step 2)	1000	Worst-case default value
DT_{50} sediment [d] (Step 2)	1000	Worst-case default value
Sum of max. observed occurrence in water/sediment [%]	10.9	Study on aerobic mineralization in surface water

Table 9.2.5-12: Summary of FOCUS input parameters for BF 500-6

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	305.8	0.5 of molar mass as metabolite is a dimer
Water solubility at 20°C [mg L^{-1}]	0.003	Phys.-chem. properties
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	321.0	Bias-corrected geometric mean from pooled laboratory and field studies
Max. observed occurrence in soil [%]	31.0	Maximum in laboratory study
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	107301	Median (n=11)
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	1000	Worst-case default value
DT_{50} water [d] (Step 2)	1000	Worst-case default value
DT_{50} sediment [d] (Step 2)	1000	Worst-case default value
Sum of max. observed occurrence in water/sediment [%]	6.5	Dark water/sediment study

Table 9.2.5-13: Summary of FOCUS input parameters for BF 500-7

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	297.8	0.5 of molar mass as metabolite is a dimer
Water solubility at 20°C [mg L^{-1}]	0.005	Phys.-chem. properties
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	312.0	Bias-corrected geometric mean from pooled laboratory and field studies
Max. observed occurrence in soil [%]	19.0	Maximum in field degradation study
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	149900	Median (n=11)
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	1000	Worst-case default value
DT_{50} water [d] (Step 2)	1000	Worst-case default value
DT_{50} sediment [d] (Step 2)	1000	Worst-case default value
Sum of max. observed occurrence in water/sediment [%]	6.3	Dark water/sediment study

Table 9.2.5-14: Summary of FOCUS input parameters for BF 500-11

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	277.3	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	1000	Worst-case default value
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	1	Worst-case default value
Max. observed occurrence in soil [%]	0.01	Worst-case default value
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	1×10^{-10}	Worst-case default value
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	22.6	System DT_{50} level M-I, irradiated water/sediment study
DT_{50} water [d] (Step 2)	22.6	System DT_{50} level M-I, irradiated water/sediment study
DT_{50} sediment [d] (Step 2)	22.6	System DT_{50} level M-I, irradiated water/sediment study
Sum of max. observed occurrence in water/sediment [%]	12.0	Irradiated water/sediment study

Table 9.2.5-15: Summary of FOCUS input parameters for BF 500-13

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	247.3	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	1000	Worst-case default value
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	1	Worst-case default value
Max. observed occurrence in soil [%]	0.01	Worst-case default value
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	1 x 10^{-10}	Worst-case default value
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	1000	Worst-case default value
DT_{50} water [d] (Step 2)	61.3	Aqueous photolysis study
DT_{50} sediment [d] (Step 2)	1000	Worst-case default value
Sum of max. observed occurrence in water/sediment [%]	17.8	Irradiated water/sediment study

Table 9.2.5-16: Summary of FOCUS input parameters for BF 500-14

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	387.8	Phys.-chem. properties
Water solubility at 20°C [mg L^{-1}]	1000	Worst-case default value
DEGRADATION IN SOIL		
DT_{50} (soil) [d]	1	Worst-case default value
Max. observed occurrence in soil [%]	0.01	Worst-case default value
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	1 x 10^{-10}	Worst-case default value
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total aquatic system [d] (Step 1)	17.3	System DT_{50} level M-I, irradiated water/sediment study
DT_{50} water [d] (Step 2)	17.3	System DT_{50} level M-I, irradiated water/sediment study
DT_{50} sediment [d] (Step 2)	17.3	System DT_{50} level M-I, irradiated water/sediment study
Sum of max. observed occurrence in water/sediment [%]	12.1	Irradiated water/sediment study

II. RESULTS AND DISCUSSION

All calculated PEC_{sw} and PEC_{sed} values are available in the study report. For the sake of compactness, only the maximum concentrations of pyraclostrobin and its metabolites in surface water (PEC_{sw}) and in sediment (PEC_{sed}) are reported below. Actual and time-weighted average concentrations of pyraclostrobin in surface water are presented exemplarily for Tier 1 as it provides the worst-case PECs.

SURFACE WATER

Global maximum concentrations – pyraclostrobin

Step 1 and 2

Table 9.2.5-17: Steps 1-2: $PEC_{sw,max}$ of pyraclostrobin following application to potatoes

FOCUS$_{sw}$ crop	Tier	Pyraclostrobin $PEC_{sw,max}$ [µg L^{-1}]					
		Step 1		Step 2			
				North Europe		South Europe	
		Single	Multiple	Single	Multiple	Single	Multiple
Potatoes	Tier 1	0.579	2.316	0.156	0.128	0.156	0.164
	Tier 2	0.579	2.316	0.156	0.112	0.156	0.144

Step 3

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-18: Step 3: $PEC_{sw,max}$ of pyraclostrobin following application of 17 g a.s. ha^{-1} to potatoes (Tier 1)

Location	Water body		Potatoes	
			Single application	Multiple application
D3	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.088 Drift	0.059 Drift
D4	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.004 Drift	0.005 Drift
D4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.069 Drift	0.051 Drift
D6 (1st)	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.088 Drift	0.059 Drift
D6 (2nd)	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.087 Drift	0.060 Drift
R1	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.004 Runoff	0.010 Drift
R1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.061 Drift	0.041 Drift
R2	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.081 Drift	0.055 Drift
R3	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.086 Drift	0.058 Drift

Tier 2 (parameters from irradiated water/sediment study)

Table 9.2.5-19: Step 3: $PEC_{sw,max}$ of pyraclostrobin following application of 17 g a.s. ha^{-1} to potatoes (Tier 2)

Location	Water body		Potatoes	
			Single application	Multiple application
D3	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.088 Drift	0.059 Drift
D4	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.004 Drift	0.003 Drift
D4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.069 Drift	0.051 Drift
D6 (1st)	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.088 Drift	0.059 Drift
D6 (2nd)	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.087 Drift	0.059 Drift
R1	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.004 Drift	0.007 Drift
R1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.061 Drift	0.041 Drift
R2	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.081 Drift	0.055 Drift
R3	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.086 Drift	0.058 Drift

Global maximum concentrations – metabolites of pyraclostrobin

Table 9.2.5-20: Steps 1-2: $PEC_{sw,max}$ of the metabolites of pyraclostrobin following application to potatoes

Compound	Potatoes					
	$PEC_{sw,max}$ [µg L^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
BF 500-5	0.009	0.035	0.009	0.016	0.009	0.016
BF 500-11	0.014	0.055	0.013	0.024	0.013	0.024
BF 500-13	0.018	0.072	0.018	0.041	0.018	0.041
BF 500-14	0.020	0.078	0.019	0.031	0.019	0.031

Actual and time-weighted average concentrations – pyraclostrobin

Step 1 and 2

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-21: Step 1: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following application of 17 g a.s. ha^{-1} to potatoes (Tier 1)

Time[a] [d]	Step 1			
	Potatoes			
	Single application		Multiple application	
	PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
	Actual	TWA	Actual	TWA
0	0.579	-	2.316	-
1	0.423	0.501	1.690	2.003
2	0.411	0.459	1.644	1.835
4	0.389	0.429	1.555	1.717
7	0.358	0.405	1.431	1.621
14	0.295	0.365	1.179	1.461
21	0.243	0.333	0.971	1.331
28	0.200	0.305	0.799	1.219
42	0.136	0.258	0.542	1.033
50	0.109	0.237	0.434	0.946
100	0.027	0.148	0.109	0.590

[a]Time: days following maximum concentration (Actual) or time interval (TWA)

Table 9.2.5-22: Step 2: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following application of 17 g a.s. ha^{-1} to potatoes (Tier 1)

Time[a] [d]	Potatoes							
	Step 2							
	North Europe (Mar-May)				South Europe (Mar-May)			
	Single application		Multiple application		Single application		Multiple application	
	PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
0	0.156	-	0.128	-	0.156	-	0.164	-
1	0.060	0.108	0.062	0.095	0.060	0.108	0.153	0.158
2	0.030	0.077	0.041	0.073	0.030	0.077	0.149	0.155
4	0.040	0.052	0.098	0.063	0.063	0.055	0.142	0.150
7	0.032	0.044	0.082	0.073	0.054	0.056	0.131	0.144
14	0.027	0.037	0.069	0.074	0.045	0.053	0.109	0.132
21	0.022	0.033	0.058	0.071	0.038	0.049	0.091	0.121
28	0.019	0.030	0.048	0.066	0.031	0.045	0.076	0.112
42	0.013	0.025	0.034	0.058	0.022	0.039	0.053	0.096
50	0.011	0.023	0.027	0.053	0.018	0.036	0.043	0.088
100	0.003	0.014	0.008	0.034	0.005	0.023	0.012	0.056

[a]Time: days following maximum concentration (Actual) or time interval (TWA)

Step 3

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-23: Step 3: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of pyraclostrobin following application of 17 g a.s. ha^{-1} to potatoes (Tier 1)

		Potatoes			
		Step 3 (edge-of-field)			
		Single application		Multiple application	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
Location	Time[a] [d]	Actual	TWA	Actual	TWA
D3, ditch	0	0.088	-	0.059	-
	1	0.031	0.064	0.024	0.045
	2	0.002	0.038	0.002	0.029
	4	<0.001	0.019	<0.001	0.015
	7	<0.001	0.011	<0.001	0.009
	14	<0.001	0.006	<0.001	0.008
	21	<0.001	0.004	<0.001	0.006
	28	<0.001	0.003	<0.001	0.006
	42	<0.001	0.002	<0.001	0.005
	50	<0.001	0.002	<0.001	0.005
	100	<0.001	0.001	<0.001	0.002
D4, pond	0	0.004	-	0.005	-
	1	0.003	0.003	0.005	0.005
	2	0.003	0.003	0.005	0.005
	4	0.003	0.003	0.004	0.005
	7	0.003	0.003	0.004	0.004
	14	0.002	0.003	0.004	0.004
	21	0.002	0.003	0.003	0.004
	28	0.002	0.002	0.003	0.004
	42	0.001	0.002	0.002	0.003
	50	0.001	0.002	0.002	0.003
	100	<0.001	0.001	0.001	0.003
D4, stream	0	0.069	-	0.051	-
	1	<0.001	0.003	<0.001	0.005
	2	<0.001	0.001	<0.001	0.003
	4	<0.001	0.001	<0.001	0.001
	7	<0.001	<0.001	<0.001	0.001
	14	<0.001	<0.001	<0.001	<0.001
	21	<0.001	<0.001	<0.001	<0.001
	28	<0.001	<0.001	<0.001	<0.001
	42	<0.001	<0.001	<0.001	<0.001
	50	<0.001	<0.001	<0.001	<0.001
	100	<0.001	<0.001	<0.001	<0.001

		Potatoes			
		Step 3 (edge-of-field)			
		Single application		Multiple application	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
Location	**Time[a] [d]**	**Actual**	**TWA**	**Actual**	**TWA**
D6, ditch (1st)	0	0.088	-	0.059	-
	1	0.029	0.065	0.036	0.051
	2	0.001	0.037	0.006	0.037
	4	<0.001	0.019	<0.001	0.020
	7	<0.001	0.011	<0.001	0.012
	14	<0.001	0.005	<0.001	0.011
	21	<0.001	0.004	<0.001	0.007
	28	<0.001	0.003	<0.001	0.006
	42	<0.001	0.002	<0.001	0.005
	50	<0.001	0.002	<0.001	0.005
	100	<0.001	0.001	<0.001	0.003
D6, ditch (2nd)	0	0.087	-	0.060	-
	1	<0.001	0.029	0.045	0.052
	2	<0.001	0.014	0.021	0.043
	4	<0.001	0.007	0.003	0.026
	7	<0.001	0.004	0.001	0.015
	14	<0.001	0.002	<0.001	0.011
	21	<0.001	0.001	<0.001	0.008
	28	<0.001	0.001	<0.001	0.007
	42	<0.001	0.001	<0.001	0.005
	50	<0.001	0.001	<0.001	0.004
	100	<0.001	<0.001	<0.001	0.002
R1, pond	0	0.004	-	0.010	-
	1	0.004	0.004	0.010	0.010
	2	0.004	0.004	0.010	0.010
	4	0.004	0.004	0.009	0.010
	7	0.003	0.004	0.009	0.009
	14	0.003	0.004	0.008	0.009
	21	0.003	0.003	0.009	0.009
	28	0.002	0.003	0.008	0.009
	42	0.002	0.003	0.006	0.008
	50	0.002	0.003	0.005	0.008
	100	0.001	0.003	0.003	0.006
R1, stream	0	0.061	-	0.041	-
	1	<0.001	0.013	<0.001	0.016
	2	<0.001	0.007	<0.001	0.010
	4	<0.001	0.003	0.018	0.007
	7	<0.001	0.002	<0.001	0.005
	14	<0.001	0.002	<0.001	0.004
	21	<0.001	0.001	<0.001	0.003
	28	0.005	0.001	<0.001	0.002
	42	<0.001	0.001	<0.001	0.002
	50	<0.001	0.001	<0.001	0.002
	100	<0.001	<0.001	<0.001	0.001

		Potatoes			
		Step 3 (edge-of-field)			
		Single application		Multiple application	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
Location	**Time[a] [d]**	**Actual**	**TWA**	**Actual**	**TWA**
R2, stream	0	0.081	-	0.055	-
	1	<0.001	0.007	<0.001	0.011
	2	<0.001	0.004	<0.001	0.006
	4	<0.001	0.002	<0.001	0.003
	7	<0.001	0.001	<0.001	0.003
	14	<0.001	0.001	0.055	0.001
	21	0.003	<0.001	<0.001	0.001
	28	<0.001	<0.001	<0.001	0.001
	42	<0.001	<0.001	<0.001	0.001
	50	<0.001	<0.001	<0.001	0.001
	100	<0.001	<0.001	<0.001	<0.001
R3, stream	0	0.086	-	0.058	-
	1	<0.001	0.028	<0.001	0.030
	2	<0.001	0.014	<0.001	0.016
	4	<0.001	0.007	0.037	0.010
	7	<0.001	0.005	<0.001	0.009
	14	<0.001	0.003	<0.001	0.006
	21	<0.001	0.002	<0.001	0.005
	28	<0.001	0.001	<0.001	0.005
	42	<0.001	0.001	<0.001	0.004
	50	<0.001	0.001	<0.001	0.004
	100	<0.001	0.001	<0.001	0.002

[a] Time: days after maximum concentration (Actual) or time interval (TWA)

SEDIMENT

Only global maximum values are reported, which can also be considered as worst-case estimates of short-term and long-term exposure.

Global maximum concentrations – pyraclostrobin

Step 1 and 2

Table 9.2.5-24: Steps 1-2: $PEC_{sed,max}$ of pyraclostrobin following application to potatoes

FOCUS$_{sw}$ crop	Tier	Pyraclostrobin $PEC_{sed,max}$ [µg kg^{-1}]					
		Step 1		Step 2			
				North Europe		South Europe	
		Single	Multiple	Single	Multiple	Single	Multiple
Potatoes	Tier 1	39.330	157.319	3.084	7.990	5.224	14.142
	Tier 2	39.330	157.319	2.823	6.852	4.962	13.003

Step 3

Tier 1 (parameters from dark water/sediment study)

Table 9.2.5-25: Step 3: $PEC_{sed,max}$ of pyraclostrobin following application to potatoes (Tier 1)

Location	Water body	$PEC_{sed,max}$ [µg kg^{-1}]	
		Potatoes	
		Single application	Multiple application
D3	ditch	0.054	0.071
D4	pond	0.025	0.057
D4	stream	0.002	0.005
D6 (1st)	ditch	0.053	0.080
D6 (2nd)	ditch	0.021	0.095
R1	pond	0.063	0.139
R1	stream	0.709	1.550
R2	stream	0.207	1.052
R3	stream	0.165	0.964

Tier 2 (parameters from irradiated water/sediment study)

Table 9.2.5-26: Step 3: $PEC_{sed,max}$ of pyraclostrobin following application to potatoes (Tier 2)

Location	Water body	$PEC_{sed,max}$ [µg kg^{-1}]	
		Potatoes	
		Single application	Multiple application
D3	ditch	0.049	0.044
D4	pond	0.011	0.014
D4	stream	0.002	0.004
D6 (1st)	ditch	0.047	0.044
D6 (2nd)	ditch	0.020	0.064
R1	pond	0.025	0.059
R1	stream	0.516	1.327
R2	stream	0.188	0.999
R3	stream	0.112	0.710

Global maximum concentrations – metabolites of pyraclostrobin

Table 9.2.5-27: Steps 1-2: $PEC_{sed,max}$ of the metabolites of pyraclostrobin following application of pyraclostrobin to potatoes

Compound	Potatoes					
	$PEC_{sed,max}$ [µg kg^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
BF 500-3 (dark)	0.681	2.724	0.676	1.805	0.676	1.805
BF 500-3 (irradiated)	0.221	0.885	0.211	0.511	0.211	0.511
BF 500-6	10.368	41.471	0.673	2.535	1.286	4.912
BF 500-7	6.221	24.885	0.423	1.569	0.790	2.989

III. CONCLUSION

Predicted environmental concentrations (PEC) were calculated for pyraclostrobin and its metabolites in surface water (BF 500-5, BF 500-11, BF 500-13, BF 500-14) and in sediment (BF 500-3, BF 500-6, BF 500-7) following application of the formulated product BAS 516 07 F to potatoes under European conditions.

For the parent compound a tiered approach using endpoints from dark (Tier 1) and irradiated (Tier 2) water/sediment studies was considered for the calculations. While this had almost no effect on maximum PEC_{sw}, the maximum PEC_{sed} in Tier 2 decreased slightly (with approximately 17% for, fourfold application, R1–stream). Time weighted average (TWA) values did not differ significantly between the two tiers. Despite the large discrepancy between the degradation rates in the water compartment between the two tiers, the results show that sorption from the water phase to sediment is the dominating process. This is in agreement with the behavior observed in aqueous metabolism studies. Although the selection of endpoints has no major effect on the PEC_{sw} values, it considerably affects the behavior in sediment. Since pyraclostrobin breakdown under irradiated conditions is a relevant pathway and degradation occurs rapidly these Tier 2 endpoints should be considered for further usage.

The predicted concentrations in surface water are appropriate to be used for the subsequent risk assessment for aquatic organisms.

Report: CP 9.2.5/2
Kallweit W., 2014b
Predicted environmental concentrations of BAS 510 F - Boscalid in soil, groundwater, surface water and sediment following application to potatoes in Europe
2014/1001461

Guidelines: SANCO/321/2000, FOCUS Groundwater (2012) Generic guidance for FOCUS groundwater scenarios v 2.1, FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011, SANCO/4802/2001 rev. 2 (FOCUS surface water scenarios) May 2003, FOCUS Surface Water (2012) SANCO/4802/2001 v1.1, FOCUS groundwater (2009): SANCO/13144/2009

GLP: no

Executive Summary

Predicted environmental concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) were calculated for boscalid, according to the recommendations of the FOCUS working group on surface water scenarios (2001, 2012) in a stepwise approach considering the entry pathways spray drift, drainage and runoff. Calculations were carried out for single and fourfold application of boscalid to potatoes taking into account the maximum application rate (67 g a.s. ha^{-1}) and the minimum application interval (10 days).

Calculations were carried out at Step 1 to Step 3 level. The software packages STEPS1-2 (version 2.1) for Step 1 and Step 2 and SWASH 3.1 in combination with MACRO 4.4.2, PRZM 1.5.6 and TOXSWA 3.3.1 for Step 3 were used for the calculations. At Step 2 of the assessment, the regions 'North Europe' and 'South Europe' were taken into account. At Step 3 all FOCUS surface water scenarios relevant for potatoes were considered for the calculations.

The maximum concentrations of boscalid in surface water and sediment are summarized in the following table.

Table 9.2.5-28: Summary of highest global PEC_{sw} and PEC_{sed} values of boscalid following application to potatoes

FOCUS level	SURFACE WATER	SEDIMENT
	Max. PEC_{sw} [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	46.515	348.754
Step 2	5.554	41.843
Step 3	2.064 D6 d (2nd), multiple	3.189 R2 s, multiple

d = ditch, s = stream
multiple = multiple application

I. MATERIAL AND METHODS

Application scenarios

According to Good Agricultural Practice (GAP), boscalid is scheduled for up to four applications to potatoes at a maximum application rate equivalent to 67 g a.s. ha^{-1} per treatment and a minimum interval of 10 days. The worst-case application scenarios considered for PEC_{sw} and PEC_{sed} calculations are given in Table 9.2.5-29.

Table 9.2.5-29: Worst-case application scenarios of boscalid applied to potatoes considered for PEC_{sw} and PEC_{sed} calculations

Potatoes		
Max. no. of applications [-]	1	4
Crop growth stage at first application [BBCH]	51	51
Minimum application interval [d]	-	10
Application rate [g a.s. ha^{-1}]	67	67
Total yearly application rate [g a.s. ha^{-1}]	67	268

Environmental fate parameters

Fate and behavior in soil

Details about the degradation behavior of boscalid in soil can be found in M-CP 9.1.3. The geometric mean DT_{50} of 130.0 days that was calculated from field DT_{50} normalized to 20°C (Q_{10} = 2.2) was used for the calculations in surface water. Simulations were carried out using a Q_{10} value of 2.58 as recommended by EFSA [*EFSA (2007)*]. The combination of Q_{10} = 2.2 for normalization and Q_{10} = 2.58 for simulation leads to worse-case degradation conditions for temperatures below 20°C, which are predominant in the FOCUS scenarios. $PEC_{sw,sed}$ calculations can therefore be considered conservative regarding the use of the Q_{10} value.

Details about the sorption behavior of boscalid to soil can be found in M-CP 9.2.4. The arithmetic mean $K_{f,oc}$ of 771 mL g^{-1} and the arithmetic mean Freundlich exponent of 0.864 were used for the $PEC_{sw,sed}$ calculations.

Fate and behavior in aquatic systems

The dissipation behavior of boscalid in water was investigated in two water/sediment studies under different test conditions. The first study was performed under standard conditions in the laboratory in the dark [*Ebert – BASF DocID 2000/1000135*]. To reflect a more realistic environmental scenario, the second water/sediment study was conducted under outdoor conditions with the influence of natural sunlight [*Fent – BASF DocID 2000/1017038*].

The standard water/sediment study [*Ebert – BASF DocID 2000/1000135*] was conducted in the laboratory at 20°C under dark conditions. Two aquatic test systems were investigated representing a pond and a river system. The dissipation of boscalid in the water phase of both systems was mainly based on sorption processes. The highest amount of boscalid in sediment was observed in the river system with 78.8% TAR. The amount of bound residues in the sediment was low; a maximum value of 12.9% was observed the pond system at day 100 after treatment. The best-fit (non single first-order) DT_{50} values describing dissipation from the water phase of both systems were determined graphically; they were estimated to be 9 days and 3 days, respectively. It was not possible to estimate single first-order DT_{50} values describing degradation in water and sediment that are necessary input parameters for FOCUS surface water models.

The outdoor study [*Fent – BASF DocID 2000/1017038*] was accomplished in a pond water/sediment system (location "Kellmetschweiher"). The maximum concentration in sediment was observed at day 103 with 28.2% TAR.

In a kinetic evaluation [*Platz – BASF DocID 2000/1017047*] the observed residues of boscalid in the water and in the sediment phase were fitted with the help of a compartment model that considered the dissipation of boscalid in water and in sediment, and sorption/desorption processes in the sediment phase. Residues up to day 58 with fairly constant temperature conditions of about 20°C were considered. The biotic/abiotic degradation rate in water (separated from partitioning) of 32 days was used in the assessment, which is considered as a realistic worst-case estimate of degradation in the aquatic phase of water/sediment systems.

Degradation in the sediment and the total water/sediment system could not be estimated [*Platz – BASF DocID 2000/1017047*]. Therefore, conservative default values of 1000 days were considered in the calculations for the degradation of boscalid in the total system and in sediment.

Surface water assessment according to FOCUS

The calculations were performed in a stepwise approach according to the recommendations of the FOCUS working group on surface water scenarios (2001, 2012).

For all calculations the following model versions were used: STEPS1-2 in FOCUS version 2.1, FOCUS-PRZM version 1.5.6, FOCUS-MACRO version 4.4.2 and FOCUS-TOXSWA version 3.3.1. The modeling runs were set up with SWASH version 3.1.

Setup of FOCUS surface water runs

Calculations for boscalid were carried out at Step 1 to Step 3, according to the recommendations of the FOCUS working group on surface water scenarios and considering the entry routes spray drift, drainage and runoff. The scenario settings for Step 1 – 3 are equal to the ones for pyraclostrobin.

Summary of input parameters

A summary of the environmental fate parameters of boscalid used for modeling is given in Table 9.2.5-30.

Table 9.2.5-30: Summary of FOCUS input parameters for boscalid for PEC_{sw} and PEC_{sed} calculations

Parameter	Value	Remarks
Entry routes into surface water	Spray drift Runoff Drainage	-
Molecular weight [g mol^{-1}]	343.2	Phys.-chem. properties
Water solubility [mg L^{-1}] (20°C)	4.6	Phys.-chem. properties
Vapor pressure [Pa] (20°C)	1 x 10^{-10}	Default value
DEGRADATION IN SOIL		
DT_{50} soil [d]	130.0	Geometric mean of field studies (n=3, standardized to 20°C)
Temperature correction function Reference temperature [°C] MACRO temp. exponent [K^{-1}] PRZM Q_{10} [-]	 20 0.095 2.58	EFSA opinion
Moisture correction function Reference moisture [-] PRZM / MACRO moisture exponent [-]	 pF 2 0.7	FOCUS recommendation
SORPTION TO SOIL		
K_{oc} [mL g^{-1}]	771	Arithmetic mean (n=6)
1/n [-]	0.864	
DEGRADATION IN AQUATIC SYSTEMS		
DT_{50} total system [d] (Step 1)	1000	Conservative default value FOCUS recommendation
DT_{50} water [d] (Step 2, Step 3)	32.0	Single first-order DT_{50} from water/sediment study
DT_{50} sediment [d] (Step 2, Step 3)	1000	Conservative default value FOCUS recommendation
DT_{50} crop [d] (Step 3)	10	FOCUS recommendation
Temperature correction function Reference temperature [°C] TOXSWA: activation energy [J mol^{-1}]	 20 65400	EFSA opinion
MANAGEMENT RELATED PARAMETERS		
Crop uptake factor	0.5	FOCUS recommendation
Wash off coefficient PRZM: [cm^{-1}] MACRO: [mm^{-1}] [mm^{-1}]	 0.5 0.05	

II. RESULTS AND DISCUSSION

Predicted concentrations in surface water

Global maximum concentrations

Step 1 and 2

Predicted environmental concentrations in surface water of boscalid at Step 1 and 2 are given in Table 9.2.5-31.

Table 9.2.5-31: Steps 1-2: $PEC_{sw,max}$ of boscalid following application of 67 g a.s. ha^{-1} to potatoes

$FOCUS_{sw}$ crop	$PEC_{sw,max}$ [µg L^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Potatoes	11.629	46.515	0.990	3.162	1.637	5.554

Step 3

Predicted environmental concentrations in surface water of boscalid at Step 3 are given in Table 9.2.5-32.

Table 9.2.5-32: Step 3: $PEC_{sw,max}$ of boscalid following application of 67 g a.s. ha^{-1} to potatoes

Location	Water body		Potatoes	
			Single application	Multiple application
D3	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.350 Drift	0.236 Drift
D4	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.067 Drainage	0.282 Drainage
D4	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.277 Drift	0.578 Drainage
D6 (1st)	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.351 Drift	0.997 Drainage
D6 (2nd)	ditch	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.440 Drainage	2.064 Drainage
R1	pond	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.081 Runoff	0.180 Runoff
R1	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.368 Runoff	0.901 Runoff
R2	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.321 Drift	0.495 Runoff
R3	stream	$PEC_{sw,max}$ [µg L^{-1}] main entry route	0.421 Runoff	1.592 Runoff

Actual and time-weighted average concentrations

Step 1 and 2

Table 9.2.5-33: Step 1: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of boscalid following application of 67 g a.s. ha^{-1} to potatoes

Time[a] [d]	PEC_{sw} [µg L^{-1}]			
	Step 1			
	Single application		Multiple application	
	Actual	TWA	Actual	TWA
0	11.629	-	46.515	-
1	11.309	11.469	45.234	45.874
2	11.301	11.387	45.203	45.546
4	11.285	11.340	45.140	45.359
7	11.262	11.311	45.046	45.245
14	11.207	11.273	44.828	45.091
21	11.153	11.242	44.611	44.967
28	11.099	11.213	44.395	44.851
42	10.992	11.157	43.967	44.628
50	10.931	11.126	43.723	44.502
100	10.559	10.935	42.234	43.738

[a] Time: days after maximum concentration (Actual) or time interval (TWA)

Table 9.2.5-34: Step 2: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of boscalid following application of 67 g a.s. ha^{-1} to potatoes

Time[a] [d]	PEC_{sw} [µg L^{-1}]							
	Step 2							
	North Europe				South Europe			
	Single application		Multiple application		Single application		Multiple application	
	Actual	TWA	Actual	TWA	Actual	TWA	Actual	TWA
0	0.990	-	3.162	-	1.637	-	5.554	-
1	0.915	0.952	2.973	3.067	1.548	1.592	5.315	5.434
2	0.905	0.931	2.941	3.012	1.531	1.566	5.257	5.360
4	0.885	0.913	2.877	2.960	1.498	1.540	5.142	5.280
7	0.856	0.895	2.784	2.905	1.449	1.511	4.976	5.185
14	0.793	0.860	2.578	2.792	1.342	1.453	4.608	4.987
21	0.734	0.827	2.387	2.688	1.243	1.399	4.267	4.803
28	0.680	0.797	2.211	2.591	1.151	1.348	3.951	4.629
42	0.583	0.742	1.896	2.410	0.987	1.254	3.388	4.307
50	0.534	0.712	1.736	2.315	0.904	1.205	3.104	4.137
100	0.309	0.562	1.003	1.826	0.522	0.950	1.793	3.263

[a] Time: days after maximum concentration (Actual) or time interval (TWA)

Step 3

Table 9.2.5-35: Step 3: $PEC_{sw,act}$ and $PEC_{sw,twa}$ of boscalid following application of 67 g a.s. ha^{-1} to potatoes

		Potatoes			
		Step 3 (edge-of-field)			
		Single application		Multiple application	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
Location	Time[a] [d]	Actual	TWA	Actual	TWA
D3, ditch	0	0.350	-	0.236	-
	1	0.123	0.253	0.096	0.179
	2	0.011	0.152	0.010	0.114
	4	0.002	0.078	0.001	0.059
	7	0.001	0.045	0.001	0.034
	14	<0.001	0.023	<0.001	0.032
	21	<0.001	0.015	0.001	0.022
	28	<0.001	0.011	<0.001	0.024
	42	<0.001	0.008	<0.001	0.022
	50	<0.001	0.006	<0.001	0.018
	100	<0.001	0.003	<0.001	0.009
D4, pond	0	0.067	-	0.282	-
	1	0.067	0.067	0.281	0.282
	2	0.067	0.067	0.280	0.282
	4	0.067	0.067	0.275	0.281
	7	0.065	0.067	0.267	0.281
	14	0.060	0.066	0.247	0.277
	21	0.056	0.064	0.230	0.271
	28	0.053	0.062	0.239	0.264
	42	0.054	0.060	0.220	0.254
	50	0.050	0.059	0.203	0.251
	100	0.031	0.052	0.128	0.222
D4, stream	0	0.277	-	0.578	-
	1	0.003	0.119	0.159	0.450
	2	0.003	0.102	0.462	0.390
	4	0.002	0.090	0.110	0.349
	7	0.002	0.073	0.130	0.290
	14	0.003	0.050	0.230	0.206
	21	0.004	0.046	0.123	0.195
	28	0.004	0.041	0.061	0.175
	42	0.007	0.030	0.048	0.133
	50	0.003	0.029	0.106	0.125
	100	<0.001	0.018	0.005	0.079

		Potatoes			
		Step 3 (edge-of-field)			
		Single application		Multiple application	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
Location	**Time[a] [d]**	**Actual**	**TWA**	**Actual**	**TWA**
D6, ditch (1st)	0	0.351	-	0.997	-
	1	0.115	0.257	0.266	0.639
	2	0.008	0.149	0.123	0.484
	4	0.003	0.077	0.061	0.315
	7	0.002	0.050	0.274	0.257
	14	0.001	0.037	0.042	0.198
	21	0.001	0.030	0.013	0.143
	28	0.001	0.024	0.008	0.118
	42	0.001	0.020	0.004	0.102
	50	0.001	0.017	0.077	0.088
	100	0.004	0.014	0.013	0.076
D6, ditch (2nd)	0	0.440	-	2.064	-
	1	0.124	0.287	0.624	1.378
	2	0.069	0.207	0.360	0.989
	4	0.041	0.155	0.225	0.747
	7	0.023	0.122	0.127	0.604
	14	0.006	0.097	0.033	0.479
	21	0.004	0.078	0.026	0.384
	28	0.003	0.062	0.017	0.304
	42	0.018	0.045	0.096	0.235
	50	0.015	0.038	0.073	0.204
	100	0.012	0.029	0.056	0.155
R1, pond	0	0.081	-	0.180	-
	1	0.078	0.079	0.173	0.178
	2	0.075	0.078	0.176	0.176
	4	0.070	0.076	0.164	0.172
	7	0.065	0.072	0.149	0.167
	14	0.060	0.068	0.154	0.164
	21	0.050	0.064	0.137	0.157
	28	0.042	0.060	0.115	0.150
	42	0.031	0.053	0.084	0.139
	50	0.027	0.052	0.071	0.137
	100	0.014	0.039	0.040	0.116
R1, stream	0	0.368	-	0.901	-
	1	0.007	0.291	0.002	0.478
	2	0.001	0.147	0.001	0.302
	4	<0.001	0.074	0.001	0.198
	7	<0.001	0.059	<0.001	0.155
	14	<0.001	0.038	<0.001	0.093
	21	0.067	0.029	<0.001	0.078
	28	<0.001	0.030	<0.001	0.062
	42	<0.001	0.023	<0.001	0.050
	50	<0.001	0.020	<0.001	0.042
	100	<0.001	0.010	0.001	0.023

		Potatoes			
		Step 3 (edge-of-field)			
		Single application		Multiple application	
		PEC_{sw} [µg L^{-1}]		PEC_{sw} [µg L^{-1}]	
Location	**Time[a] [d]**	**Actual**	**TWA**	**Actual**	**TWA**
R2, stream	0	0.321	-	0.495	-
	1	<0.001	0.096	0.136	0.493
	2	<0.001	0.051	0.002	0.262
	4	<0.001	0.026	0.001	0.131
	7	<0.001	0.015	<0.001	0.078
	14	<0.001	0.009	<0.001	0.040
	21	0.103	0.006	<0.001	0.028
	28	<0.001	0.006	0.002	0.032
	42	<0.001	0.005	<0.001	0.022
	50	<0.001	0.005	<0.001	0.019
	100	<0.001	0.003	<0.001	0.013
R3, stream	0	0.421	-	1.592	-
	1	0.002	0.269	0.261	1.269
	2	0.001	0.142	0.031	0.670
	4	0.341	0.072	0.006	0.425
	7	0.001	0.060	0.003	0.255
	14	<0.001	0.041	0.005	0.200
	21	<0.001	0.035	0.001	0.182
	28	0.001	0.027	0.782	0.142
	42	<0.001	0.027	0.002	0.125
	50	<0.001	0.026	0.001	0.115
	100	<0.001	0.018	<0.001	0.073

[a] Time: days after maximum concentration (Actual) or time interval (TWA)

Predicted concentrations in sediment

Only global maximum values are reported, which can also be considered as worst-case estimates of short-term and long-term exposure.

Step 1 and 2

Global maximum concentrations in sediment of boscalid at Step 1 and 2 are given in Table 9.2.5-36.

Table 9.2.5-36: Steps 1-2: $PEC_{sed,max}$ of boscalid following application of 67 g a.s. ha^{-1} to potatoes

FOCUS$_{sw}$ crop	$PEC_{sed,max}$ [µg kg^{-1}]					
	Step 1		Step 2			
			North Europe		South Europe	
	Single	Multiple	Single	Multiple	Single	Multiple
Potatoes	87.188	348.754	7.201	23.409	12.185	41.843

Step 3

Global maximum concentrations in sediment of boscalid at Step 3 are given in Table 9.2.5-37.

Table 9.2.5-37: Step 3: $PEC_{sed,max}$ of boscalid following application of 67 g a.s. ha^{-1} to potatoes

Location	Water body	$PEC_{sed,max}$ [µg kg^{-1}]	
		Potatoes	
		Single application	Multiple application
D3	ditch	0.185	0.234
D4	pond	0.697	2.648
D4	stream	0.303	1.170
D6 (1st)	ditch	0.285	1.334
D6 (2nd)	ditch	0.579	2.743
R1	pond	0.508	1.905
R1	stream	1.066	2.451
R2	stream	0.754	3.189
R3	stream	0.478	2.819

III. CONCLUSION

Predicted environmental concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) were calculated for boscalid following application of the formulated product BAS 516 07 F to potatoes under European pedo-climatic conditions.

The predicted concentrations in surface water are appropriate to be used for the subsequent risk assessment for aquatic organisms.

CP 9.3 Fate and behaviour in air

CP 9.3.1 Route and rate of degradation in air and transport via air

No studies were performed with BAS 516 07 F. The route and rate of degradation in air as well as transport via air is sufficiently addressed by information given in M-CA 7.3.

Predicted environmental concentrations from airborne transport

No risk assessment needs to be performed, because both active substances in BAS 516 07 F, pyraclostrobin and boscalid, have a very low volatilisation potential and a short DT_{50} in air. Consequently no PECs have been calculated.

CP 9.4 Estimation of concentrations for other routes of exposure

No other routes of exposure are relevant for the use of BAS 516 07 F in potatoes.

BASF
The Chemical Company

BAS 516 07 F

DOCUMENT M-CP, Section 10

ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-July-2014		BASF DocID 2014/1162346 (version 1)
09-June-2015	One study was moved from M-CP 10.7 to M-CP 10.4 and another study from M-CP 10.7 to M-CP 10.5. The three chapters have been adapted accordingly. New or changed text is marked in yellow.	BASF DocID 2015/1107662 (version 2)
27-Feb-2017	Update of 10.1.3 reflecting the information of two additional amphibian studies that are described in detail in M-CP 10 of BAS 500 06 F Update of 10.3.1 reflecting the information of a new bumblebee study that is described in detail in M-CP 10 of BAS 500 06 F New or changed text is marked in blue.	BASF DocID 2017/1033586 (version 3)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 10 ECOTOXICOLOGICAL STUDIES ON PLANT PROTECTION PRODUCT

BAS 516 07 F is a new representative formulation, which has not been evaluated during a previous Annex I inclusion process.

This document reviews the ecotoxicological studies for BAS 516 07 F, a WG formulation containing 67 g/kg pyraclostrobin and 267 g/kg boscalid. In addition, risk assessments are provided, which show that BAS 516 07 F is safe for aquatic and terrestrial non-target organisms.

CP 10.1 Effects on birds and other terrestrial vertebrates

The risk assessment on birds and mammals considers the principles given in the latest guidance document by EFSA (*Anonymous 2009: Guidance Document on risk assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. European Food Safety Authority*), hereafter cited as EFSA/2009/1438. EU agreed endpoints and endpoints from new studies are used for the risk assessments on birds and mammals together with data from new additional studies.

The representative formulation BAS 516 07 F is containing pyraclostrobin and boscalid. No separate risk assessment for boscalid will be presented in the following chapter, as the active substance in focus of the evaluation is pyraclostrobin. Boscalid is solely addressed in the context of the assessment of combined effects of formulations containing more than one active substance (i.e. dietary risk assessment for a virtual compound and the formulation).

In addition, the potential risk to amphibians and reptiles will be addressed following the new requirement under Regulation 1107/2009.

Study conducted for the avian risk assessment

For the avian risk assessment the following new information on formulation toxicity is used (Table 10.1-1). The study summary is given in M-CP 10.1.1.2.

Table 10.1-1: New information used for the avian risk assessment

Data point	Reference (BASF DocID)	Year	Title
M-CP 10.1.1.2/1	2006/1030309	2006	BAS 516 07 F - Acute toxicity in the bobwhite quail (*Colinus virginianus*) after single oral administration (LD_{50})

CP 10.1.1 Effects on birds

In this chapter, data on BAS 516 07 F are evaluated and the risk assessments are presented for the active substance pyraclostrobin and for the evaluation of combined effects of formulations containing more than one active substance based on the already registered use pattern.

Table 10.1.1-1: Endpoints for birds

Test system	Test species	Study endpoints	Endpoints used in present risk assessment [4]
Acute toxicity	*Colinus virginianus*	LD_{50} > 2000 mg a.s./kg b.w. [1]	LD_{50} (geometric mean) = 1701 mg a.s./kg b.w. [5]
	Serinus canaria	LD_{50} > 1446 mg a.s./kg b.w. [2]	
Reproductive toxicity (long-term)	*Colinus virginianus*	NOEL = 105 mg a.s./kg b.w./d [1] [3]	NOEC = 1000 mg a.s./kg diet NOEL = 105 mg a.s./kg b.w./d [2]

1 Endpoints confirmed in the ecotoxicology section of the Review Report for the active substance pyraclostrobin, SANCO/1420/2001-Final. 8. September 2004
2 Based on data from a new study and therefore not evaluated during the previous Annex I process. For the detailed study summary please refer to M-CA 8.1.1.1/1
3 Daily Dose [mg/kg b.w./d] calculated based on study data for food consumption and body weight
4 Endpoints according to EFSA/2009/1438
5 For calculation of LD_{50} (geometric mean) please refer to Table 10.1.1-3

RISK ASSESSMENT

Summary of avian toxicity testing

The avian toxicity studies with pyraclostrobin relevant for the dietary risk assessment for birds are summarized in Table 10.1.1-2. The listed studies have been already evaluated during the previous Annex I listing process except for the acute toxicity study in the canary (BASF DocID 2013/1400375). The two available and already evaluated short term toxicity studies in bobwhite quail and mallard duck are not listed below, because they are no longer part of the core data package according to EFSA/2009/1438.

Table 10.1.1-2: Summary of avian studies on the acute and reproductive toxicity of pyraclostrobin

Test system	Test species	Results	Reference (BASF DocID)
Acute oral toxicity	*Colinus virginianus*	LD_{50} > 2000 mg a.s./kg b.w. [1)]	1997/11136
	Serinus canaria	LD_{50} > 1446 mg a.s./kg b.w.	2013/1400375
Sub-chronic toxicity and reproduction	*Colinus virginianus*	NOEC = 1000 mg a.s./kg diet NOEL = 105 mg a.s./kg b.w./d [1) 2)]	1999/11207
	Anas platyrhynchos	NOEC = 1000 mg a.s./kg diet NOEL = 128 mg a.s./kg b.w./d [2)]	1999/11206

1 Endpoints confirmed in the ecotoxicology section of the Review Report for the active substance pyraclostrobin (SANCO/1420/2001-Final. 8. September 2004).
2 Daily Dose [mg/kg b.w./d] calculated based on study data for food consumption and body weight

Calculated avian toxicity endpoint for use in the acute tier 1 risk assessment

For pyraclostrobin acute oral studies in the bobwhite quail and the canary are available (Table 10.1.1-2). No mortality occurred in any of the two acute oral toxicity bird studies in the highest dose tested. However, in the study with the bobwhite quail treatment related effects were observed at the two highest dose groups (i.e. dose groups of 1000 and 2000 mg a.s./kg b.w.) and therefore no extrapolation of the LD_{50} >2000 mg a.s./kg b.w. for the bobwhite quail is possible according to EFSA/2009/1438 (2.1.2, p.16). In the study with the canary no clear signs of toxicity were seen up to the highest dose tested of 1446 mg a.s./kg b.w.. It would be thus permissible to extrapolate an LD_{50} value upwards. However, for a conservative evaluation an extrapolation of the LD_{50} >1446 mg a.s./kg b.w. for the canary is not conducted.

According EFSA/2009/1438 a geometric mean of the endpoints can be used in the acute dietary risk assessment as two different species were tested and the endpoint of the most sensitive species (i.e. the canary) is not by a factor of 10 below the overall geometric mean. The most sensitive endpoint with an LD_{50} > 1446 mg a.s./kg b.w./d in the canary is clearly higher than the 'assessment factor LD_{50}' of 170.1 mg a.s./kg b.w./d (Table 10.1.1-3). Hence, the geometric mean of both acute toxicity endpoints will be used.

The overall geometric mean LD_{50} value, i.e. the **LD_{50} (geometric mean) = 1701 mg a.s./kg b.w.** is the relevant endpoint to be used for the acute avian risk assessment.

Table 10.1.1-3: Calculation of the relevant avian toxicity endpoint for the acute risk assessment of pyraclostrobin

Characteristic	*Colinus virginianus*	*Serinus canaria*
Experimentally obtained LD_{50} [mg a.s./kg b.w.]	> 2000 [1)]	> 1446 [2)]
Birds tested/group [no.]	10	10
Mortality at highest dose level [%]	0	0
Extrapolation factor [3)]	--	--
LD_{50} (extrapolated) [4)] [mg a.s./kg b.w.]	--	--
LD_{50} (overall geometric mean) [5)] [mg a.s./kg b.w.]	1701	
Assessment factor LD_{50} [6)]: LD_{50} (overall geometric mean) / 10 [mg a.s./kg b.w.]	170.1	

1 BASF DocID 1997/11136
2 BASF DocID 2013/1400375
3 Extrapolation factor can be applied to an LD_{50} value in case 10 birds were tested and no mortalities occurred at the highest dose tested (EFSA GD 2009, 2.1.2, p.16). For a conservative evaluation an extrapolation of the endpoints derived is not conducted.
4 LD_{50} multiplied by the extrapolation factor
5 Determination of the geometric mean out of the LD_{50} values of >2000 and >1446 mg a.s./kg b.w. of the acute oral toxicity studies (EFSA/2009/1438, 2.4.2)
6 'Assessment factor LD_{50}' of all the tested species, geometric mean divided by a factor of 10 (EFSA/2009/1438, 2.4.2)

Calculated avian toxicity endpoint for use in the <u>reproductive</u> tier 1 risk assessment

In order to obtain the relevant toxicity endpoint to be used in the screening and tier 1 reproductive risk assessment, the lowest NOEL from available bird reproduction studies and the 'assessment factor LD_{50}' [LD_{50} (overall geometric mean) / 10] from acute toxicity studies (EFSA/2009/1438, p. 33 and 35) have to be considered.

The lowest toxicity value from the avian reproduction study for pyraclostrobin is NOEL = 105 mg a.s./kg b.w./d derived from the study with bobwhite quail (BASF DocID 1999/11207). The acute toxicity endpoint was calculated to LD_{50} = 1701 mg a.s./kg b.w. (Table 10.1.1-3).

Following EFSA/2009/1438 (p. 33 and 35), either the overall lowest NOEL or the 'assessment factor LD_{50}' is to be selected as relevant reproductive toxicity endpoint. Since the lowest of both is the NOEL, the value of **NOEL = 105 mg a.s./kg b.w./d** will be used in the screening step and the tier 1 reproductive risk assessment for pyraclostrobin.

Results from available toxicity testing with the formulation

The avian toxicity study with BAS 516 07 F (BASF DocID 2006/1030309) relevant for the risk assessment for birds is summarized in Table 10.1.1-4. Please note that the study was erroneously included in the Application submitted in January 2014 in M-CP 10.1.1.1 and is now given in M-CP 10.1.1.2.

Table 10.1.1-4: Summary of avian study on the acute toxicity of BAS 516 07 F

Test system	Test species	Results	Reference (BASF DocID)
Acute oral toxicity	*Colinus virginianus*	LD_{50} > 3200 mg/kg b.w.	2006/1030309

This study was not evaluated during previous Annex I listing processes. Therefore, the study is submitted within this dossier together with a detailed study summary provided in chapter 10.1.1.2. In addition, an acute risk assessment for BAS 516 07 F considering the **LD_{50} > 3200 mg/kg b.w. for BAS 516 07 F** is provided below.

Metabolites of pyraclostrobin

The metabolism of pyraclostrobin in potential food items of wild living birds or mammals (i.e. green plant matter, fruits, or seeds) was investigated in plant metabolism studies in grapes (BASF DocIDs 1998/10988 and 2000/1000201), potatoes (BASF DocIDs 1999/11419 and 2000/1000048) and wheat (BASF DocID 1999/11137). Most metabolites occurred only at trace amounts far below 10% TRR in the potential food items. The only metabolite that occurred at higher levels in potential food items was the desmethyl metabolite (BF 500-3, synonym: 500M07). It was found at levels of up to 15.3% TRR in grapes, 21.4% TRR in green matter of potatoes, 13.1% TRR in wheat forage, and 10.5% TRR in wheat grains. However, this desmethyl metabolite was also detectable in rats (BASF DocID 1999/11781), goats (BASF DocID 2000/1000004) and hens (BASF DocID 1999/11480). Hence, it can be concluded that the mammalian toxicity studies with pyraclostrobin cover this metabolite, and that the dietary risk assessment for pyraclostrobin provided for birds and mammals covers the potential risk from this metabolite.

Water metabolites are of minor importance for the risk assessment for wild living birds and mammals, considering the predominant route of exposure via food items like plants, seeds, or arthropods. Water uptake itself or exposure via the aquatic compartment, however, can play a role in the drinking water and the secondary poisoning risk assessment for fish-eaters. Hence in the following the risk from relevant surface water metabolites to birds and mammals is considered. The metabolites BF 500-5, BF 500-11, BF 500-13, BF 500-14 were found at levels slightly above 10% TAR in surface water (for details see M-CA 7.2). However, the risk from these metabolites to birds and mammals is considered to be covered by the presented risk assessments for the parent compound pyraclostrobin due to the following reasons:

i) PEC values of the metabolites are by a factor of ~30-66 below the one of the parent (highest global PEC_{sw}, max [µg/L] following FOCUS Step1 for the use in potatoes are for the metabolites BF 500-5 = 0.035 µg/L, BF 500-11 = 0.055 µg/L, BF 500-13 = 0.072 µg/L, BF 500-14 = 0.078 µg/L, and for the parent BAS 500 F = 2.316 µg/L; for details see M-CP 9.2)

ii) There are no specific toxicity studies with the metabolites BF 500-5, BF 500-11, BF 500-13, BF 500-14 available in birds or mammals, however, the low toxicity of the metabolites was confirmed in standard acute fish toxicity studies with *O. mykiss*.

Summary of relevant avian toxicity endpoints to be used in the screening and tier 1 risk assessments

The avian toxicity endpoints relevant for the screening and tier 1 risk assessments are given in Table 10.1.1-5.

Table 10.1.1-5: Avian toxicity endpoints for screening and tier 1 risk assessments of pyraclostrobin and BAS 516 07 F

Test substance	Test system	Relevant toxicity endpoint
Pyraclostrobin	Acute oral toxicity	LD_{50} (geometric mean) = 1701 mg a.s./kg b.w.
	Sub-chronic toxicity and reproduction	NOEL = 105 mg a.s./kg b.w./d
BAS 516 07 F	Acute oral toxicity	LD_{50} > 3200 mg a.s./kg b.w.

Relevant exposure scenarios

The relevant scenarios for the intended representative use patterns of the formulation BAS 516 07 F (Table 10.1.1-6) for potatoes are given in Table 10.1.1-7.

Table 10.1.1-6: Critical use pattern for BAS 516 07 F

Crop	EFSA/2009/ 1438 crop group	Application time (BBCH growth stage)	No. of applications	Interval [days]	Maximum application rate per treatment		
					Pyraclostrobin [kg/ha]	Boscalid [1)] [kg/ha]	BAS 516 07 F [kg/ha]
Potatoes	Potatoes	51-89	4	10	0.017	0.067	0.25

1 Not relevant for renewal of approval of pyraclostrobin. Therefore, the a.s. boscalid is not evaluated here.

Table 10.1.1-7: Summary of exposure scenarios to be considered for risk assessments for spray applications of BAS 516 07 F in potatoes at BBCH 51 – 89

Scenario	Generic focal species
Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail"
Potatoes BBCH ≥ 40	Small omnivorous bird "lark"

CP 10.1.1.1 Acute oral toxicity

In this section, the dietary TER acute values for the screening step and tier 1 assessment obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) are presented in Table 10.1.1.1-1.

Table 10.1.1.1-1: Pyraclostrobin: Acute dietary risk assessment for birds in potatoes - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Potatoes	0.017	4	10	10.0	1701	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	158.8	2.7	1.5	4.05	420.1	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	**No refinement required**
	Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail" ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods			25.2	2647.1	
	Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods			7.2	9264.7	

In conclusion, even under the conservative assumptions of the screening step and the tier 1 acute dietary risk assessment all TER_A values for pyraclostrobin exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for birds from the use of BAS 516 07 F in potatoes according to the recommended use pattern.

CP 10.1.1.2 Higher tier data on birds

Please note that this section, albeit suggested by its header, does not only include higher tier data on birds. The calculations presented in Table 10.1.1.2-1 are the dietary TER reproductive values of the screening step and tier 1 assessment obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) for potatoes.

Table 10.1.1.2-1: Pyraclostrobin: Reproductive dietary risk assessment for birds in potatoes - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Repro End Point (mg/kg b.w./d)**	**Time weighted average (TWA)**
	Potatoes	0.017	4	10	10.0	105	0.53
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	64.8	1.10	1.9	1.11	94.7	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	**No refinement required**
	Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail" ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods			9.7	632.3	
	Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods			3.3	1858.6	

In conclusion, even under the conservative assumptions of the screening and the tier 1 reproductive risk assessments, all TER_{LT} values for pyraclostrobin exceed the trigger values set by Commission regulation (EU) 546/2011 for acceptability of effects, hence indicating a low reproductive risk for birds from the use of BAS 516 07 F in potatoes according to the recommended use pattern.

Acute toxicity of the formulation

An acute oral toxicity study with the formulation BAS 516 07 F in the bobwhite quail is available available and summarized below. Please note that the study was erroneously included in the Application submitted in January 2014 in chapter M-CP 10.1.1.1.

The results of the study show no increased formulation toxicity as the formulation is practically acutely non-toxic to birds (LD_{50} > 3 200 mg BAS 516 07 F/kg b.w.).

A detailed study summary is presented in the following. Further, an acute risk assessment for BAS 516 07 F considering the LD_{50} from formulation toxicity testing is provided below.

Report:	CP 10.1.1.2/1 [redacted], 2006b BAS 516 07 F - Acute toxicity in the bobwhite quail (Colinus virginianus) after single oral administration (LD50) 2006/1030309
Guidelines:	EPA 71-1, EPA 850.2100, EPA 540/9-85-007
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

A standard guideline study has been carried out to determine the effect of administration of BAS 516 07 F via gavage on the bobwhite quail (*Colinus virginianus*) and to determine the acute median lethal dose (LD_{50}) as well as the no observed effect level (NOEL). The study complies with the data requirements of US EPA FIFRA § 71-1 1982 and OPPTS 850.2100.

Bobwhite quails (*Colinus virginianus*) were approximately 5 months old, and before their first egg-laying season. The dose groups consisted of 5 male and 5 female birds. After fasted for about 22 hours the birds were administered doses of 0 (control), 800, 1 600 and 3 200 mg BAS 516 07 F / kg body weight in 0.5% aqueous carboxymethylcellulose (CMC) suspension by gavage into the crop. Mortalities and signs of toxicity were recorded for 14 days after administration.

No toxic signs were observed in the control and all dose groups. The highest dose causing no mortality was 3 200 mg BAS 516 07 F/kg b.w. No treatment-related impairment of food uptake or of body weights was observed in any of the dose groups. The gross post-mortem examination showed no treatment-related abnormalities in surviving birds after sacrifice. The highest dose tested without toxic signs was 3 200 mg BAS 516 07 F / kg b.w. The "No Observed Effect Level" (NOEL) was ≥ 3200 mg BAS 516 07 F / kg b.w. The acute median lethal dose (LD_{50}) was > 3 200 mg BAS 516 07 F / kg b.w.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 516 07 F, batch no. 1789; content of a.s.: Boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); Pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).
Test species:	Bobwhite quail (*Colinus virginianus*), indistinguishable from wild birds; age: approx. 5 months: source: Küberich, Wiesentheid/Geesdorf, Germany.

B. STUDY DESIGN

Test design:	Birds were treated once by administering the test item in aqueous CMC-dispersion by gavage (5 males / 5 females per dose group) into the crop. Observation period: 14 days. Assessment for mortality and clinical signs was carried out three times on day of dosing, thereafter daily. Assessment of body weight was carried out on the day of dosing and on days 7 and 14.
Endpoints:	LD_{50}, mortality, clinical signs, feed consumption, body weight (b.w.), gross pathological examinations were conducted on all surviving birds sacrificed at the termination of the definitive test.
Test concentrations:	Control, 800, 1 600 and 3 200 mg BAS 516 07 F / kg body weight (nominal).
Test conditions:	Birds fasted for 22 hours before administration of the test item; temperature 21°C ± 2°C, relative humidity: 35% - 70%, photoperiod 8 hours light : 16 hours dark during the test period, light intensity: 53 lux - 135 lux.
Analytics:	The test item concentrations were analyzed using HPLC with UV-detection.
Statistics:	Descriptive statistics, ANOVA followed by Dunnett-test for weight data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements:
The results of the analytical verification of the test item concentration were within a range of 94% to 98% for Boscalid and 96% to 100% for pyraclostrobin of the nominal concentrations during the test. The biological results are therefore based on the nominal values.

Biological results:
No toxic signs were observed in the control and all dose groups. The highest dose causing no mortality was 3 200 mg BAS 516 07 F / kg b.w. No treatment-related impairment of feed uptake or of body weights was observed in any of the dose groups. The gross post-mortem examination showed no treatment-related abnormalities in surviving birds after sacrifice.
The highest dose tested without toxic signs was 3 200 mg BAS 516 07 F / kg b.w. The "No Observed Effect Level" (NOEL) was ≥ 3 200 mg BAS 516 07 F / kg b.w. The acute median lethal dose (LD_{50}) was > 3 200 mg BAS 516 07 F / kg b.w. The results are summarized below.

Acute toxicity of BAS 516 07 F to the bobwhite quail (*Colinus virginianus*)

Mortality	Dose [mg BAS 516 07 F/kg b.w.]
Highest dose causing no treatment-related mortality	3 200
LD_{50} (14 d)	> 3 200
NOEL	≥ 3 200

III. CONCLUSION

In an acute toxicity test with the bobwhite quail, the 14 day LD_{50} of BAS 516 07 F was > 3 200 mg BAS 516 07 F / kg b.w. The NOEL was ≥ 3 200 mg BAS 516 07 F / kg b.w.

Acute risk assessment considering the LD_{50} from toxicity testing with BAS 516 07 F

Toxicity endpoint of the formulation for birds

Please note that the use of the endpoint from the acute toxicity study (BASF DocID 2006/1030309) of BAS 516 07 F on bobwhite quails (*i.e.* setting LD_{50} to 3200 mg/kg b.w. in the tier 1 calculations with EFSA calculator tool) will lead to an over-conservative approach as no substance-related mortality occurred throughout the study period, and it can thus be expected that the true LD_{50}, which would be actually relevant, is clearly higher.

A tier 1 acute dietary exposure assessment and TER calculation with the LD_{50} > 3200 mg BAS 516 07 F /kg b.w. from the acute oral bobwhite quail study with the formulation will be carried out (see below). However, due to the points mentioned above, the risk is adequately and more realistically assessed using the LD_{50} mix for a virtual compound in Table 10.1.1.2-10.

Exposure

BAS 516 07 F is intended to be used in potatoes with four applications at a maximum single application rate of 0.25 kg as BAS 516 07 F/ha, taking into account the density of the formulation of 1.04 g/cm^3 (see Table 10.1.1-6 for the critical use pattern). The relevant scenarios for the critical use pattern are given in Table 10.1.1-7.

Calculation of the screening and tier 1 risk assessment for BAS 516 07 F according to EFSA/2009/1438

The screening and tier 1 TER values for the acute dietary risk assessment for birds as obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) are presented for in Table 10.1.1.2-2.

Table 10.1.1.2-2: BAS 516 07 F: Acute risk assessment for birds in potatoes - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg formulation/ ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Potatoes	0.25	4	10	10.0	>3200.0	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	158.8	39.7	1.5	59.55	53.7	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**		**Shortcut value**	**TER**	**No refinement required**	
	Potatoes BBCH ≥ 20	Small insectivorous bird “wagtail” ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods		25.2	> 338.6		
	Potatoes BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods		7.2	> 1185.2		

In conclusion, even under the conservative assumptions of the screening step and the tier 1 acute dietary risk assessment all TER_A values for the formulation exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for birds from the use of BAS 516 07 F in potatoes according to the recommended use pattern.

Effects of secondary poisoning

The log P_{ow} of the active substance pyraclostrobin is 3.99 (SANCO/1420/2001-final), which triggers an assessment for the potential risk through secondary poisoning according to EFSA/2009/1438.

Risk assessment for earthworm-eating birds

The risk assessment for earthworm-eating birds will be based on the worst-case PEC_{soil} (twa, 21 days) derived from M-CP 9.1. The calculations and the resulting TER_{LT} values are summarized in Table 10.1.1.2-3.

Table 10.1.1.2-3: Risk assessment for pyraclostrobin concerning earthworm-eating birds (tier 1) – dry soil approach

Parameter	Pyraclostrobin	Reference
PEC_{soil} (twa, 21 days) [1] [mg/kg soil]	0.013	M-CP 9.1
K_{ow}	9772	SANCO/1420/2001-final
K_{oc} (arithmetic mean)	9304	SANCO/1420/2001-final
f_{oc} (default)	0.02	EFSA/2009/1438
BCF [2]	0.635	--
PEC_{worm} [mg/kg] [3]	0.008	--
Daily dose [mg/kg b.w./d] [4]	0.009	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	105.0	Table 10.1.1-1
TER_{LT} [5]	**12119.70**	**--**

1 PEC_{soil} (twa, 21 days) value calculated for four applications of BAS 516 07 F in potatoes. For details see chapter 9.1.
2 Bioconcentration factor (BCF) = $(0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
3 $PEC_{worm} = PEC_{soil} \times BCF$
4 Daily dose = $1.05 \times PEC_{worm}$
5 TER_{LT} = NO(A)EL / Daily dose.

Risk assessment for fish-eating birds

The risk assessment for fish-eating birds will be based on the worst-case PEC_{sw} (twa, 21 days) derived from M-CP 9.2. The calculations and the resulting TER_{LT} values are summarized in Table 10.1.1.2-4.

Table 10.1.1.2-4: Risk assessment for pyraclostrobin concerning fish-eating birds (tier 1)

Parameter	Pyraclostrobin	Reference
$PEC_{sw,}$ (twa, 21 days) [mg/L] [1)]	$0.121 * 10^{-3}$	M-CP 9.2
BCF fish (max. worst case)	736	SANCO/1420/2001-final
PEC_{fish} [mg/kg] [2)]	0.089	--
Daily dose [mg/kg b.w./d] [3)]	0.014	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	105.0	Table 10.1.1-1
TER_{LT} [4)]	**7415.30**	--

1 Highest PEC_{sw} (twa, 21 days) from FOCUS Step 2 (South Europe) calculation for a multiple application scenario of BAS 516 07 F in potatoes with parameters from dark water/sediment study as worst-case. For details see chapter M-CP 9.2.
2 PEC_{fish} = PEC_{sw} (twa, 21 days) x BCF
3 Daily dose = 0.159 * PEC_{fish}
4 TER_{LT} = NO(A)EL / Daily dose

In the above risk assessments for earthworm- and fish-eating birds, the TER values exceed the trigger of 5 set by Commission regulation (EU) 546/2011 for acceptability of effects. It can therefore be concluded that the application of BAS 516 07 F in potatoes does not provide reason for concern regarding a potential accumulation of the active substance pyraclostrobin in the food chain or for concern of secondary poisoning.

Biomagnification in terrestrial food chains

No evidence was found for potential of accumulation of pyraclostrobin in animal tissue (Review Report for the active substance pyraclostrobin. Appendix II, endpoints and related information. 1. Toxicology and metabolism. September 2004).

Based on this it can be concluded that the potential for bioaccumulation in animal tissue for pyraclostrobin is low and thus no further assessment on biomagnification is required.

Risk for birds through drinking water

EFSA/2009/1438 proposes an assessment methodology for the risk to birds from active substances in drinking water using small granivorous birds as indicator species in tier 1. Out of the two scenarios, i.e. the leaf and the puddle scenario, the leaf scenario is not relevant for use in potatoes. Consequently, in a drinking water risk assessment for birds only the 'puddle scenario' would need to be considered for the application of BAS 516 07 F in potatoes.

According to EFSA/2009/1438 no specific calculations of exposure and TER values are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg b.w./d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg). The ratio calculations for effective application rate to relevant endpoint are detailed in a screening step in Table 10.1.1.2-5. The ratio for acute and reproductive endpoints for pyraclostrobin (0.02 and 0.4, respectively) do not exceed the threshold value of 3000 as given by EFSA/2009/1438 for more sorptive substances (Koc > 500 L/kg), thus no specific calculations of exposure for birds through drinking water for the puddle scenario are necessary (Table 10.1.1.2-5).

In conclusion, the risk through drinking water from the use of BAS 516 07 F in potatoes is acceptable.

Table 10.1.1.2-5: Screening step for drinking water risk assessment - ratio of effective application rate to relevant endpoint for birds

Parameter	Pyraclostrobin	Reference
K_{oc} [L/kg]	9304	SANCO/1420/2001-final
DT_{50} (soil) [d]	18	M-CP 9.2
Number of applications	4	Table 10.1.1-6
Interval [d]	10	Table 10.1.1-6
MAF_m 1)	2.46	--
Max use rate [g/ha]	17	Table 10.1.1-6
AR_{eff} [g/ha] 2)	41.82	--
LD_{50} [mg/kg b.w.]	1701	Table 10.1.1-1
Ratio (acute) 3)	0.02	--
Reproductive endpoint [mg/kg b.w./d]	105	Table 10.1.1-1
Ratio (repro) 3)	0.4	--
Trigger 4)	3000	--
Drinking water assessment required [Yes/No] 5)	No	--

1 $MAFm = (1-e^{-nki}) / (1-e^{-ki})$ with $k = ln(2)/DT_{50}$ (rate constant), n = number of applications and i = application interval [d]
2 AR_{eff} = Application rate (g/ha) x MAF_m
3 Ratio of AR_{eff} and relevant toxicity endpoint
4 Trigger according to EFSA/2009/1438
5 Drinking water risk assessment is not necessary when trigger value is not exceeded.

A quantitative drinking water risk assessment is not triggered for the recommended use pattern of BAS 516 07 F in potatoes according to EFSA/2009/1438 criteria. Therefore, the risk to birds via drinking water is acceptable.

Exposure to several active substances

In the following the risk of birds being exposed to a formulated product containing more than one active substance is addressed using the **EFSA/2009/1438 approach on "Combined effects of simultaneous exposure to several active substances"**. The simultaneous exposure of animals to residues of two potential toxicants of BAS 516 07 F, i.e. pyraclostrobin and boscalid, was estimated according to information given in Appendix B of EFSA/2009/1438.

Acute risk assessment for a virtual compound with surrogate LD_{50} (=LD_{50} mix)

Calculation of surrogate LD_{50} values for acute effects (mortality)

The following equation has been used for deriving a surrogate LD_{50} (mix) for a mixture of active substances based on the assumption of dose additivity.

Equation 1:

$$LD_{50(mix)} = \left(\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

With:

$X(a.s._i)$ = fraction of active substance [i] in the mixture; ($\Sigma X(a.s._i) = 1$)
$LD_{50}(a.s._i)$ = acute toxicity value for active substance [i]
LD_{50}(mix) is also called 'surrogate LD_{50}'

The acute risk assessment for a virtual compound is assessed for the formulation BAS 516 07 F.

According to the label, BAS 516 07 F contains 67 g/kg pyraclostrobin and 267 g/kg boscalid. The relative amounts of pyraclostrobin and boscalid for the formulated product are given in Table 10.1.1.2-6.

Table 10.1.1.2-6: Relative amounts of pyraclostrobin and boscalid in BAS 516 07 F

Formulation	BAS 516 07 F	
Active substance	**Amount a.s. in product [g/kg]**	**Fraction of active substance in product**
Boscalid	267	0.80
Pyraclostrobin	67	0.20
Sum	334	1

Avian acute oral toxicity studies have been carried out with the active substances pyraclostrobin (Table 10.1.1-1) and boscalid (Table 10.1.1.2-7). Equation 1 has been used for deriving a surrogate LD50 for a mixture of active substances as presented in Table 10.1.1.2-8.

Table 10.1.1.2-7: EU agreed acute endpoint of boscalid for birds

Study type	Test species	EU agreed endpoints	Endpoints used in risk assessment
Boscalid			
Acute toxicity to birds	*Colinus virginianus*	LD_{50} > 2000 mg a.s. / kg b.w.[1)]	LD_{50} > 2000 mg a.s. / kg b.w.[1)]

1 Endpoint from Review Report given under '3 Ecotoxicology' for the active substance boscalid, SANCO/3919/2007-rev. 5, 17.01.08

Table 10.1.1.2-8: Calculation of surrogate LD_{50} values for acute dietary risk assessment calculated from acute endpoints of active substances

Active substance	Acute LD_{50} [mg a.s./kg b.w.]	Fraction of a.s. in product: X ($a.s._i$)	X ($a.s._i$)/$LD_{50\ i}$	Sum [1)]	Surrogate LD_{50} [2)] [mg virtual compound/kg b.w.]
Boscalid	>2000	0.8	0.00040	0.00052	1931.9
Pyraclostrobin	1701	0.2	0.00012		

1 $\sum$ (X ($a.s._i$) / extrapolated $LD_{50\ i}$)
2 Surrogate LD_{50} = 1/($\sum$ X ($a.s._i$) / extrapolated $LD_{50\ of\ a\ s}$)

The surrogate LD_{50}, or LD_{50} (mix), for the mixture of active substances in BAS 516 07 F is 1931.9 mg virtual compound/kg b.w.

According to EFSA/2009/1438 the calculated LD_{50} (mix) can be used in the risk assessment if the deviation between the 'tox per fraction' of the active substance to the tox per fraction of the mixture are greater than 10% for each active substance. This is the case for the tox per fraction of pyraclostrobin and boscalid as detailed in Table 10.1.1.2-9. The tox per fraction for boscalid deviates by 30% to the tox per fraction of the mixture and for pyraclostrobin it deviates by 339% to the tox per fraction of the mixture. Therefore, the calculated LD_{50} (mix) can be used in the risk assessment using the combination toxicity approach.

Table 10.1.1.2-9: Deviation of the tox per fraction comparing pyraclostrobin and boscalid to the tox per fraction of the mixture

Active substance	Tox per fraction (a.s.) [1)] [mg a.s./kg b.w.]	LD_{50} (mix) [2)] [mg virtual compound/kg b.w.]	Deviation [3)] [%]
Boscalid	2501.9	1931.9	30
Pyraclostrobin	8479.6		339

1 Extrapolated LD_{50} ($a.s._i$) /X ($a.s._i$)
2 LD50 (mix) is the "tox per fraction (mix)" and calculated with LD_{50} (mix) = surrogate LD_{50}) /Σ X($a.s._i$). This means that the LD_{50} (mix) is equal to the surrogate LD_{50}, since the sum of the fractions of a.s. in the product must equal 1.
3 (100/Tox per fraction (mix) x Tox per fraction ($a.s._i$))-100

In conclusion, the acute ecotoxicological endpoint, estimating mixture toxicity of pyraclostrobin and boscalid as contained in the formulation BAS 516 07 F is LD_{50} (mix) = 1931.9 mg virtual compound/kg b.w.

Exposure

BAS 516 07 F (containing the active substances pyraclostrobin and boscalid) is intended to be used in potatoes from BBCH 51-89 (Table 10.1.1-6). The potential exposure of birds to pyraclostrobin and boscalid was estimated following four applications of BAS 516 07 F at the maximum use rate of 0.25 kg product/ha, corresponding to 0.017 kg/ha pyraclostrobin and 0.067 kg/ha boscalid. For details please refer to the use pattern in Table 10.1.1-6.

According to the EFSA/2009/1438 an endpoint for a mixture of active substances calculated assuming dose additivity should be conceived as an endpoint of a single virtual compound. Therefore, the exposure calculation for the risk assessment is based as well on this assumption. Content in the formulation and application rate per hectare should thus be expressed in terms of this virtual compound. Therefore, the overall application rate for active substances combined of 0.084 kg virtual compound/ha is considered for the acute risk assessment. The relevant scenarios are given in Table 10.1.1-7.

Calculation of the screening and tier 1 risk assessment for a virtual compound according to EFSA/2009/1438

The avian screening and tier 1 TER values for the acute dietary risk assessment of the virtual compound as obtained by means of the EFSA calculator tool (version of 9 July 2010, http:// www.efsa. europa.eu /de /efsajournal/pub/1438.htm) are presented in Table 10.1.1.2-10.

Table 10.1.1.2-10: Virtual compound: Acute risk assessment for birds in potatoes - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (Kg/ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Potatoes	0.084	4	10	10.0	1931.9	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small omnivorous bird	158.8	13.34	1.5	20.01	96.6	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	**No refinement required**
	Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail" ground invertebrates with interception 50% ground arthropods, 50% foliar arthropods			25.2	608.4	
	Potatoes BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates without interception) 25% crop leaves 25% weed seeds 50% ground arthropods			7.2	2129.5	

In conclusion, even under the conservative assumptions of the screening step and the tier 1 acute dietary risk assessment all TER values for the virtual compound exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for birds from the use of BAS 516 07 F in potatoes.

Consideration of mixture toxicity for sublethal effects and effects on reproduction

Please note that it is currently not recommended to consider the use of predicted toxicity values as surrogates in the reproductive risk assessment. For a detailed discussion please refer to Appendix B (p.4) of EFSA/2009/1438.

Conclusion

In conclusion, even under the conservative assumptions of the screening step and the tier 1 acute dietary risk assessments for the virtual compound and the formulation, all TER_A values, from the risk assessment addressing the combined effects of simultaneous exposure to two active substances, exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for birds from the use of BAS 516 07 F in potatoes according to the recommended use pattern.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

Data on BAS 516 07 F are evaluated and the risk assessments are presented for the active substance pyraclostrobin and for the evaluation of combined effects of formulations containing more than one active substance based on the already registered use pattern.

Table 10.1.2-1: Endpoints for mammals

Test system	Test species	EU agreed endpoints	Endpoints used in present risk assessment
Acute toxicity	Rat	LD_{50} > 5000 mg a.s./kg b.w. [1)]	LD_{50} > 5000 mg a.s./kg b.w.
Reproductive toxicity (long-term)	Rat	--	NOEL = 3 mg a.s./kg b.w./d [2)]

1 Endpoints confirmed in the Ecotoxicology section of the Review report for the active substance pyraclostrobin, September 2004.

2 Daily Dose [mg/kg b.w./d] calculated based on study data for food consumption and body weight.

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Summary of mammalian toxicity testing

The mammalian toxicity studies with pyraclostrobin relevant for a tier 1 dietary risk assessment for wild mammals are summarized in Table 10.1.2-2.

Table 10.1.2-2: Summary of studies relevant for the mammalian risk assessment for pyraclostrobin

Test system	Test species	Results	Reference (BASF DocID)
Acute oral toxicity	Rat	LD_{50} > 5000 mg a.s./kg b.w. [1)]	1998/10965
Reproductive toxicity	Rat	$NOEL_{Reproduction}$ = 32.6 mg a.s./kg b.w./d [2)] $NOEL_{Offspring}$ = 8.2 mg a.s./kg b.w./d [2)] $NOEL_{Parents}$ = 8.2 mg a.s./kg b.w./d [2)]	1999/11869
Developmental toxicity	Rat	$NOEL_{Rel\ Maternal}$ = 10 mg a.s./kg b.w./d [2)] $NOEL_{Rel\ Developmental}$ = 50 mg a.s./kg b.w./d [2)]	1999/11511
	Rabbit	$NOEL_{Rel\ Maternal}$ = 3 mg a.s./kg b.w./d [2)] $NOEL_{Rel\ Developmental}$ = 5 mg a.s./kg b.w./d [2)]	2001/1003803 and 1999/11512

1 Endpoints confirmed in the Ecotoxicology section of the Review report for the active substance pyraclostrobin, September 2004.

2 Daily Dose [mg/kg b.w./d] calculated based on study data for food consumption and body weight.

Mammalian toxicity endpoint for use in the acute tier 1 risk assessment

There is one acute oral toxicity test with pyraclostrobin available in rats (BASF DocID 1998/10965). This study resulted in an **LD_{50} > 5000 mg a.s./kg b.w.**, which is the relevant endpoint to be used in the acute risk assessment.

Mammalian toxicity endpoint for use in the reproductive tier 1 risk assessment

In order to obtain the relevant toxicity endpoint for the tier 1 reproductive risk assessment, the lowest NOAEL from the two-generation rat study and the lowest relevant endpoint from the developmental studies have to be considered.

For pyraclostrobin, the lowest toxicity endpoint from these studies (Table 10.1.2-2) is the **NOEL = 3 mg a.s./kg b.w./d**, derived from the developmental rabbit study. This value is the endpoint used for the screening and tier 1 reproductive risk assessment.

Results from available toxicity testing with the formulation

The mammalian toxicity study with BAS 516 07 F relevant for the risk assessment for mammals is summarized in Table 10.1.2-3.

Table 10.1.2-3: Summary of mammalian study on the acute toxicity of BAS 516 07 F

Test system	Test species	Results	Reference (BASF DocID)
Acute oral toxicity	Rat	LD_{50} > 2000 mg/kg b.w.	2008/1004838 [1)]

1 For detailed study summary please refer to M-CP 7.1.1.

A full summary of this study is given in M-CP 7.1.1. An acute risk assessment for BAS 516 07 F considering the **LD_{50} > 2000 mg/kg b.w. for BAS 516 07 F** is provided further below.

Metabolites of pyraclostrobin

A full assessment of the relevance of pyraclostrobin metabolites for the birds and mammals risk assessment is given in M-CP 10.1.1. Please refer to M-CP 10.1.1 for details.

Summary of relevant mammalian toxicity endpoints to be used in the screening and tier 1 risk assessments

The mammalian endpoints relevant for the screening step and for the tier 1 risk assessments are given in Table 10.1.2-4.

Table 10.1.2-4: Mammalian toxicity endpoints for screening and tier 1 risk assessments of pyraclostrobin and BAS 516 07 F

Test substance	Test system	Relevant toxicity endpoint
Pyraclostrobin	Acute oral toxicity	LD_{50} > 5 000 mg a.s./kg b.w.
	Sub-chronic toxicity and reproduction	NOEL = 3 mg a.s./kg b.w./d
BAS 516 07 F	Acute oral toxicity	LD_{50} > 2000 mg a.s./kg b.w.

Relevant exposure scenarios

The relevant scenarios for the already registered use pattern (Table 10.1.2-5) are given in Table 10.1.2-6.

Table 10.1.2-5: Critical use pattern for BAS 516 07 F

Crop	Crop group according to EFSA/2009/ 1438	Application time (BBCH growth stage)	Number of applications	Interval [days]	Maximum application rate per treatment		
					Pyraclostrobin [kg/ha]	Boscalid[1)] [kg/ha]	BAS 516 07 F [L/ha]
Potatoes	Potatoes	51-89	4	10	0.017	0.067	0.25

1 Not relevant for the renewing the approval of pyraclostrobin. Therefore, the a.s. boscalid is not evaluated here.

Table 10.1.2-6: Summary of exposure scenarios to be considered for risk assessments for spray applications of BAS 516 07 F in potatoes

Scenario	Generic focal species
Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew”
Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph”
Potatoes BBCH ≥ 40	Small herbivorous mammal "vole"
Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse”

CP 10.1.2.1 Acute oral toxicity to mammals

In this section, the dietary TER acute values for the screening step and tier 1 assessment obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) are presented for pyraclostrobin in Table 10.1.2.1-1.

Table 10.1.2.1-1: Pyraclostrobin: Acute dietary risk assessment for mammals in potatoes - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Potatoes	0.017	4	10	10.0	>5000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small herbivorous mammal	118.4	2.01	1.5	3.02	1656.1	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**		**Shortcut value**	**TER**	**No refinement required**	
	Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew” ground dwelling invertebrates with interception 100% ground arthropods		5.4	>36310.8		
	Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs		10.5	>18674.1		
	Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass		40.9	>4794.1		
	Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods		5.2	>37707.4		

In conclusion, even under the conservative assumptions of the screening and tier 1 acute dietary risk assessment all TER_A values for pyraclostrobin exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for mammals from the use of BAS 516 07 F in potatoes according to the recommended use pattern.

CP 10.1.2.2 Higher tier data on mammals

Please note that this section, albeit suggested by its header, does not only include higher tier data on mammals. The calculations presented for potatoes in Table 10.1.2.2-1 are the **dietary TER reproductive values for the screening step and tier 1 assessment** obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu /de/efsajournal/pub/1438.htm).

Table 10.1.2.2-1: Pyraclostrobin: Reproductive risk assessment for mammals in potatoes - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (Kg a.s./ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**Repro End Point (mg/kg bw/d)**	**Time weighted average (TWA)**
	Potatoes	0.017	4	10	10	3	0.53
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF mean**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small herbivorous mammal	48.3	0.82	.19	0.83	3.63	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**			**Shortcut value**	**TER**	**No refinement required**
	Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew” ground dwelling invertebrates with interception 100% ground arthropods			1.9	92.2	
	Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs			4.3	40.8	
	Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass			21.7	8.1	
	Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods			2.3	76.2	

In conclusion, even under the conservative assumptions of the tier 1 reproductive dietary assessments, all TER_{LT} values for pyraclostrobin exceed the trigger values set by Commission regulation (EU) 546/2011 for acceptability of effect. This indicates a low reproductive risk for mammals from the use of BAS 516 07 F in potatoes according to the recommended use pattern.

Acute oral toxicity of the preparation

An acute oral toxicity study is available for the formulation BAS 516 07 F. The results of the study show no increased formulation toxicity as the formulation is practically acutely non-toxic to mammals (LD_{50} > 2000 mg BAS 516 07 F/kg b.w.). A detailed summary of this study is given in M-CP 7.1.1.

Please note, an acute risk assessment for BAS 516 07 F with LD_{50} from formulation toxicity testing is provided further below.

Acute risk assessment considering the LD_{50} from toxicity testing with BAS 516 07 F

Toxicity endpoint of the formulation for mammals

Acute oral toxicity studies of formulations are not specifically designed for use in wild mammal risk assessments but are conducted for the purpose of classification and labelling and include testing of rates with single doses of the intact formulation administered by oral gavage. The type and pattern of exposure of wild mammals following a foliar spray in the field is very different than the exposure pattern tested in acute oral gavage tests.

Hence, the results of such acute oral toxicity tests are of limited relevance for the acute wild mammal risk assessment. Nevertheless, for a conservative estimate, the endpoint of the acute oral toxicity study with BAS 516 07 F on rats (i.e. LD_{50} > 2000 mg/kg b.w.) will be used for a tier 1 acute risk assessment of the formulation. The results of the tier 1 acute dietary exposure assessment and TER calculation will be presented below.

However, note that the risk of the formulation is considered to be adequately and more realistically assessed using the LD_{50} mix for a virtual compound in Table 10.1.2.2-10.

Exposure

BAS 516 07 F is intended to be used in potatoes with four applications at a maximum single application rate of 0.25 kg as BAS 516 07 F/ha (see Table 10.1.1-6 for the critical use pattern). The relevant scenarios for the critical use pattern are given in Table 10.1.1-7.

Calculation of the screening and tier 1 risk assessment for BAS 516 07 F according to EFSA/2009/1438

The screening and tier 1 TER values for the acute dietary risk assessment for mammals as obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) are presented in Table 10.1.2.2-2.

Table 10.1.2.2-2: BAS 516 07 F: Acute risk assessment for mammals - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg formulation/ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Potatoes	0.25	4	10	10	>2000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement required**
	Small herbivorous mammal	118.4	29.60	1.5	44.40	>45.0	

Tier 1 Risk Assessment:					
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**	**Shortcut value**	**TER**	**No refinement required**
	Potatoes BBCH ≥ 20	Small insectivorous mammal “shrew” ground dwelling invertebrates with interception 100% ground arthropods	5.4	>987.7	
	Potatoes BBCH ≥ 40	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs	10.5	>507.9	
	Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	40.9	>130.4	
	Potatoes BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	5.2	>1025.6	

In conclusion, even under the conservative assumptions of the screening step and the tier 1 acute dietary risk assessment all TER values for the formulation exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for mammals from the use of BAS 516 07 F in potatoes according to the recommended use pattern.

Effects of secondary poisoning

The log P_{ow} of the active substance pyraclostrobin was determined to be 3.99 (SANCO/1420/2001-final) which triggers an assessment for the potential risk through secondary poisoning according to EFSA/2009/1438.

Risk assessment for earthworm-eating mammals

The risk assessment for earthworm-eating mammals will be based on the worst-case PEC_{soil} (twa, 21 days) derived from M-CP 9.1. The calculations and the resulting TER_{LT} values are summarized in Table 10.1.2.2-3.

Table 10.1.2.2-3: Risk assessment for pyraclostrobin concerning earthworm-eating mammals – dry soil approach

Parameter	Pyraclostrobin	Reference
PEC_{soil} (twa, 21 days) [1] [mg/kg soil]	0.013	M-CP 9.1
K_{ow}	9772	SANCO/1420/2001-final;
K_{oc} (arithmetic mean)	9304	SANCO/1420/2001-final;
f_{oc} (default)	0.02	EFSA/2009/1438
BCF [2]	0.635	--
PEC_{worm} [mg/kg] [3]	0.008	--
Daily dose [mg/kg b.w./d] [4]	0.011	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	3	Table 10.1.2-1
TER_{LT} [5]	284.06	--

1 PECsoil (twa, 21 days) value calculated for four applications of BAS 516 07 F in potatoes. For details see chapter 9.1.
2 Bioconcentration factor (BCF) = $(0.84 + 0.012 \times K_{ow}) / f_{oc} \times K_{oc}$
3 $PEC_{worm} = PEC_{soil} \times BCF$
4 Daily dose = $1.28 \times PEC_{worm}$
5 TER_{LT} = NO(A)EL / Daily dose.

The risk assessment for fish-eating mammals will be based on the worst-case PEC_{sw} (twa, 21 days) derived from M-CP 9.2. The calculations and the resulting TER_{LT} values are summarized in Table 10.1.2.2-4.

Table 10.1.2.2-4: Risk assessment for pyraclostrobin concerning fish-eating birds (tier 1)

Parameter	Pyraclostrobin	Reference
$PEC_{sw,}$ (twa, 21 days) [mg/L] 1)	$0.121 * 10^{-3}$	M-CP 9.2
BCF fish (max. worst case)	736	SANCO/1420/2001-final
PEC_{fish} [mg/kg] 2)	0.089	--
Daily dose [mg/kg b.w./d] 3)	0.013	EFSA/2009/1438
NO(A)EL [mg/kg b.w./d]	3.0	Table 10.1.2-1
TER_{LT} 4)	237.23	--

1 Highest PECsw (twa, 21 days) from FOCUS Step 2 (South Europe) calculation for a multiple application scenario of BAS 516 07 F in potatoes with parameters from dark water/sediment study as worst-case.
2 $PEC_{fish} = PEC_{sw}$ x BCF
3 Daily dose = 0.142 x PEC_{fish}
4 TER_{LT} = NO(A)EL / Daily dose

In the above risk assessments for earthworm- and fish-eating mammals, the TER values exceed the trigger of 5 set by Commission regulation (EU) 546/2011 for acceptability of effects. **It can therefore be concluded that the application of BAS 516 07 F in potatoes does not provide reason for concern regarding a potential accumulation of the active substance pyraclostrobin in the food chain or for concern of secondary poisoning.**

Biomagnification in terrestrial food chains

No evidence was found for potential of accumulation of pyraclostrobin in animal tissue (Review report for the active substance pyraclostrobin. Appendix II, endpoints and related information. September, 2004).

Based on this it can be concluded that the potential for bioaccumulation in animal tissue for pyraclostrobin is low and thus no further assessment on biomagnification is required**.**

Risk for mammals through drinking water

EFSA/2009/1438 proposes an assessment methodology for the risk to mammals from active substances in drinking water using small granivorous mammals as indicator species in tier 1. Out of the two scenarios, i.e. the leaf and the puddle scenario, the leaf scenario is not relevant for small mammals. Consequently, in the following drinking water risk assessment for mammals only the ‘puddle scenario’ will be considered for the application of BAS 516 07 F in potatoes.

According to EFSA/2009/1438 no specific calculations of exposure and TER values are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg b.w./d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg). The ratio calculations for effective application rate to relevant endpoint are detailed in a screening step in Table 10.1.2.2-5. The ratio for acute and reproductive endpoints for pyraclostrobin (<0.01 and 13.9, respectively) and do not exceed the threshold value of 3000, as given by EFSA/2009/1438 for more sorptive substances (Koc < 500 L/kg). Thus no specific calculations of exposure and TER for mammals through drinking water for the puddle scenario are necessary**. In conclusion, the risk through drinking water from the intended use of BAS 516 07 F in potatoes is acceptable.**

Table 10.1.2.2-5: Screening step for drinking water risk assessment - ratio of effective application rate to relevant endpoint for mammals

Parameter	Pyraclostrobin	Reference
Koc [L/kg] (arithmetic mean)	9304	SANCO/1420/2001-final
DT_{50} (soil) [days]	18	M-CP 9.2
Number of applications	4	Table 10.1.2-5
Interval [days]	10	Table 10.1.2-5
MAF_m 1)	2.46	--
Max use rate [g/ha]	17	Table 10.1.2-5
AR_{eff} [g/ha] 2)	41.82	--
LD_{50} [mg/kg b.w.]	> 5 000	Table 10.1.2-1
Ratio (acute) 3)	<0.01	--
NO(A)EL [mg/kg b.w./d]	3	Table 10.1.2-1
Ratio (repro) 3)	13.9	--
Trigger 4)	3 000	--
Drinking water assessment required [Yes/No] 5)	No	--

1 $MAFm = (1-e^{-nki}) / (1-e^{-ki})$ with $k = ln(2)/ DT_{50}$ (rate constant), n = number of applications and i = application interval [d]
2 AR_{eff} = Application rate (g/ha) x MAF_m
3 Ratio of AR_{eff} and relevant toxicity endpoint
4 Trigger according to EFSA/2009/1438
5 Drinking water risk assessment is not necessary when trigger value is not exceeded

Exposure to several active substances

In the following the risk of mammals being exposed to a formulated product containing more than one active substance is addressed using the **EFSA/2009/1438 approach on "Combined effects of simultaneous exposure to several active substances"**. The simultaneous exposure of animals to residues of two potential toxicants of BAS 516 07 F, i.e. pyraclostrobin and boscalid, was estimated according to information given in Appendix B of EFSA/2009/1438.

Acute risk assessment for a virtual compound with surrogate LD_{50} (=LD_{50} mix)

Calculation of surrogate LD_{50} values for acute effects (mortality)

The following equation has been used for deriving a surrogate LD_{50} (mix) for a mixture of active substances based on the assumption of dose additivity.

Equation 1:

$$LD_{50(mix)} = \left(\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} \right)^{-1}$$

With:

$X(a.s._i)$ = fraction of active substance [i] in the mixture; ($\Sigma X(a.s._i) = 1$)
$LD_{50}(a.s._i)$ = acute toxicity value for active substance [i]
LD_{50} (mix) is a synonym for 'surrogate LD_{50}'

The acute risk assessment for a virtual compound is assessed for the formulation BAS 516 07 F.

According to the label, BAS 516 07 F contains 67 g/kg pyraclostrobin and 267 g/kg boscalid. The relative amounts of pyraclostrobin and boscalid for the formulated product are given in Table 10.1.2.2-6.

Table 10.1.2.2-6: Relative amounts of pyraclostrobin and boscalid in BAS 516 07 F

Formulation	**BAS 516 07 F**	
Active substance	**Amount in product [g/kg]**	**Fraction of active substance in product**
Boscalid	267	0.80
Pyraclostrobin	67	0.20
Sum	334	1

Mammalian acute oral toxicity studies have been carried out with the active substances pyraclostrobin (s. Table 10.1.2-1) and boscalid (s. Table 10.1.2.2-7). Equation 1 has been used for deriving a surrogate LD50 for a mixture of active substances as presented in Table 10.1.2.2-8.

Table 10.1.2.2-7: EU agreed acute endpoint of boscalid for mammals

Study type	Test species	EU agreed endpoints	Endpoints used in risk assessment
Boscalid			
Acute toxicity to mammals	Rat	LD_{50} > 5000 mg. a.s. / kg b.w.[1)]	LD_{50} > 5000 mg. a.s. / kg b.w.[1)]

1 Endpoint from Review Report given under '3 Ecotoxicology' for the active substance boscalid, SANCO/3919/2007-rev. 5, 17.01.08

Table 10.1.2.2-8: Calculation of surrogate LD_{50} values for acute dietary risk assessment based on acute endpoints of active substances

Active substance	LD_{50} [mg a.s./kg b.w.]	Fraction of a.s. in product: X ($a.s._i$)	X ($a.s._i$)/$LD_{50\ i}$	Sum[1)]	Surrogate LD_{50} [2)] [mg a.s./kg b.w.]
Boscalid	>5000	0.80	0.00016	0.0002	5000
Pyraclostrobin	>5000	0.20	0.00004		

1 $\sum$ (X (a.s.i)/LD50 i)
2 LD_{50} (mix) (= surrogate LD_{50}) = $1/(\sum X\ (a.s._i)/LD_{50\ of\ a\ s})$

The surrogate LD_{50}, or LD_{50} (mix), for the mixture of active substances in BAS 516 07 F is 5000mg a.s./kg b.w.

According to EFSA/2009/1438 the calculated LD_{50} (mix) can be used in the risk assessment if the deviation between the 'tox per fraction' of the active substance to the tox per fraction of the mixture are greater than 10% for each active substance. This is the case for the tox per fraction of pyraclostrobin and boscalid as detailed in Table 10.1.2.2-9. The tox per fraction values for pyraclostrobin and boscalid deviate by 25% and 399% from the tox per fraction value of the mixture, respectively. Therefore, the calculated LD_{50} (mix) can be used in the risk assessment using the combination toxicity approach.

Table 10.1.2.2-9: Deviation of the tox per fraction comparing pyraclostrobin and boscalid to the tox per fraction of the mixture

Active substance	Tox per fraction (a.s.) [1)] [mg a.s./kg b.w.]	Tox per fraction (mix) [2)] [mg a.s./kg b.w.]	Deviation [3)] [%]
Boscalid	6254.7	5000	25
Pyraclostrobin	24925.4		399

1 $LD_{50}\ (a.s._i)/X\ (a.s._i)$
2 LD_{50} (mix) (= surrogate LD_{50})/Σ X($a.s._i$). This means that acute tox per fraction (mix) = LD_{50} (mix) (= surrogate LD_{50}), since the sum of the fractions must be equal to 1.
3 (100/Tox per fraction (mix) x Tox per fraction ($a.s._i$))-100

In conclusion, the acute ecotoxicological endpoint, estimating mixture toxicity of pyraclostrobin and boscalid is LD_{50} mix = 5000.0 mg virtual compound/kg b.w.

Exposure

BAS 516 07 F (containing the active substances pyraclostrobin and boscalid) is intended to be used in potatoes from BBCH 51-89 (Table 10.1.1-6). The potential exposure of birds to pyraclostrobin and boscalid was estimated following four applications of BAS 516 07 F at the maximum use rate of 0.25 kg product/ha, corresponding to 0.017 kg/ha pyraclostrobin and 0.067 kg/ha boscalid. For details please refer to the use pattern in Table 10.1.1-6. The relevant scenarios are given in Table 10.1.1-7.

According to the EFSA/2009/1438 an endpoint for a mixture of active substances calculated assuming dose additivity should be conceived as an endpoint of a single virtual compound. Therefore, the exposure calculation for the risk assessment is based as well on this assumption. Content in the formulation and application rate per hectare should thus be expressed in terms of this virtual compound. Therefore, the overall application rate for active substances combined of 0.084 kg virtual compound/ha is considered for the acute risk assessment.

Calculation of the screening and tier 1 risk assessment for a virtual compound according to EFSA/2009/1438

The screening and tier 1 TER values for the acute dietary risk assessment for mammals as obtained by means of the EFSA calculator tool (version of 9 July 2010, http://www.efsa.europa.eu/de/efsajournal/pub/1438.htm) are presented in Table 10.1.2.2-10.

Table 10.1.2.2-10: Virtual compound: Acute dietary risk assessment for mammals - results from EFSA calculator tool

Data from Data_Entry worksheet	**Crop**	**Application rate (kg/ha)**	**Number of applications**	**Application Interval**	**DT_{50}**	**LD_{50}**	
	Potatoes	0.084	4	10	10.0	5000	
Screening step:							
Acute risk assessment screening step	**Indicator species**	**Shortcut value**	**Daily Dietary Dose (single)**	**MAF (90)**	**Daily Dietary Dose (Multiple)**	**TER**	**No refinement step required**
	Small herbivorous mammal	118.4	9.95	1.5	14.92	335.2	
First Tier Risk Assessment:							
Calculate TER for each generic focal species selected	**Crop**	**Generic focal species**		**Shortcut value**	**TER**	**No refinement required**	
	Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods		5.4	7348.6		
	Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs		10.5	3779.3		
	Potatoes BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass		40.9	970.2		
	Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods		5.2	7631.3		

In conclusion, even under the conservative assumptions of the screening step and the tier 1 acute dietary risk assessment all TER values for a virtual compound exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for mammals from the use of BAS 516 07 F in potatoes according to the recommended use pattern.

Consideration of mixture toxicity for sublethal effects and effects on reproduction

Note that it is currently not recommended to consider the use of predicted toxicity values as surrogates in the risk assessment. For a detailed discussion please refer to Appendix B (p.4) of EFSA/2009/1438.

Conclusion

In conclusion, even under the conservative assumptions of the screening step and the tier 1 acute dietary risk assessment for the virtual compound and the formulation, all TER_A values, from the risk assessment addressing the combined effects of simultaneous exposure to two active substances, exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects for BAS 516 07 F. This indicates a low acute risk for mammals from the use of BAS 516 07 F in potatoes according to the recommended use pattern.

CP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

In a literature review a few references on amphibian studies with pyraclostrobin, respectively pyraclostrobin-containing formulations were identified. Summaries of these publications are provided in M-CA.8.1.4. Please refer to this part of the dossier for details. There are no specific investigations or findings indicating impacts on reptiles.

Specific targeted GLP investigations have been conducted with amphibians using the solo formulation BAS 500 06 F (for details please see M-CP 10.1.3 of BAS 500 06 F).

Risk assessment - other terrestrial vertebrate wildlife: reptiles and amphibians

The laboratory studies conducted under worst-case conditions, using sensitive life stages (metamorphs) and maximizing exposure via direct overspray over animals and bare soil (Belden et al. 2010; Bruehl et al. 2013; BASF DocID 2013/1375098), indicate inherent toxicity of pyraclostrobin, respectively pyraclostrobin containing formulations to amphibians. The results from the two investigations by Belden et al. (2010) and Bruehl et al. (2013) (non-GLP studies, for more details see M-CA 8.1.4) on *Rana temporaria* and *Bufo cognatus* show no impact of the test substance at 22 g a.s./ha, whereas 220 g a.s./ha (reflecting the field rate) caused high mortality (100 and 70% at 220 g a.s./ha for *Rana temporaria* and Bufo cognatus, respectively). The BASF laboratory study with *Rana temporaria* provides comparable results while narrowing down the toxicity range. Results are shown in Table 10.1.3-1.

The above-mentioned laboratory studies show that young, juvenile frogs and toads can be sensitive if oversprayed directly with EC formulated pyraclostrobin (containing organic solvents). BASF conducted additionally a study (BASF DocID 2014/1221859) investigating the impact of the same product when not overspraying the toads directly, but exposing them to bare soil that has been oversprayed before. The toxicity to animals exposed to the soil residues shortly after spraying was significantly reduced as compared to direct overspray, and following a time interval of about four hours between spraying and animals moving onto the contaminated soil, no effects on the toads were observed even at elevated rates of 310 g a.s./ha.

Table 10.1.3-1: List of endpoints obtained in studies with amphibians

Study	Test species	Test substance	Formulation type	Appl. rate [g a.s./ha]	Effect (% mortality)
Belden et al. (2010)	*Bufo cognatus*	BAS 500 00 F (Headline®)	EC (emulsifiable concentrate)	22	0
				220	70
				2200	100
Brühl et al. (2013)	*Rana temporaria*	BAS 500 00 F (Headline®)	EC	22	0
				220	100
		BAS 500 18 F	CS (capsule suspension)	2200	no significant effect
BASF DocID 2013/1375098	*Rana temporaria*	BAS 500 06 F	EC	40	0
				63	0 sublethal symptoms
				99	70
BASF DocID 2014/1375098	*Bufo bufo*	BAS 500 06 F	EC	155 / 15 min 219 / 15 min 310 / 15 min 310 / 60 min 310 / 4 h[1)]	0 0 (20% sublethal) 20 (60% sublethal) 20 (40% sublethal) (20)[2)] (0% sublethal)

[1)] Animals were exposed to the respective rates either shortly (within 15 minutes) or 1 h or 4 h after spraying.
[2)] The observed toxicity in this case is not considered treatment-related.

BAS 516 07 F is a WG-formulation containing no organic solvents. It is assumed that the toxicity of this product is lower than the toxicity of the solo EC-formulation BAS 500 06 F. However, additional studies with BAS 516 07 F have not been conducted as the risk assessment shown later on indicates low risk of BAS 516 07 F applications to amphibians even when using the data from the EC-formulation.

In previous chapters it has been demonstrated that there is a low risk of pyraclostrobin to bird and mammal species. Yet, pyraclostrobin and certain pyraclostrobin containing formulations can be toxic to amphibians. The particular sensitivity of amphibians is most likely due to their specific skin morphological and physiological differences (dermal toxicity). The amphibian skin not only constitutes a much lesser barrier as compared to the skin of mammals and birds (Quaranta et al., 2009), however, it is itself a sensitive organ relevant for respiration and uptake of water and regulation of minerals in amphibians (see Wells, 2007 for a review). It seems likely that pyraclostrobin, in case sufficiently large surfaces of the skin are exposed, impacts mitochondrial respiration in the skin and thus disturbs a vital process for respiration and function of the skin. However, if the vital function is not sufficiently (largely) disturbed, the substance being rapidly metabolized and excreted (compare M-CA 5.1 and M-CA 6.2) will not cause any further sub-lethal or chronic latency effects. This is in agreement with the observed finding: effects are observed rather rapidly and if there are no rapid effects or once recovery from initial effects occurs, then there is no delayed or long-term toxicity.

The laboratory studies indicate the potential intrinsic toxicity of pyraclostrobin. However, conditions as simulated in these worst-case laboratory studies bear little relevance to field situations.

In general, amphibians need both terrestrial and aquatic habitats. In Europe, most amphibian species depend on aquatic breeding sites and use the terrestrial habitat within 300 - 1000 m of the surrounding area (e.g. Semlitsch and Bodie 2003, Schabetsberger et al. 2004, Sinsch et al. 2012, Berger et al. 2011). Agricultural landscapes, too, may constitute habitats used by amphibians, if these are in the range of the aquatic breeding sites (summary in Berger et al. 2011).
However, agricultural landscapes are generally perceived as low quality environments for most amphibian species for several reasons, posing high resistance to movement for aquatic breeding amphibians, which is corroborated by studies showing lower abundance and association of amphibians with arable land (Pagetti et al. 2006, e.g. *Bufo bufo*: Kyek et al. 1997, Piha et al. 2007; *Epidalea calamita*: Miaud and Sanuy 2005; *Triturus carnifex, T. marmoratus*: Jehle and Arntzen 2000). In particular, agricultural fields with no or low plant cover are avoided since amphibians have to face high water losses in anthropogenically disturbed areas without sufficient plant cover (Rothermel and Semlitsch 2002, Mazerolle and Desrochers 2005, Cosentino et al. 2011). The risk of desiccation and the lack of cover against predators are likely the major factors leading to avoidance of amphibians of such areas. Mazerolle and Vos (2006) observed that *Rana esculenta* opted to take migration routes with higher cover rather than taking shortcuts through bare field. Similar behaviour was reported by Berger et al. (2011) during telemetry and mark-and recapture studies with *Bufo bufo, Triturus cristatus* and *Pelobates fuscus* in agricultural fields in Germany: individuals migrated either fast through the field taking the shortest route, and subsequently stayed in off-crop habitats, which offer more plant cover and structure; or they returned into cover offered by the pond's surrounding. Individuals of *B. bufo* continued to stay in the arable field when favourable conditions, i.e. burrows, stubble field with clods and sufficient plant material, were found (see also Kneitz 1998).

Thus, habitat use and behaviour of amphibians in agricultural landscapes highly depend on availability of cover in the respective area (Berger et al. 2011).

The dependence on a moist and the preference for a structured environment with high plant cover is especially crucial for juvenile amphibians. Due to their smaller size and volume to surface ratio, juvenile amphibians are more sensitive to environmental influences, especially desiccation, than adult life stages (Rittenhouse et al. 2008). Accordingly, Vos et al. (2007) found an even more pronounced preference for meadows in contrast to arable land in *Rana temporaria* juveniles than in adults (individuals found in meadows: arable land; 9.6:1 (juveniles), 4.3:1 (adults)). Crop fields might therefore be used by these sensitive stages only if they offer sufficient plant cover and therefore a favourable microclimate and protection against predators.

Habitat use, of course, depends on biology and ecology of the respective species. A few amphibian species, which are physiologically and behaviourally adapted to arid conditions, may also use agricultural fields with low plant cover as habitats (e.g. *P. fuscus, Epidalea calamita*). However, also for these species availability of shelter and cover are of crucial importance (Seebacher and Alford 2002). By their fossorial behaviour and staying in moist shelters (e.g. stone embankments, burrows) during the day, postponing search for food to the night and using short rainy events for rehydration (Nöllert and Nöllert 1992, Miaud et al. 2000, Eggert 2002) they avoid dehydration and extreme temperatures (Seebacher and Alford 2002).

This predominantly nocturnal activity (and hiding behaviour during the day) can be found in many European amphibian species (e.g. *Rana temporaria*, *B. bufo, Lissotriton vulgaris*, see Günther 1996). Furthermore, nocturnal activity, the use of shelter and plant cover also limits visual encounter by predators, a strong selective force shaping behaviour in organisms (Lima and Dill 1990). At the same time this behaviour reduces significantly potential exposure to pesticide applications generally.

In summary, a number of aspects will significantly reduce potential exposure and thus the risk to pesticides under realistic field conditions:

- avoidance of agricultural sites, particularly those with low plant cover
- behavioral aspects: burrowing, hiding, nocturnal activity
- more sensitive juveniles will migrate into or through agricultural fields later in the year, when there is either dense plant cover and/or no pesticide use.

The risk assessment has to consider the likely exposure next to the toxicity data. In order to do this appropriately it is essential to consider behavioural aspects in addition to simple interception calculations. Therefore, semi-field studies with *Rana temporaria* and *Bufo bufo* were designed and conducted. These studies cover realistic (worst-case) exposure and behavior. In addition, they cover all routes of exposure: direct spraying, contact to treated surfaces and uptake of contaminated feed.

Higher tier semi-field study with juvenile Rana temporaria under realistic worst-case conditions

Test item	Application rate	affected (%)
BAS 500 06 F	0.75 x max field rate (0.94 L/ha) 1.0 x max field rate (1.25 L/ha) 1.25 x max field rate (1.56 L/ha)	0 0 0[#]

[#] One single individual (out of 16) showed a short term, transient behavioural impact

Higher tier semi-field study with juvenile Bufo bufo under realistic worst-case conditions

Test item	Application rate	affected (%)
BAS 500 06 F	1.0 x max field rate (1.25 L/ha) 1.5 x max field rate (1.875 L/ha) 10 x max field rate (12.5 L/ha)	0 0 50 (75)[#]

[#] One animal was moribund (if counted as dead mortality results in 75%)

The semi-field studies demonstrate that applications of BAS 500 06 F under realistic conditions will be of low risk to amphibians. Even elevated rates of 1.56 L/ha (study with *Rana temporaria*) and 1.875 L/ha (study with *Bufo bufo*), corresponding to over 313 and 375 g a.s./ha did not cause any mortality under realistic worst-case conditions in the field.

Considering in the first tier just the results from the laboratory studies it is demonstrated that application rates of less than 40 to 60 g pyraclostrobin/ha are of low risk to amphibians even under absolute worst-case conditions. The rate of pyraclostrobin applied in the formulated product BAS 516 07 F is only 17 g/ha. The second active substance in this product (boscalid) is generally of significantly lower toxicity to all groups of non-target organisms (e.g. compare fish endpoints of LC_{50} = 0.0062 mg/L for pyraclostrobin and LC_{50} =2.7 mg/L for boscalid). There is neither any information that boscalid or related compounds would cause significant toxicity to amphibians.
Considering the results from the worst-case laboratory studies, i.e. no mortality at 63 g pyraclostrobin/ha and comparing this to the application rate of 17 g pyraclostrobin/ha it can be concluded that applications of BAS 516 07 F to potatoes will be of low risk to amphibians. Furthermore, under realistic conditions exposure will be significantly reduced and accordingly toxicity will be much less as also demonstrated in the semi-field studies, which showed no mortality even at application rates of 313 and 375 g pyraclostrobin/ha, which is about 20-fold higher than the highest application rate of pyraclostrobin by the use of BAS 516 07 F in potatoes.

In conclusion, there will be very low risk to amphibians from BAS 516 07 F applications in potatoes according to label recommendations and good agricultural practice.

In the case of reptiles there is even less information available than for amphibians (see the revision by Fryday and Thompson, 2009). The risk from dietary exposure can be assumed to be lower for reptiles than for birds and mammals (Fryday and Thompson 2009). This is because reptiles are poikilotherm (i.e., do not maintain a constant body temperature) and as a result feeding activity will peak at warm days and will be zero during hibernation or at cold days. In contrast, birds and mammals will have to maintain a constant body temperature and, hence, will need to feed every day (Fryday and Thompson 2009). Uncertainties remain on the contribution of dermal exposure to the overall exposure to reptiles. However, in contrast to amphibians the skin of reptiles is much less permeable; it functions in general as protection and barrier and it is not an organ used for respiration or water/mineral exchange with the environment. Thus, reptiles are considered less vulnerable to dermal exposure as compared to amphibians. Nevertheless, some uncertainty with respect to the risk to reptiles, i.e. whether they are sufficiently covered by other (more standard) ecotoxicological data will remain and further research is needed.

However, pyraclostrobin containing products have been used for many years in many countries worldwide and usually at much higher rates compared to the use of BAS 516 07 F in potatoes. So far, there are no publications indicating a potential risk of this compound to reptiles and despite the long term use worldwide the applicant is not aware of findings or (incidence) reports that amphibians or reptiles were harmfully affected by correct applications of pyraclostrobin containing fungicides.

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CP 10.2 Effects on aquatic organisms

The EU agreed endpoints for the active substance pyraclostrobin (BAS 500 F) and its major metabolites plus endpoints from new studies and revised endpoints from EU agreed studies, respectively, are used for the risk assessment on aquatic organisms (see Table 10.2-1). The new representative formulation BAS 516 07 F was neither evaluated as part of the previous Annex I inclusion process of pyraclostrobin nor the one of boscalid. The respective data are presented within this chapter. A comprehensive risk assessment for the active substance, its metabolites and the formulation based on the already registered use pattern is provided below. The risk assessment is based on the "Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters", EFSA Journal 2013; 11(7):3290.

For the evaluation of potential effects of boscalid (BAS 510 F), the second active substance in the formulation, the considered EU agreed endpoints can be found in: Monograph (Vol. 3, Annex B.9, 2002), Addendum 2 to DAR (Vol. 3, Annex B.9, 2006) and EU Review Report (SANCO/3919 /2007-rev.5, 2008). The relevant endpoints used in the risk assessments for boscalid are included in Table 10.2-1.

In general, different code numbers of different numbering systems are assigned to the metabolites of pyraclostrobin. In this chapter all metabolites are identified by the BF-code and synonym metabolite codes are given in brackets only where deemed to be helpful.

Table 10.2-1: EU agreed and new /revised endpoints for aquatic species

Test substance	Test species	EU agreed endpoints	Endpoints used in standard and refined risk assessment
Acute toxicity to fish			
Pyraclostrobin	*Oncorhynchus mykiss*	96 h LC_{50} = 0.00616 mg/L	96 h LC_{50} = 0.00616 mg/L
Pyraclostrobin	*O. mykiss* [1)]	--	96 h LC_{50} = 0.0062 mg/L
Pyraclostrobin	*Cyprinodon variegatus* [1), 2)]	--	96 h LC_{50} = 0.0769 mg/L
Pyraclostrobin	*Cyprinus carpio*	96 h LC_{50} = 0.0177 mg/L	96 h LC_{50} = 0.0177 mg/L
Pyraclostrobin	*Danio rerio* [3)]	96 h LC_{50} = 0.0619 mg/L	96 h LC_{50} = 0.0619 mg/L
Pyraclostrobin	*Lepomis macrochirus*	96 h LC_{50} = 0.0254 mg/L	96 h LC_{50} = 0.0254 mg/L
Pyraclostrobin	*L. macrochirus* [3)]	--	96 h LC_{50} = 0.0114 mg/L
Pyraclostrobin	*Leuciscus idus melanotus* [4)]	96 h LC_{50} = 0.0191 mg/L	96 h LC_{50} = 0.0191 mg/L
Pyraclostrobin	*Oryzias latipes* [3)]	96 h LC_{50} = 0.0533 mg/L	96 h LC_{50} = 0.0533 mg/L
Pyraclostrobin	*Pimephales promela* [3)]	96 h LC_{50} = 0.0161 mg/L	96 h LC_{50} = 0.0161 mg/L
BF 500-3	*O. mykiss* [1)]	--	96 h LC_{50} > 0.0948 mg/L
BF 500-5	*O. mykiss* [1)]	--	96 h LC_{50} =11.3 mg/L
BF 500-11	*O. mykiss*	96 h LC_{50} > 100 mg/L	96 h LC_{50} > 100 mg/L
BF 500-13	*O. mykiss*	96 h LC_{50} > 50 < 100 mg/L	96 h LC_{50} > 50 < 100 mg/L
BF 500-14	*O. mykiss*	96 h LC_{50} > 39.4 < 82.6 mg/L	96 h LC_{50} > 39.4 < 82.6 mg/L

Test substance	Test species	EU agreed endpoints	Endpoints used in standard and refined risk assessment
Boscalid	*O. mykiss*	96 h $LC_{50} \cong 2.7$ mg/L	96 h $LC_{50} \cong 2.7$ mg/L
BAS 516 07 F *	*O. mykiss*	--	96 h $LC_{50} = 0.088$ mg/L
Chronic toxicity to fish			
Pyraclostrobin	*O. mykiss* (ELS study)	98 d NOEC = 0.00235 mg/L	98 d NOEC = 0.00235 mg/L
Pyraclostrobin	*C. variegatus* [1), 2)] (ELS study)	36 d NOEC = 0.0108	36 d NOEC = 0.0108
Pyraclostrobin	*P. promelas* [1)] (ELS study)	36 d NOEC = 0.00414	36 d NOEC = 0.00414
Boscalid	*O. mykiss* (ELS)	97 d NOEC = 0.125 mg/L	97 d NOEC = 0.125 mg/L
Acute toxicity to aquatic invertebrates			
Pyraclostrobin	*Daphnia magna*	48 h $EC_{50} = 0.0157$ mg/L	48 h $EC_{50} = 0.0157$ mg/L
BF 500-3	*D. magna* [1)]	--	48 h $EC_{50} > 0.100$ mg/L
BF 500-5	*D. magna* [1)]	--	48 h $EC_{50} > 10.0$ mg/L
BF 500-11	*D. magna*	48 h $EC_{50} > 100$ mg/L	48 h $EC_{50} > 100$ mg/L
BF 500-13	*D. magna*	48 h $EC_{50} > 100$ mg/L	48 h $EC_{50} > 100$ mg/L
BF 500-14	*D. magna*	48 h $EC_{50} > 60.9$ mg/L	48 h $EC_{50} > 60.9$ mg/L
Boscalid	*D. magna*	48 h $EC_{50} = 5.33$ mg/L	48 h $EC_{50} = 5.33$ mg/L
BAS 516 07 F	*D. magna*	--	48 h $EC_{50} = 0.210$ mg/L
Chronic toxicity to aquatic invertebrates			
Pyraclostrobin	*D. magna*	21 d NOEC = 0.004 mg/L	21 d NOEC = 0.004 mg/L
Boscalid	*D. magna*	21 d NOEC = 1.31 mg/L	--
		--	21 d NOEC = 0.80 mg/L [1)]
Sub-lethal toxicity to sediment dwelling aquatic invertebrates			
Pyraclostrobin	*Leptocheirus plumulosus* [1), 2)] (spiked sediment)	--	10 d $LC_{50} = 4.41$ mg/kg dry sediment
Chronic toxicity to sediment dwelling aquatic invertebrates			
Pyraclostrobin	*Chironomus riparius* (spiked water)	28 d NOEC = 0.040 mg/L	28 d NOEC = 0.040 mg/L
Pyraclostrobin	*C. riparius* [1)] (spiked sediment)	--	28 d NOEC = 1.37 mg/kg dry sediment
BF 500-3	*C. riparius* [1)] (spiked sediment)	--	28 d NOEC $\geq$ 16.0 mg/kg dry sediment
BF 500-6	*C. riparius* [1)] (spiked sediment)	--	28 d NOEC 1.2 mg/kg dry sediment
BF 500-7	*C. riparius* [1)] (spiked sediment)	--	28 d NOEC $\geq$ 123.5 mg/kg dry sediment
Boscalid	*C. riparius* (spiked water)	28 d NOEC = 1.0 mg/L	28 d NOEC = 1.0 mg/L
Boscalid	*C. riparius* (spiked sediment)	28 d NOEC = 23.26 mg/kg dry sediment	28 d NOEC = 23.26 mg/kg dry sediment

Test substance	Test species	EU agreed endpoints	Endpoints used in standard and refined risk assessment
Toxicity to algae			
Pyraclostrobin	*Pseudokirchneriella subcapitata*	96 h E_rC_{50} > 0.843 mg/L	72 h E_rC_{50} > 0.843 mg/L [4]
		96 h E_bC_{50} = 0.152 mg/L	--
BF 500-3	*P. subcapitata* [1]	--	72 h E_rC_{50} > 1.17 mg/L [5]
BF 500-5	*P. subcapitata* [1]	--	72 h E_rC_{50} = 5.33 mg/L
BF 500-11	*Scenedesmus subspicatus*	72 h E_rC_{50} > 100 mg/L	72 h E_rC_{50} > 100 mg/L
		72 h E_bC_{50} > 100 mg/L	--
BF 500-13	*S. subspicatus*	72 h E_rC_{50} > 100 mg/L	72 h E_rC_{50} > 100 mg/L
		72 h E_bC_{50} = 66 mg/L	--
BF 500-14	*S. subspicatus*	72 h E_rC_{50} > 100 mg/L	72 h E_rC_{50} > 100 mg/L
		72 h E_bC_{50} = 46.6 mg/L	--
Boscalid	*P. subcapitata*	96 h E_rC_{50} = 3.75 mg/L	96 h E_rC_{50} = 3.75 mg/L
		96 h E_yC_{50} = 1.34 mg/L	--
BAS 516 07 F	*P. subcapitata*	--	72 h E_rC_{50} = 10.8 mg/L
Toxicity to aquatic macrophytes			
Pyraclostrobin	*Lemna gibba* [1]	--	14 d E_bC_{50} = 1.72 mg/L
Higher tier studies / calculations			
Pyraclostrobin	*O. mykiss* (TTE study; different exposure durations [1]	--	96 h LC_{50} > 0.027 mg/L (0.5 h peak exposure) [6]
		--	96 h LC_{50} = 0.022 mg/L (2 h) [6]
		--	96 h LC_{50} = 0.015 mg/L (8 h) [6]
Pyraclostrobin	SSD (based on 96 h NOECs for 8 fish species)	--	HC_5 = 0.00338 mg/L
Pyraclostrobin	*O. mykiss* (ELS study with multiple exposure)	97 d NOEC = 0.005 mg/L	97 d NOEC = 0.005 mg/L
Pyraclostrobin	outdoor mesocosm (multiple spray application) [3]	6 mo NOEC = 0.008 mg/L	6 mo NOEC = 0.008 mg/L
		6 mo NOEAEC > 0.0080 < 0.0240 mg/L	6 mo NOEAEC > 0.008 < 0.024 mg/L

ELS = early life stage; NO(E)AEC = No observed (ecologically) adverse effect concentration; LC = life cycle; TTE = Time-To-Effect/Event; SSD = Species Sensitivity Distribution; HC_5 = 5% hazardous concentration

* Study was conducted with the minor change formulation BAS 516 00 F (reference is made to Document JCP 1.4.1 of the BAS 516 07 F dossier).

[1] Study not submitted during the previous Annex I inclusion process of pyraclostrobin.

[2] marine / saltwater species

[3] Study was performed with the previous representative solo-formulation BAS 500 00 F; however, results are given in mg a.s./L.

[4] In accordance to recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints of the EU agreed study on the green alga have been (re-)calculated from original data. The re-calculated values are presented here and are used in the aquatic risk assessment; for details on these calculations please refer to the respective supplement. A summary of this study and the re-calculations is provided in M-CA 8.2.

[5] In accordance to recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints obtained in the 96 h / 120 h alga studies are considered as relevant endpoint and are presented here.

[6] Endpoint obtained in the TTE study after exposure over respective duration, i.e. the value in brackets (0.5, 2 or 8 hours exposure time).

Risk assessment for aquatic organisms

Toxicity

An overview on all available toxicity data for all aquatic organisms is provided in Table 10.2-2 for the active substance and the formulated product and in Table 10.2-3 for the metabolites of pyraclostrobin.

Studies with the formulated product BAS 516 07 F are summarized in this chapter, while summaries of the new studies with the active substance and its major metabolites are provided in M-CA 8.2. For completeness this includes some older studies that had not been submitted during the previous Annex I inclusion process (e.g. because there is no respective data requirement in the EU) and also EU agreed studies that have been amended in the meantime (e.g. due to recalculations of endpoints or a new evaluation according to current guidelines). Further information on the EU agreed studies, which have been already evaluated within the previous Annex I inclusion process of pyraclostrobin, can be found in the Monograph (Vol. 3, Annex B.9, August 2001) and the EU Review Report (SANCO/1420/2001-final, September 2004). Furthermore, the reports of these studies will be provided to RMS, co-RMS and EFSA and are available upon request for other member states.

Active substance pyraclostrobin and formulated product

Table 10.2-2: List of studies and endpoints for aquatic organisms and the active substance pyraclostrobin (BAS 500 F) and the formulated product BAS 516 07 F

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)
Active substance: pyraclostrobin			
Fish			
Oncorhynchus mykiss	96 h LC_{50}	**0.00616** #	1999/11414
O. mykiss [1)]	96 h LC_{50}	0.0062	2000/5034
Cyprinodon variegatus [1), 2)]	96 h LC_{50}	0.0769	2000/5032
Cyprinus carpio	96 h LC_{50}	0.0177 (> 0.0121 < 0.0252) *	1998/11580
Danio rerio [3)]	96 h LC_{50}	0.0619 (0.0558 - 0.0680) +	1999/11834
Lepomis macrochirus	96 h LC_{50}	0.0254 (0.0233 - 0.0275)	1998/10951
L. macrochirus [1)]	96 h LC_{50}	0.0114	2000/5033
Leuciscus idus melanotus [3)]	96 h LC_{50}	0.0191 (> 0.0135 < 0.0270)	1999/11835
Oryzias latipes [3)]	96 h LC_{50}	0.0533 [5)] (0.0444 - 0.0622)	1999/11821
Pimephales promelas [3)]	96 h LC_{50}	0.0161 [5)] (0.0141 - 0.0181)	1999/11833
O. mykiss	28 d NOEC	0.00464	1999/11249

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)
O. mykiss	98 d NOEC (ELS study)	0.00235 #	1999/11343
C. variegatus [1), 2)]	36 d NOEC (ELS study)	0.0108	2000/5247
P. promelas [1)]	36 d NOEC (ELS study)	0.00414	2000/5053
Aquatic invertebrates			
Daphnia magna	48 h EC_{50}	**0.0157** #	1999/10444 + amendment: 1999/10739
Americamysis bahia [1), 2)] (former name: *Mysidopsis bahia*)	48 h LC_{50}	> 0.00597 [4)]	2000/5031
Crassostrea virginica [1), 2)]	96 h EC_{50}	0.0125	2000/5042
D. magna	21 d NOEC	**0.004** #	1999/11864
A. bahia [1), 2)]	28 d NOEC (LC study)	0.0005	2004/5000004
A. bahia [1), 2)]	31 d NOEC (LC study)	(0.000365) 0.00128 [14)]	2013/7002075
Sediment dwelling aquatic invertebrates			
Leptocheirus plumulosus [1), 2)]	10 d LC_{50} (spiked sediment)	4.41 mg/kg dry sediment	2013/7000055
Chironomus riparius	28 d NOEC (spiked water study)	0.040	2000/1000010
C. riparius [1)]	28 d NOEC (spiked sediment)	1.37 mg/kg dry sediment	2012/1185699
Algae			
Pseudokirchneriella subcapitata (syn. *Selenastrum capricornutum*)	72 h E_rC_{50}	> **0.843** [5)] #	1999/11020 + supplement: 2009/1037148
	72 h E_yC_{50}	0.148 [5)]	
Navicula pelliculosa [1)]	72 h E_rC_{50}	0.0158 [6)]	2000/5046
	72 h E_bC_{50}	0.00165 [6)]	
Anabaena flos-aquae [1)]	96 h E_rC_{50}	1.41 [7), 8)]	2000/5036
	96 h E_bC_{50}	0.367 [7), 8)]	
Skeletonema costatum [1), 2)]	72 h E_rC_{50}	0.0962 [6), 8)]	2000/5035
	72 h E_bC_{50}	< 9.73 [6), 8)]	
Aquatic macrophytes			
Lemna gibba [1)]	14 d E_bC_{50}	> 1.72 [9)] / 1.72 [10)]	2000/5037

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)
Higher tier studies / calculations			
O. mykiss	96 h LC_{50} (TTE study)	> 0.027 (0.5 h) [11)]	2000/1014919
		0.022 (2 h) [11)]	
		0.015 (8 h) [11)]	
SSD (based on 96 h NOECs for 8 fish species) [16)]	median HC_5	**0.00338**	--
geometric mean (based on ELS NOECs for 3 fish species) [16)]	$geomean_{chronic}$	**0.00472**	--
O. mykiss	97 d NOEC (ELS study with multiple exposure) [13)]	0.005	1999/11537
outdoor mesocosm (multiple spray application) [3)]	NOEC	0.008	2000/1000011 + supplement: 2012/1357084
	NOEAEC	> 0.008 < 0.024	
Formulated product: BAS 516 07 F			
Fish			
O. mykiss [1), 15)]	96 h LC_{50}	0.088	2000/1018726
Aquatic invertebrates			
D. magna [1)]	48 h EC_{50}	0.120 [16)]	2007/1008605
D. magna [1)]	48 h EC_{50}	0.210 [16)]	2009/1117877
Algae			
P. subcapitata [1)]	72 h E_rC_{50}	10.8	2007/1005074
	72 h E_yC_{50}	3.0	

Bold figures: Where several endpoints are available for the same group or where several endpoints are available for one study based on different effect parameters (e.g. for algae and macrophytes), the relevant endpoint is used in the standard TER calculations.

ELS = early life stage; NO(E)AEC = No observed (ecologically) adverse effect concentration; LC = life cycle; TTE = Time-To-Effect/Event; SSD = Species Sensitivity Distribution; HC_5 = 5% hazardous concentration

[#] At tier I level, only the endpoints for the standard aquatic test species are considered even though studies on additional invertebrate/alga species are available partly resulting in lower endpoints. This is considered appropriate as a mesocosm study is available covering the risk to a great number of different aquatic invertebrate and alga species (for detailed justifications see below).

[+] Spearman-Karber estimate of LC_{50} with confidence limits

[*] Geometric mean with corresponding LC_0 (>) and LC_{100} (<) values

[1)] Study has not been submitted during the previous Annex I inclusion process of pyraclostrobin (summaries for the studies with pyraclostrobin and its metabolites are provided in M-CA 8.2; summaries for the studies with the formulation are provided below).

[2)] Marine / saltwater species

[3)] Study was performed with the solo-formulation BAS 500 00 F (containing 250 g pyraclostrobin/L, nominally)

[4)] The 48-h LC_{50} obtained in the 96 h study is used in the risk assessment according to EU Regulation 283/2013 (European Commission, 2013) on the data requirements for active substances and the EFSA Aquatic Guidance Document (EFSA, 2013).

[5)] In accordance to recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints of the EU agreed study on the green alga have been (re-)calculated from original data. The re-calculated values are presented here and are used in the aquatic risk assessment; for details on these calculations please refer to the supplement. A summary of this study and the re-calculations is provided below.

[6)] In accordance to recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints obtained in the 96 h / 120 h alga studies are considered as relevant endpoint and are presented here

7) In this study the 24, 48, and 72 hour effective concentrations were not calculated because sufficient growth had not yet occurred in any of the test groups to allow for these calculations; thus the 96 h endpoints obtained in the 120 h alga studies are presented here.
8) Due to the reasoning given below, the study on this alga species is considered to be not valid.
9) Based on frond numbers 10) Based on dry weight
11) Endpoint obtained in the TTE study after peak exposure over respective duration, i.e. the value in brackets (0.5, 2 or 8 h exposure time).
12) For details on SSD / geometric mean calculations and derivation of respective RAC values please refer to the refined risk assessment presented below.
13) During the peer review this substance had been considered to be not valid; however, this was done apparently based on a misunderstanding of the data; more explanation in the respective text on the fish RA.
14) A statistically significant impact based on a small reduction in male weight was given at 0.676 µg/L; however, a biologically relevant impact (based on female weight reduction and a corresponding reduced reproduction level) was observed at the highest test concentration only (but which had no impact on the F1 generation). Accordingly, the NOEC is 1.28 µg/L.
15) Study was conducted with the minor change formulation BAS 516 00 F (reference is made to Document JCP 1.4.1 of the BAS 516 07 F dossier).
16) Two acute studies on *D. magna* were performed with BAS 516 07 F. The first study showed some shortcomings (i.e. measured concentrations of boscalid were > 120% of nominal concentrations at test initiation and test end indicating an overdose over the whole study period). Therefore, and as the study results could not be based on mean measured concentrations (formulation contains two active substances) the study was repeated and the results of the second study are considered as relevant endpoints for the aquatic risk assessment.

Metabolites of pyraclostrobin (BAS 500 F)

The results from toxicity tests on representative freshwater species with the major aquatic metabolites are summarized in Table 10.2-3.

In the dark water/sediment study the metabolites BF 500-3, BF 500-6 and BF 500-7 occurred as major metabolites (see M-CP 9.1 - 9.2). Additionally, BF 500-3, BF 500-11, BF 500-13 and BF 500-14 occurred in relevant amounts in the irradiated water/sediment study and BF 500-5 was detected as major metabolite in the study on aerobic mineralization in surface water.

The pyraclostrobin metabolite BF 500-3 was not observed in standard aerobic soil metabolism studies, but only in soil under anaerobic conditions (see M-CP 9.1 - 9.2), however, aerobic conditions are predominant in soil. The sorption behavior of BF 500-3 was investigated in the context of the EU review of pyraclostrobin showing a mean K_{oc} of 9315 mL/g (see M-CP 9.2). Due to its non-occurrence under more relevant aerobic conditions and its high K_{oc}, this metabolite will not be relevant for drainage or runoff entry into surface water. BF 500-3 was observed in sediment with a maximum occurrence of 16.9% TAR in an irradiated water/sediment study. The highest amount of BF 500-3 in the water phase never exceeded 5.0% TAR and thus, this metabolite is only relevant in the sediment phase. Nevertheless, toxicity studies were performed for BF 500-3 on fish, daphnids and algae (on the request of Canadian authorities) that were not included in the previous pyraclostrobin EU review; summaries for these studies are provided in M-CA 8.2. These studies (see Table 10.2-3) showed no toxicity up to the solubility level of this metabolite. The evaluation above shows that both theoretical considerations and also the evidence from toxicity studies demonstrate a very low risk to aquatic organisms resulting from water exposure to metabolite BF 500-3. Therefore, the results from these studies are not considered further in the risk assessment. However, as a major sediment metabolite it has also been tested in a 28 d spiked sediment study with *Chironomus riparius* and the results are used in the respective TER calculations (see below).

The aerobic soil metabolites BF 500-6 and BF 500-7 show very high sorption indicating that they are non-mobile in soil (BF 500-6: median K_{oc} of 107301 mL/g; BF 500-7: median K_{oc} of 149900 mL/g; see M-CP 9.2). Considering the high sorption, the low mobility in soil and the very low water solubility, it can be concluded that BF 500-6 and BF 500-7 will not enter surface water to any ecotoxicologically relevant amount. Furthermore, studies with these metabolites and soil organisms indicate very low toxicity and overall low ecotoxicological potential. To assess the risk to sediment dwelling organisms, 28 d spiked sediment studies on *C. riparius* have been conducted with both metabolites. Study summaries are provided in M-CA 8.2 and the results are considered for the risk assessment below.

The metabolite BF 500-5 was found in relevant amounts in the OECD 309 study (dark) in water and was not detected in the sediment phase of the suspended solid test. This metabolite was therefore considered not to be relevant for the sediment.

The metabolites BF 500-11, BF 500-13 and BF 500-14 were observed in the water phase of irradiated water/sediment systems. The highest amount of the metabolites BF 500-11, BF 500-13 and BF 500-14 in the sediment phase of water/sediment systems never exceeded 0.6, 2.1 and 0.7% TAR, respectively; these metabolites were therefore considered not to be relevant for the sediment.

Further information on the metabolites of pyraclostrobin can be found in document N3.

Table 10.2-3: Summary of toxicity values for aquatic organisms of the major metabolites of pyraclostrobin

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)
Metabolite: BF 500-3 (Reg. No. 340266; 500M07)			
Fish			
O. mykiss [1)]	96 h LC_{50}	> 0.0948	2007/1010836
Aquatic invertebrates			
D. magna [1)]	48 h EC_{50}	> 0.100	2006/1038907
Sediment dwelling organisms			
C. riparius [1)]	28 d NOEC (spiked sediment)	≥ 16.0 mg/kg dry sediment	2013/1237446
Algae			
P. subcapitata [1)]	72 h E_rC_{50} / E_yC_{50}	> 1.17 [2)]	2006/1038445
Metabolite: BF 500-5 (Reg. No. 298327; 500M04)			
Fish			
O. mykiss [1)]	96 h LC_{50}	11.3	2013/1349200
Aquatic invertebrates			
D. magna [1)]	48 h EC_{50}	> 10.0	2013/1349201
Algae			
P. subcapitata [1)]	72 h E_rC_{50}	**5.33**	2013/1349202
	72 h E_yC_{50}	2.03	
Metabolite: BF 500-6 (Reg. No. 364380; 500M01) [3)]			
Sediment dwelling organisms			
C. riparius [1)]	28 d NOEC (spiked sediment)	1.2 mg/kg dry sediment	2014/1001481
Metabolite: BF 500-7 (Reg. No. 369315; 500M02) [3)]			
Sediment dwelling organisms			
C. riparius [1)]	28 d NOEC (spiked sediment)	≥ 123.5 mg/kg dry sediment	2014/1001482
Metabolite: BF 500-11 (Reg. No. 411847; 500M60)			
Fish			
O. mykiss	96 h LC_{50}	> 100	1999/11909
Aquatic invertebrates			
D. magna	48 h EC_{50}	> 100	1999/11917
Algae			
Scenedesmus subspicatus	72 h E_rC_{50} / E_bC_{50}	> 100	1999/11918

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)
Metabolite: BF 500-13 (Reg. No. 412785; 500M62)			
Fish			
O. mykiss	96 h LC_{50}	> 50 < 100	1999/11913
Aquatic invertebrates			
D. magna	48 h EC_{50}	> 100	1999/11921
Algae			
S. subspicatus	72 h E_rC_{50}	**> 100**	1999/11922
	72 h E_bC_{50}	66.0	
Metabolite: BF 500-14 (Reg. No. 413039; 500M76)			
Fish			
O. mykiss	96 h LC_{50}	> 39.4 < 82.6	1999/11837
Aquatic invertebrates			
D. magna	48 h EC_{50}	> 60.9	1999/11910
Algae			
S. subspicatus	72 h E_rC_{50}	**> 100**	1999/11914
	72 h E_bC_{50}	46.6	

1) Study has not been submitted during the previous Annex I inclusion process of pyraclostrobin (study summaries are provided in M-CA 8.2).

2) In accordance to recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints obtained in the 96 h alga study are considered as relevant endpoint and are presented here.

3) The pyraclostrobin metabolites BF 500-6 and BF 500-7 do not occur to any significant extent in water; they are thus considered to be only of relevance in sediment.

Exposure

The critical use pattern of BAS 516 07 F considered in the risk assessment below is presented in Table 10.2-4.

Table 10.2-4: Critical use pattern

Crop	Application time (BBCH growth stage)	Number of applications	Minimum interval [days]	Application rate per treatment		
				boscalid [kg a.s./ha]	pyraclostrobin [kg a.s./ha]	BAS 516 07 F [kg/ha]
potatoes	51 - 89	4	10	0.067	0.017	0.25

Aquatic organisms may be exposed to BAS 516 07 F through spray drift and to the active substances and their metabolites through spray drift, run-off and drainage from the application site into adjacent water bodies. Exposure of aquatic organisms from these routes was estimated by calculating Predicted Environmental Concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) for the active substance and its major metabolites using the FOCUS surface water models. The PEC values were calculated for single and fourfold application of BAS 516 07 F in potatoes at a use rate of 0.25 kg/ha (equivalent to 17 g pyraclostrobin/ha). A stepwise approach has been followed, starting with simple worst-case assumptions in the first two steps and proceeding to more realistic worst-case conditions in the third step of the exposure assessment. PEC values were calculated with endpoints derived from a dark water/sediment study (Tier 1) and additionally with parameters derived from an irradiated water/sediment study (Tier 2).

However, only tier 1 PEC values were considered for the following aquatic risk assessment, except for the metabolites BF 500-11, BF 500-13 und BF 500-14 that were mainly observed in irradiated water/sediment studies. Accordingly, only PEC values derived from irradiated water/sediment studies are reported for these metabolites.

The resulting PEC_{sw} and PEC_{sed} values for pyraclostrobin and its major metabolites are presented in Table 10.2.3-1 and Table 10.2.3-2. Only the worst-case PEC values are presented, either resulting from calculations for single or multiple applications. The pyraclostrobin metabolites BF 500-3, BF 500-6 and BF 500-7 do not occur to any significant extent in water. They are thus considered to be of relevance only in sediment and only PEC_{sed} values have been calculated for these metabolites. The highest amount of the metabolites BF 500-11, BF 500-13 and BF 500-14 in the sediment phase of water/sediment systems never exceeded 0.6, 2.1 and 0.7% TAR, respectively; the metabolite BF 500-5 was not detected in the sediment phase of the suspended solid test. These metabolites were therefore considered to be not relevant for the sediment and no PEC_{sed} values were calculated.

A summary of the FOCUS $PEC_{sw, max}$ values for pyraclostrobin and its major metabolites used for the aquatic risk assessment are provided in the Appendix at the end of this chapter. For full details of the assumptions used in the exposure calculations, please see M-CP 9.2.5.

Toxicity exposure ratios

The following TER calculations were conducted based on application of BAS 516 07 F according to the already registered use in potatoes. The initial risk assessments were carried out by comparing the $PEC_{sw, max}$ and $PEC_{sed, max}$ values with the acute and long-term toxicity endpoints.

According to the EFSA Aquatic Guidance Document (EFSA Journal 2013;11(7):3290) measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following the concentration addition (CA) model is used as proposed by EFSA (see table below).
To determine the respective formulation effect, EFSA proposed to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity ($LC_{50}/EC_{50\ mix\text{-}CA}$) by the measured mixture toxicity ($LC_{50}/EC_{50\ PPP}$). Ecotoxicity studies are biological test systems which underlie a certain natural biological variability when repeating a study. Hence, a threshold has to be defined, when an increased/ decreased mixture toxicity effect cannot be seen as only additive any longer. EFSA proposes a factor of 5, i.e. if the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement.

The calculated MDR values are between 0.51 and 1.10 for all organisms (see Table 10.2-5), indicating that the formulation does not cause an (unexpected) increased toxicity compared to the active substances. For example, the measured and calculated toxicity for the most sensitive group, i.e. fish is nearly identical with the LC_{50} values of 0.088 and 0.091 mg/L. Accordingly, the formulation causes no synergisms or additional toxicity.

Table 10.2-5: Comparison of the measured toxicity of the formulated product BAS 516 07 F and the calculated mixture toxicity of the active substances

Test species	Endpoint & Test system	Measured toxicity of BAS 516 07 F (EC_{XPPP}) [mg/L]	Calculated mixture toxicity ($EC_{Xmix\text{-}CA}$) § [mg/L]	MDR ($EC_{Xmix\text{-}CA}$ / EC_{XPPP})
O. mykiss	LC_{50}, acute, 96 h	0.088	0.091	1.03
D. magna	EC_{50}, acute, 48 h	0.210	0.232	1.10
P. subcapitata #	E_rC_{50}, static, 72 h	10.8	> 6.64	0.61
	E_yC_{50}, static, 72 h	3.0	1.53	0.51

PPP = Plant Protection Product; CA = concentration addition; MDR = model deviation ratio

§ The mixture toxicity of the formulation was re-calculated assuming concentration addition based on the measured toxicity data of pyraclostrobin and boscalid and their nominal contents within the formulation (i.e. 67 g pyraclostrobin/kg and 267 g boscalid/kg).

As no clear growth rate endpoint was obtained in the study with pyraclostrobin on green algae (i.e. E_rC_{50} > 0.843 mg/L), also the endpoint yield was used for comparison of the toxicity data; for boscalid the 96 h endpoints are used as no 72 h endpoints are available for green algae.

With regard to the mixture risk assessment EFSA further states that if the toxicity of the mixture is largely explained by the toxicity of a single active substance, a sufficient protection level might be achieved by simply basing the RA on the toxicity data for that single 'driver'.

Regarding BAS 516 07 F, the active substance pyraclostrobin is clearly driving the risk for the most sensitive organism groups fish and *Daphnia.* In acute studies, boscalid was two orders of magnitude less toxic to fish and daphnia as compared to pyraclostrobin (2.7 mg/L / 0.0062mg/L ~factor 440 for fish and 5.33 mg/L / 0.0157 mg/L for daphnids ~factor 340). Similarly, in chronic studies pyraclostrobin provides 53 and 200 times lower endpoints for fish and *Daphnia,* respectively, as compared to boscalid. Thus, despite the about 4 times higher content of boscalid in the formulated product, the toxicity is clearly driven by the active substance pyraclostrobin.
This is reflected in calculations of fish toxicity to the product based on pyraclostrobin alone (0.092 mg/L) as compared to the mixture calculation (0.091 mg/L) in relation to the actually measured value of 0.088 mg/L (see also above). These values are nearly identical and well within the limit of experimental variation. This is also stated by the new EFSA guidance document on aquatics: "*it is assumed that a difference in toxicity is relevant to RA, if the relevant NOEC or ECx values differ by a factor of more than three in studies with the same species conducted under comparable test conditions*" (EFSA 2013, p. 83).

Furthermore, chronic exposure to the product is highly unlikely (pyraclostrobin has a dissipation half-life in water of 4.47 days).

In conclusion, boscalid has no significant contribution to the observed toxicity of the product to the most sensitive aquatic species and there is no indication of combined or unexpected toxicity. Therefore, the aquatic risk assessment presented for the active substance pyraclostrobin also cover the risk to aquatic organisms following the proposed uses of BAS 516 07 F and no additional TER calculations were performed for the formulated product.

TER_A for fish

Two acute toxicity endpoints for *O. mykiss* are available for the active substance; a study performed under static conditions and an additional study performed for the US under flow-through conditions. Both studies provide nearly identical endpoints based on mean measured concentrations, i.e. of 6.16 and 6.2 µg/L, respectively. In the following risk assessment of pyraclostrobin the rounded value of 6.2 µg/L is used. TER_A values were calculated using worst-case FOCUS Step 1 and 2 $PEC_{sw, max}$ values. The results are presented in Table 10.2-6. The acute risk to fish from the product BAS 516 07 F is addressed by the risk assessment for the active substance pyraclostrobin (see reasoning above).

Table 10.2-6: Fish acute TER values for pyraclostrobin and its major metabolites using worst-case FOCUS Step 1 and 2 $PEC_{sw, max}$ values

Test organism	Test substance	96 h LC_{50} [µg/L]	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER_A	Trigger value
O. mykiss	pyraclostrobin	6.2	2	0.164	**38**	100
O. mykiss	BF 500-5	11300	1	0.035	322857	100
O. mykiss	BF 500-11	> 100000	1	0.055	> 1818182	100
O. mykiss	BF 500-13	> 50000 < 100000	1	0.072	> 694444 < 1388889	100
O. mykiss	BF 500-14	> 39400 < 82600	1	0.078	> 505128 < 1058974	100

TERs shown in **bold** fall below the relevant trigger.

The calculated TER_A value for pyraclostrobin is below the trigger value of 100 based on FOCUS Step 2 calculation following application of BAS 516 07 F in potatoes. Therefore, additional TER calculations considering Step 3 $PEC_{sw, max}$ values are presented in Table 10.2-7.

The TER_A values for the metabolites BF 500-5, BF 500-11, BF 500-13 and BF 500-14 exceed the trigger value of 100 by far based on worst-case FOCUS Step 1 calculations, indicating low ecotoxicological relevance of the metabolites.

Table 10.2-7: Fish acute TER values for pyraclostrobin using worst-case FOCUS Step 3 $PEC_{sw, max}$ values

FOCUS Scenarios		96 h LC_{50} [µg/L]	FOCUS Step 3	
			$PEC_{sw, max}$ [µg/L]	TER_A
D3	ditch	6.2	0.088	**70**
D4	pond	6.2	0.005	1240
	stream	6.2	0.069	**90**
D6 (1st)	ditch	6.2	0.088	**70**
D6 (2nd)	ditch	6.2	0.087	**71**
R1	pond	6.2	0.010	620
	stream	6.2	0.061	102
R2	stream	6.2	0.081	**77**
R3	stream	6.2	0.086	**72**

TERs shown in **bold** fall below the relevant trigger

Based on FOCUS Step 3 calculations the TER_A values for pyraclostrobin are below the standard trigger of 100 for some scenarios following application of BAS 516 07 F in potatoes.

For a more thorough assessment of the risk to fish additional studies and a refined risk assessment have been conducted. Besides the standard fish species *O. mykiss,* seven other fish species were tested in acute 96 h laboratory studies with pyraclostrobin (see Table 10.2-2). To ease test item application, some of the additional tests were conducted using pyraclostrobin in the form of the previous representative solo-formulation BAS 500 00 F (due to the low water solubility of pyraclostrobin) the addition of solvents is appropriate anyway). A comparison of acute toxicity data with trout tested with the solo-formulation and the active substance provided a slightly lower endpoint for the solo-formulation (4 µg/L as compared to 6.2 µg/L for the a.s.). These data were considered in the previous Annex I inclusion process. Therefore, the use of data from tests with this formulation is considered a conservative surrogate for active substance testing.
Pyraclostrobin shows a very steep concentration response relationship. Therefore, in several cases no effect was observed at the first concentration whereas 100% mortality was found at the next higher concentration. In other studies, only one mortality measurement could be made between 0 and 100% mortality. To obtain relevant figures for the following calculations, in the first case the LC_{50} was determined as geometric mean between the two concentrations, while in the latter case a Spearman-Karber estimate of the LC_{50} was calculated using the mortality and analytical data for the relevant concentrations from the original study reports. Thereby the results may deviate slightly from those given in some of the reports, where no statistical analysis of the LC_{50} had been performed. The respective results were corrected for analytically determined concentrations. These studies and the respective endpoint re-calculations have already been evaluated during the previous Annex I inclusion process.

Additional species testing reduces uncertainty in the risk assessment by addressing better capturing of inter-species variations in sensitivity. According to the proposals of the EFSA Panel on Plant Protection Products and their Residues (PPR) given in the Aquatic Guidance Document (EFSA, 2013), it is recommended to preferably apply a species sensitivity distribution (SSD) approach for refined risk assessment if data on a sufficient number of species is available for the respective group of aquatic organisms. For fish, the SSD approach should be applied if toxicity data is available for at least 5 different species (for pyraclostrobin data on 8 species are available). The PPR Panel further recommends to use acute NOEC/LC_{10} values to construct the SSD for fish, since a higher protection level is desired for vertebrates than for invertebrates and plants.

Therefore, an SSD analysis based on the 96 h NOEC values for 8 fish species was conducted and the results of these calculations are presented below. The data used for SSD calculations are summarized in Table 10.2-8. For fish species were more than one study was performed (i.e. *O. mykiss* and *L. macrochirus*), the arithmetic mean of the endpoints of these studies was considered for SSD calculations.

Table 10.2-8: Pyraclostrobin endpoints selected for calculation of the Hazardous Concentrations based on Species Sensitivity Distribution (SSD) Analysis

Test species	LC_{50} [µg a.s./L]	NOEC / LC_{10} [µg a.s./L]	Ratio (LC_{50}/NOEC)	Reference (BASF DocID)
O. mykiss Salmonidae	6.16 #	4.5	1.4	1999/11414
	6.2	3.6	1.7	2000/5034
	6.2 *	**4.1 ***	1.5	--
C. carpio Cyprinidae	17.7 +	**12.1**	1.5	1998/11580
C. variegatus Cyprinodontidae	76.9	**53.5**	1.4	2000/5032
D. rerio Cyprinidae	61.9 #	**23.4**	2.6	1999/11834
L. idus melanotus Cyprinidae	19.1 +	**13.5**	1.4	1999/11835
L. macrochirus Centrarchidae	25.4 #	10.9	2.3	1998/10951
	11.4	6.1	1.9	2000/5033
	18.4 *	**8.5 ***	2.2	--
O. latipes Adrianichthyidae	53.3 #	**16.5**	3.2	1999/11821
P. promelas Cyprinidae	16.1 #	**7.0**	2.3	1999/11833

Bold values were used for SSD calculations.
* Arithmetic mean value of two studies conducted with the same species.
\# Spearman-Karber estimate of LC_{50} (for confidence limits see endpoint tables above; Table 10.2-2).
\+ Geometric mean (for corresponding LC_0 (>) and LC_{100} (<) values see endpoint tables above).

The SSD calculations have been performed using the software tool ETX 2.0 (RIVM, 2004). The results (graphs and statistical results) are shown below. In the first step, the toxicity data (NOEC) were subjected to three different goodness of fit tests (Anderson-Darling, Kolmogorov-Smirnov and the Cramer von Mises), where the normality was checked at different significance levels. The respective analysis of the NOEC values confirms normal distribution of the data (see below).

Analysis of normality

Parameters of the normal distribution

Name	Value	Description
mean	1.114	mean of the log toxicity values
s.d.	0.341	sample standard deviation
n	8	sample size

Goodness of fit tests

Sign. level	Tests for normality n=8					
	Anderson-Darling		Kolmogorov-Smirnov		Cramer von Mises	
0.1	0.631	Accepted	0.819	Accepted	0.104	Accepted
0.05	0.752	Accepted	0.895	Accepted	0.126	Accepted
0.025	0.873	Accepted	0.995	Accepted	0.148	Accepted
0.01	1.035	Accepted	1.035	Accepted	0.179	Accepted
Statistic	0.1757		0.4090		0.0114	

Figure 10.2-1: SSD Histogram (based on 96 h NOEC values for 8 fish species)

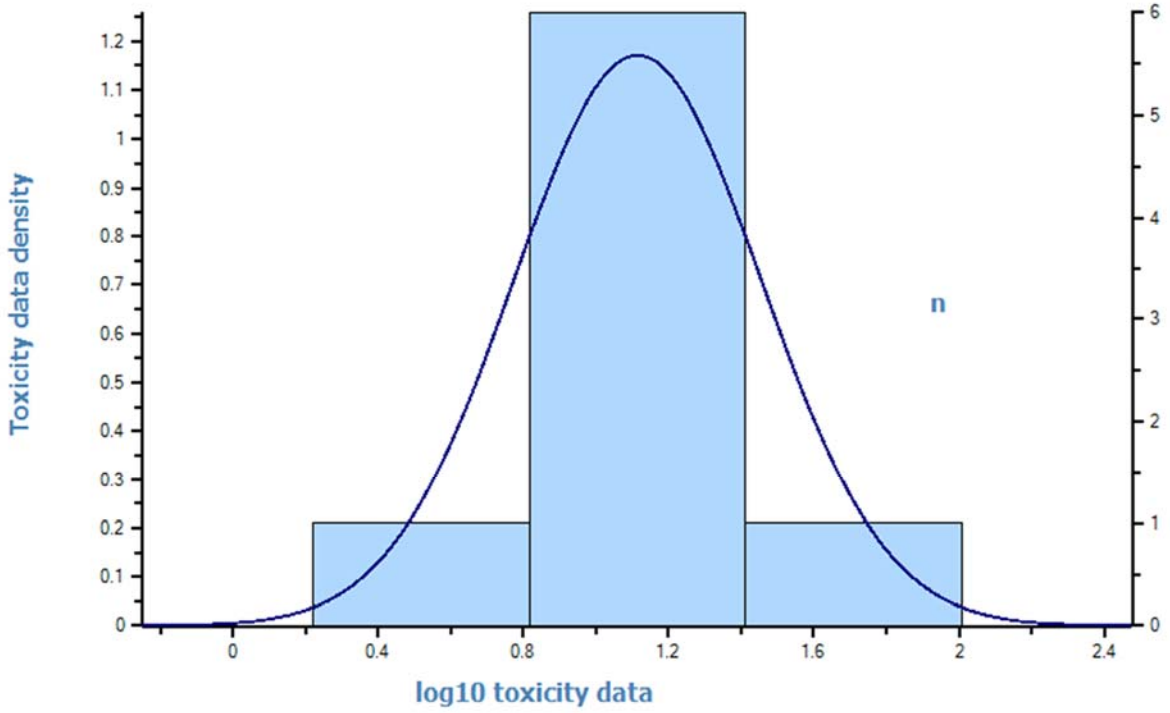
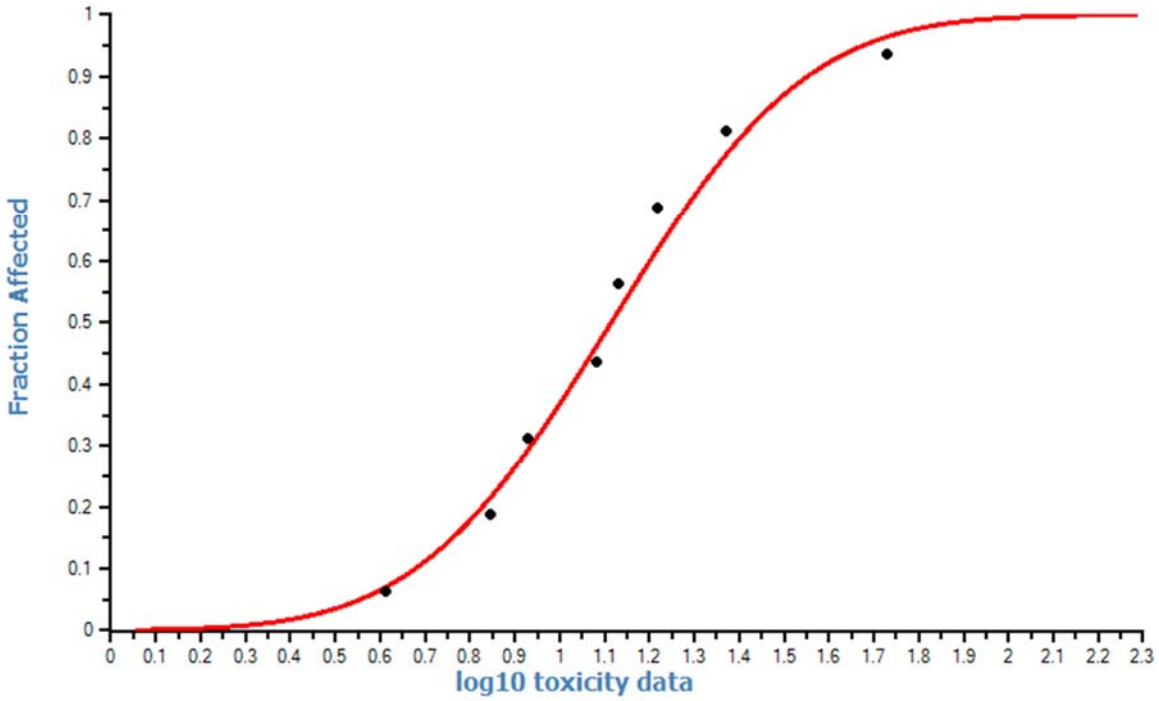


Figure 10.2-2: Species sensitivity distribution (based on 96 h NOEC values for 8 fish species)



The median HC_5 (hazardous concentration to 5% of the tested species that is predicted with 50% certainty) and also the lower limit HC_5 values ($LLHC_5$; hazardous concentration to 5% of the tested species that is predicted with 95% certainty) can be derived from the SSD curves (see table below).

HC_5 results based on acute (96 h) NOEC data for 8 fish species

Name	Value	log10(Value)	Description
LL HC_5	1.069	0.029	lower estimate of the HC_5
HC_5	**3.381**	0.529	median estimate of the HC_5
UL HC_5	6.139	0.788	upper estimate of the HC_5
sprHC_5	5.745	0.759	spread of the HC_5 estimate

The **median HC_5** for fish can thus be determined to be **3.381 µg a.s./**L.

According to the recommendation given in the EFSA Aquatic GD (2013), an assessment factor of 3 should be applied on the median HC_5 from an SSD constructed with acute NOEC values for fish for derivation of an SSD-RAC (Regulatory Acceptable Concentration), when latency of effects is not to be expected, which is clearly the case for pyraclostrobin (compare chronic risk assessment for fish below). In this case, the use of an AF of 3 is considered warranted also taking into account the various aspects mentioned in the aquatic GD:

- The quality of the acute toxicity data used to construct the SSD is high. All data are based on endpoints from GLP studies meeting all the listed criteria and including analytical support. A wide taxonomical range has been covered with eight species from five different fish families.
- The lower limit value of the HC_5 is about one third of the median HC_5, the fit of the curve is good and the relevant statistical parameters are met well.
- The SSD-RAC is not higher than the tier 3 RAC derived from effect class 1 of the mesocosm study. In fact, it is about three times lower (might indicate the appropriateness of a lower assessment factor).
- The position of the toxicity data in the lower tail of the SSD are either on the SSD curve or are positioned on the right side of the SSD curve (might indicate the appropriateness of a lower assessment factor).
- The steepness of the SSD curve is less than a factor of 100 between the lowest and the highest NOEC, however, it has to be considered that this is an SSD approach for fish only, where in general the difference between species sensitivities is much less than for the taxonomically more diverse group of invertebrates. In fact, a wide range of sensitivities has been covered in this investigation.
- Since sufficient information is available for the substance, read-across information for compounds with a similar mode of action is not needed.
- The acute to chronic ratio is significantly smaller than 10 (might indicate the appropriateness of a lower assessment factor).

Accordingly, an assessment factor of three is well justified and still conservative.

The endpoint obtained with the marine fish species (*Cyprinodon variegatus)* is twice higher than the next least sensitive species. It might be considered to omit this endpoint from the freshwater fish risk assessment as it may not be representative for freshwater species. However, omitting this higher NOEC value, the resulting median and lower limit HC_5 would be slightly higher (3.89 µg/L, respectively 1.48 µg/L as compared to 3.38 and 1.07 µg/L). As a conservative assumption the lower HC_5 value (i.e. including the higher toxicity endpoint) will be used.

Following this approach and applying the recommended assessment factor an **SSD-RAC_{acute} of 1.13 µg a.s./L** can be derived. This concentration is considered unlikely to cause unacceptable toxicity to fish, the most sensitive group of aquatic organisms.

TER_A calculations based on the HC_5 value and worst-case FOCUS Step 3 $PEC_{sw, max}$ values are shown in Table 10.2-9.

Table 10.2-9: Fish acute TER values for pyraclostrobin based on the median HC_5 and worst-case FOCUS Step 3 $PEC_{sw, max}$ values

FOCUS Scenarios		HC_5 (8 species) [µg/L]	FOCUS Step 3	
			$PEC_{sw, max}$ [µg/L]	TER_A*
D3	ditch	3.38	0.088	38
D4	pond	3.38	0.005	676
	stream	3.38	0.069	49
D6 (1st)	ditch	3.38	0.088	38
D6 (2nd)	ditch	3.38	0.087	39
R1	pond	3.38	0.010	338
	stream	3.38	0.061	55
R2	stream	3.38	0.081	42
R3	stream	3.38	0.086	39

* A TER trigger of 3 is considered appropriate for comparison to the median HC_5 (for justification see above).

Based on the SSD approach the TER_A values for pyraclostrobin indicate low acute risk to fish following application of BAS 516 07 F in potatoes with no need for any additional mitigation measures.

In addition, a time-to-effect study has been performed with pyraclostrobin on rainbow trout as the most sensitive fish species (see M-CA 8.2.1, BASF DocID 2000/1014919). The study was conducted to assess the impact of short exposure pulses typical for moving water bodies such as streams by employing different exposure durations (i.e. 0.5, 2 and 8 hours). The results of this study demonstrate that short term exposure, which might be encountered in plumes of moving water bodies, will be tolerated at much higher levels.

For short exposure times (i.e. 0.5 h), simulating average flow velocities in moving water bodies such as creeks and streams, LC_{50} values of > 27 µg/L were found. In a slower moving stream, generating exposure times of about 2 hours, concentrations of up to 18 µg/L are tolerated by the most sensitive species, and even more long-term pulses (8 hours) resulted in 2 - 3 times lower toxicity as compared to the standard 96 hour exposure period.

We propose to base the overall RAC for edge of field surface waters on the hazardous concentrations (HC_5) derived from species sensitivity distributions with fish as recommended in Table 6 of the Aquatic Guidance Document (EFSA, 2013) considering that:

- There is a large dataset from acute and chronic fish studies
- Toxicity is driven by the acute toxicity
- There are no sub-lethal effects at concentrations without mortality even in long-term chronic studies
- Latency of effects is clearly not given
- Pyraclostrobin dissipates quite rapidly from water (short exposure periods only)

Nevertheless, for completeness the chronic risk will be addressed below and TER_{LT} will be provided as well.

TER_{LT} for fish

The TER_{LT} value for pyraclostrobin and fish was calculated using the NOEC obtained in the chronic 98 day early life stage study on *O. mykiss* and the worst-case FOCUS Step 2 PEC_{sw} value. The result is shown in Table 10.2-10.

Table 10.2-10: Fish long-term TER value for pyraclostrobin using the worst-case FOCUS Step 2 $PEC_{sw, max}$ value

Test organism	98 d NOEC [µg/L]	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER_{LT}	Trigger value
O. mykiss	2.35	2	2.316	**1.0**	10

TERs shown in **bold** fall below the required trigger.

The TER_{LT} value for pyraclostrobin is below the long-term trigger value of 10 based on FOCUS Step 2 calculation for application of BAS 516 07 F in potatoes. Therefore, additional TER calculations considering Step 3 PEC values are presented in Table 10.2-11.

Table 10.2-11: Fish long-term TER values for pyraclostrobin using worst-case FOCUS Step 3 $PEC_{sw, max}$ values and the lowest chronic fish endpoint

FOCUS Scenarios		98 d NOEC [µg/L]	FOCUS Step 3	
			$PEC_{sw, max}$ [µg/L]	TER_{LT}
D3	ditch	2.35	0.088	27
D4	pond	2.35	0.005	470
	stream	2.35	0.069	34
D6 (1st)	ditch	2.35	0.088	27
D6 (2nd)	ditch	2.35	0.087	27
R1	pond	2.35	0.010	235
	stream	2.35	0.061	39
R2	stream	2.35	0.081	29
R3	stream	2.35	0.086	27

Based on FOCUS Step 3 calculations, all TER values exceed the standard long-term trigger of 10, indicating low chronic risk to fish following applications of BAS 516 07 F in potatoes with no need for additional mitigation measures.

In addition to the ELS study on *O. mykiss,* ELS studies on two other fish species have been performed (i.e. *C. variegatus* and *P. promelas,* see Table 10.2-2). The results for the three tested fish species can be used to calculate the **geometric mean of 4.72 µg a.s./L.** Considering the standard chronic assessment factor of 10 this results in a **geomean-$RAC_{chronic}$ of 0.472 µg a.s./L**. Considering the geomean of 4.72 µg/L in the above TER_{LT} calculations instead of only the lowest endpoint of the chronic studies, provide even higher TER_{LT} values (by factor of two) clearly showing low risk to fish from the use of BAS 516 07 F in potatoes.

Amphibian tadpoles

There is some limited information from literature on amphibian tadpoles, another aquatic vertebrate group. Hooser et al. (2012) (see CA 8.2.8/6) observed tadpole toxicity to the active substance pyraclostrobin (LC_{50} = 10 µg/L), which is in the range of fish data. The spacing of concentrations was rather rough (factor of three). Therefore, a more precise derivation of the LC_{50} is difficult. They observed higher toxicity for a pyraclostrobin formulated product (LC_{50} = 3.7 µg/L) with apparently slight mortality at 1.7 µg/L already. In another study of the same research group (Hartman et al. 2014, see CA 8.1.4/3) tadpoles of the same species were used again and exposed over a long term period at 1.7 µg/L. In this study they did not find any mortality at all; in contrast they found that tadpoles within this treatment group performed actually better than the control showing a shorter development time, without negative impact at all on biomass development. The slightly positive impact of the fungicide treatment is difficult to evaluate as the overall and particularly the control performance of the tadpoles (i.e. the development time) was rather poor.

Several literature evaluations compare the aquatic toxicity of amphibians to other aquatic organisms and fish in particular (Aldrich, 2009; Fryday and Thompson, 2012). The most comprehensive one was published recently (Weltje et al., 2013). A common conclusion of these data evaluations is that other aquatic endpoints (generally available for pesticides) cover the potential toxicity to amphibians in water.
The literature data shown above is not fully conclusive, however, seems to confirm in general the same trend. Accordingly, we consider that the risk assessment performed for fish as the most sensitive group of aquatic organisms should also cover the risk to amphibians.
It has to be borne in mind though that amphibian tadpoles may inhabit shallower water bodies than fish. If such sensitive water bodies are close to treated fields and if tadpoles inhabit such systems during time of application, there might be a higher risk to this group of organisms. The exact level would need to be determined by a proper and robust test on tadpoles. If for example we assume a twice lower water level, but if toxicity to tadpoles would also be twice lower as compared to the most sensitive fish species (which is roughly the case here considering the a.s. data for tadpoles (Hooser E.A. et al., 2012, see CA 8.2.8/6) and the lowest fish endpoint), then the resulting risk would be the same.

TER_A for *Daphnia magna*

TER_A values for *Daphnia magna* were calculated for pyraclostrobin and its major metabolites using worst-case FOCUS Step 1 and 2 $PEC_{sw, max}$ values and are given in Table 10.2-12.

Table 10.2-12: Acute TER values for *D. magna* exposed to pyraclostrobin and its major metabolites using worst-case FOCUS Step 1 and 2 $PEC_{sw, max}$ values

Test organism	Test substance	48 h EC_{50} [µg/L]	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER_A	Trigger value
D. magna	Pyraclostrobin	15.7	2	0.164	**96**	100
D. magna	BF 500-5	> 10000	1	0.035	> 285714	100
D. magna	BF 500-11	> 100000	1	0.055	> 1818182	100
D. magna	BF 500-13	> 100000	1	0.072	> 1388889	100
D. magna	BF 500-14	> 60900	1	0.078	> 780769	100

TERs shown in **bold** fall below the relevant trigger.

The TER_A value for pyraclostrobin is (slightly) below the trigger of 100 based on FOCUS Step 2 calculation for application in potatoes. Therefore, additional TER calculations based on FOCUS Step 3 PEC values are presented in Table 10.2-13.

The TER_A values for the metabolites BF 500-5, BF 500-11, BF 500-13 and BF 500-14 exceed the trigger value of 100 based on worst-case FOCUS Step 1 calculations, indicating low ecotoxicological relevance of the metabolites.

Table 10.2-13: Acute TER values for *D. magna* exposed to pyraclostrobin using FOCUS Step 3 $PEC_{sw, max}$ values

FOCUS Scenarios		48 h EC_{50} [µg/L]	FOCUS Step 3	
			$PEC_{sw, max}$ [µg/L]	TER_A
D3	ditch	15.7	0.088	178
D4	pond	15.7	0.005	3140
	stream	15.7	0.069	228
D6	ditch	15.7	0.088	178
D6	ditch	15.7	0.087	180
R1	pond	15.7	0.010	1570
	stream	15.7	0.061	257
R2	stream	15.7	0.081	194
R3	stream	15.7	0.086	183

The TER_A values for pyraclostrobin exceed the standard acute trigger value of 100 based on FOCUS Step 3 calculations, indicating low acute risk to aquatic invertebrates following applications of BAS 516 07 F in potatoes with no need for any additional mitigation measures.

Furthermore, a mesocosm study is available indicating low risk to aquatic invertebrates at concentrations of 8 µg a.s./L or less (for more details see also the following chapter on *Daphnia* long term risk assessment).

Comparison of the respective worst-case FOCUS Step 1 $PEC_{sw, max}$ value for the active substance pyraclostrobin (= 2.316 µg a.s./L for potatoes) to the proposed $RAC_{mesocosm}$ demonstrates low risk to aquatic invertebrates following the use of BAS 516 07 F in potatoes.

Additional studies have been conducted with the marine invertebrate species *A. bahia* and *C. virginica,* partly resulting in lower endpoints compared to EC_{50} for *D. magna* (see M-CA 8.2). However, the mesocosm study includes a great number of different and more relevant freshwater invertebrate species (for more details see below and chapter M-CA 8.2). Thus, the higher tier assessment based on the mesocosm study is most appropriate to address the risk to aquatic invertebrates.

TER_{LT} for *Daphnia magna*

The TER_{LT} value for *Daphnia magna* was calculated for pyraclostrobin using the worst-case FOCUS Step 2 $PEC_{sw, max}$ value and is given in Table 10.2-14.

Table 10.2-14: Long-term TER value for *D. magna* exposed to pyraclostrobin using the worst-case FOCUS Step 2 $PEC_{sw, max}$ value

Test organism	21 d NOEC [µg/L]	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER_{LT}	Trigger value
D. magna	4.0	2	0.164	24	10

The TER_{LT} value for pyraclostrobin exceeds the long-term trigger value of 10 based on FOCUS Step 2 calculation, indicating low chronic risk to aquatic invertebrates following the proposed use of BAS 516 07 F in potatoes with no need for any additional mitigation measures.

Furthermore, a mesocosm study is available (BASF DocID 2000/1000011 + supplement 2012/1357084) and a refined risk assessment has been performed providing more realistic information about the potential risk to aquatic invertebrates. A mesocosm study provides a complex and compelling impression of the potential effects of a test substance under realistic conditions. Thus, mesocosm data allows for a general conclusion on aquatic ecosystems since major direct effects will be quite comparable within different systems (Leeuwangh, 1994).

The Dutch Platform for Assessment of Higher-Tier Studies has produced a Guidance Document on how micro-/mesocosm data should be presented and evaluated in a uniform and transparent manner (RIVM Guidance Document; de Jong et al., 2008;). The EFSA Aquatic GD (EFSA, 2013) proposes to largely use this document to present and evaluate micro-/mesocosm studies for regulatory purposes when placing plant protection products on the European market. Consequently, the mesocosm study has been evaluated following the RIVM Guidance document; for details please refer to the supplement to this study (BASF DocID 2012/1357084). Executive summaries of the originally submitted study and the evaluation according to RIVM are provided in M-CA 8.2. The mesocosm evaluation according to this new procedure results basically in the same conclusion as previously given during the Annex I listing process, i.e. a high quality study covering a wide range of sensitive species and measurements providing robust endpoints.

The results of the extensive and complex mesocosm study using a worst-case exposure scenario (i.e. 8 applications of the test substance applied as the solo-formulation BAS 500 00 F) showed that pyraclostrobin can have effects on a few species at concentrations of 24 µg a.s./L. For all plankton species the effects were found to be reversible. These transient effects are thus not considered to pose a significant (ecologically unacceptable) risk to planktonic communities in aquatic ecosystems. However, fish and molluscs may also be affected at this concentration. No effects on any species or endpoint were observed at the second highest test concentration of 8 µg a.s./L, constituting the ecosystem NOEC. The multitude of endpoints and species and environmental conditions in this mesocosm study show clearly that at this (and lower) concentration no adverse effects on aquatic communities can be expected even after multiple applications. The ecologically acceptable concentration (NOEAEC) of pyraclostrobin is thus between 8 µg a.s./L and 24 µg a.s./L.

In addition, this study still encompassed unrealistic worst case conditions (8 applications of the test substance). The number of applications will be much less under practical conditions (1-4). It is highly unlikely, that eight times a 95% worst case situation with respect to drift will be encountered.

Considering the multitude of endpoints covered, the inclusion of sensitive and relevant species, the worst-case character of the mesocosm study related to exposure and the realistic worst-case test system applied, it can be concluded that no assessment factor to the **NOEC of 8 µg a.s./L** is warranted (which is also in line with previous recommendations, guidances). However, the new Aquatic Guidance Document (EFSA 2013) recommends using an assessment factor of two in this case for the ETO-RAC derivation. Following this approach, this results in an **$RAC_{mesocosm}$ of 4 µg a.s./L.**

A comparison of the respective worst-case FOCUS Step 1 $PEC_{sw, max}$ value for the active substance pyraclostrobin (= 2.316 µg a.s./L for potatoes) to the proposed $RAC_{mesocosm}$ demonstrates low risk to aquatic invertebrates following the proposed use of BAS 516 07 F in potatoes.

TER_A for aquatic insect

Acute tests were not conducted with an aquatic insect species. Instead, chronic studies were performed with the active substance pyraclostrobin and its soil metabolites BF 500-3, BF 500-6 and BF 500-7 on *Chironomus riparius*, which address the potential long-term risk of the active substance to aquatic insects (see below). Furthermore, a mesocosm study is available for pyraclostrobin covering various aquatic invertebrate species including aquatic insects.

TER_{LT} for aquatic insect

TER_{LT} values for *Chironomus riparius* were calculated for water and sediment exposure.

The resulting TER_{LT} value for pyraclostrobin following water exposure using the worst-case FOCUS Step 1 PEC_{sw} value is given in Table 10.2-15.

Table 10.2-15: Long-term TER value for *C. riparius* exposed to pyraclostrobin using the worst-case FOCUS Step 1 $PEC_{sw, max}$ value (water exposure)

Test organism	28 d NOEC [µg/L]	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER_{LT}	Trigger value
C. riparius (spiked water)	40	1	2.316	17	10

The TER_{LT} value for pyraclostrobin exceeds the long-term trigger of 10 based on FOCUS Step 1 calculation, indicating low chronic risk to aquatic insects resulting from water exposure following application of BAS 516 07 F in potatoes.

In order to assess similarly the risk arising from sediment exposure, TER values were calculated using the 28 d NOECs from chronic spiked-sediment studies with *C. riparius*. The resulting TER_{LT} values for pyraclostrobin and its soil sediment metabolites BF 500-3, BF 500-6 and BF 500-7 using FOCUS Step 1 and 2 $PEC_{sed, max}$ values are given in Table 10.2-16.

Table 10.2-16: Long-term TER values for *C. riparius* exposed to pyraclostrobin and its major soil metabolites using the worst-case FOCUS Step 1 and 2 $PEC_{sed, max}$ values (sediment exposure)

Test substance	Test organism	28 d NOEC [µg/kg dry sediment]	FOCUS Step	$PEC_{sed, max}$ [µg/kg dry sediment]	TER_{LT}	Trigger value
pyraclostrobin	*C. riparius* (spiked sediment)	1370	2	14.142	97	10
BF 500-3	*C. riparius* (spiked sediment)	≥ 16000	1	2.724	≥ 5874	10
BF 500-6	*C. riparius* (spiked sediment)	1200	1	41.471	29	10
BF 500-7	*C. riparius* (spiked sediment)	≥ 123500	1	24.885	≥ 4963	10

The TER_{LT} value for pyraclostrobin and its soil metabolites BF 500-3, BF 500-6 and BF 500-7 exceed the standard long-term trigger of 10 based on FOCUS Step 1 and 2 calculations, indicating low chronic risk to aquatic insects resulting from sediment exposure following application of BAS 516 07 F in potatoes.

Furthermore, a mesocosm study is available for pyraclostrobin covering various aquatic invertebrate species including aquatic insects. Based on the mesocosm results a $RAC_{mesocosm}$ of 4 µg a.s./L could be received (see text above), demonstrating low risk to aquatic organisms (at FOCUS Step 1 level; water exposure) following the proposed uses of BAS 516 07 F.

TER_A for aquatic crustacean

An acute spiked sediment study has been conducted with the estuarine amphipod *Leptocheirus plumulosus.* The sensitivity of this marine crustacean species seems to be within the range of freshwater species (NOEC = 2.74 mg/kg as compared to 1.37 mg/kg for *Chironomus* in the chronic test). Although this study is not required for registration in the EU, TER calculations based on the resulting 10-day LC_{50} value and worst-case FOCUS Step 2 $PEC_{sed, max}$ are presented in Table 10.2-17.

Table 10.2-17: Acute TER value for *L. plumulosus* exposed to pyraclostrobin using the worst-case FOCUS Step 2 $PEC_{sed, max}$ value (sediment exposure)

Test organism	10 d LC_{50} [µg/kg dry sediment]	FOCUS Step	$PEC_{sed, max}$ [µg/kg dry sediment]	TER_A	Trigger value
L. plumulosus	4410	2	14.142	312	100

The TER_A value for pyraclostrobin exceeds the standard trigger of 100 based on FOCUS Step 2 calculation, indicating low acute risk to aquatic (sediment dwelling) crustacean following application of BAS 516 07 F in potatoes. Furthermore, a mesocosm study is available for pyraclostrobin covering various aquatic invertebrate species including aquatic crustacean species (see above).

TER_{LT} for aquatic crustacean

TER_{LT} calculations for the freshwater crustacean *Daphnia magna* are presented above.

An additional chronic study has been conducted with the marine species *A. bahia* showing lower endpoints compared to the NOEC *for D. magna*. However, the mesocosm study (see above) includes a great number of different and relevant freshwater invertebrate species providing strong evidence that the mysid data are not representative for freshwater organisms. Thus, the higher tier assessment based on the mesocosm study provides the more relevant and more comprehensive data base for the assessment of the risk to freshwater invertebrates.

TER_A for aquatic gastropod mollusc

TER_A values for aquatic gastropod molluscs are not required since BAS 516 07 F is not foreseen for direct application to surface water.
An additional chronic study has been conducted for the US with the marine species *C. virginica* showing endpoints in the range of *D. magna* endpoints. However, the mesocosm study (see above) includes a great number of different and relevant freshwater invertebrate species including aquatic gastropod molluscs. Thus, the higher tier assessment based on the mesocosm study provides the more relevant and more comprehensive data base for the assessment of the risk to freshwater invertebrates.

TER_{LT} for aquatic gastropod mollusc

TER_{LT} values for aquatic gastropod molluscs are not required since BAS 516 07 F is not foreseen for direct application to surface water. Therefore, no investigations have been performed with aquatic gastropod molluscs. Furthermore, mesocosm data are available for the active substance pyraclostrobin and thus information on gastropod mollusc species.

TER for algae

Besides the study on the standard green alga *P. subcapitata*, studies on three further alga species have been conducted, partly resulting in lower endpoints compared to the endpoints for the green alga. However, following the recommendations and validity criteria defined in the current OECD guideline 201 for alga testing (OECD, 2011) the 120 h studies the blue-green alga *Anabaena flos-aquae* and marine diatom *Skeletonema costatum* are considered to be not valid because in both studies, at least one validity criterion is not met (for details please refer to chapter M-CA 8.2).
The study with the freshwater diatom *Navicula pelliculosa* showed some shortcomings, too, i.e. no clear dose-response relationship could be shown, because effects at the four highest tested concentrations are in a similar range (i.e. growth rate after 120 h at the four highest tested concentrations is between 68 and 72% of control). Moreover, a mesocosm study is available including a great number of different algae species (including *Navicula* species). Thus, the higher tier assessment based on the mesocosm study provided above also covers the risk to algae. At tier 1 level, TER calculations are thus only performed based on the re-calculated 72 hour growth rate endpoint from the 96 hour study with the standard green alga *P. subcapitata.*
The resulting TER values for pyraclostrobin and its major metabolites using FOCUS Step 1 $PEC_{sw,\ max}$ values are given in Table 10.2-18.
The risk of the product BAS 516 07 F to algae is sufficiently addressed by the risk assessment for the active substance pyraclostrobin (see reasoning given above).

Table 10.2-18: TER values for algae exposed to pyraclostrobin and its major metabolites using FOCUS Step 1 $PEC_{sw,\ max}$ values

Test organism	Test substance	72 h E_rC_{50} [µg/L]	FOCUS Step	$PEC_{sw,\ max}$ [µg/L]	TER	Trigger value
P. subcapitata	pyraclostrobin	> 843	1	2.316	> 364	10
P. subcapitata	BF 500-5	5330	1	0.035	152286	10
S. subspicatus	BF 500-11	> 100000	1	0.055	> 1818182	10
S. subspicatus	BF 500-13	> 100000	1	0.072	> 1388889	10
S. subspicatus	BF 500-14	> 100000	1	0.078	> 1282051	10

The TER values for pyraclostrobin and its metabolites BF 500-5, BF 500-11, BF 500-13 and BF 500-14 exceed the trigger of 10 based on worst-case FOCUS Step 1 calculations, indicating low risk to algae following application of BAS 516 07 F in potatoes.

TER_{LT} for aquatic plants

Additional aquatic plant testing would not be required for this fungicide, however, for registration purposes in the USA a Lemna study is available. The respective TER_{LT} values for aquatic plants (given in Table 10.2-19) were calculated for pyraclostrobin using the growth rate endpoint of the 14 day study with *Lemna gibba* and FOCUS Step 1 $PEC_{sw, max}$ values.

Table 10.2-19: TER_{LT} values for *Lemna gibba* exposed to pyraclostrobin using the worst-case FOCUS Step 1 $PEC_{sw, max}$ value

Test organism	14 d E_rC_{50} [µg/L]	FOCUS Step	$PEC_{sw, max}$ [µg/L]	TER_{LT}	Trigger value
L. gibba	1720	1	2.316	743	10

The TER_{LT} value for pyraclostrobin exceeds the trigger of 10 based on worst-case FOCUS Step 1 calculation, indicating low risk to aquatic plants following application of BAS 516 07 F in potatoes with no need for additional mitigation measures.

Summary of the Refined Risk Assessment

Pyraclostrobin is toxic to aquatic organisms. Based on standard precautionary assumptions, some risk to aquatic organisms is indicated following applications of a product containing this active substance very close to sensitive aquatic systems. In order to refine the risk a number of additional and higher tier studies were performed. These include additional species testing, tests using more realistic exposure scenarios, a time-to-effect study and a mesocosm investigation.

The mesocosm study, which covers a wide range of relevant and sensitive aquatic organisms (but only one fish species), results in a clear NOEC of 8 µg/L, even though it simulates a worst-case exposure situation. Accordingly, an ETO-RAC of 4 µg/L can be derived.
In order to further refine the risk assessment for fish besides the standard fish species *O. mykiss,* seven additional fish species were tested in acute 96 h laboratory studies with pyraclostrobin. In line with the recommendations of the Aquatic Guidance document (EFSA, 2013) an SSD analysis was conducted based on the 96 h NOEC values. The median HC_5 for fish was determined to be 3.381 µg a.s./L. An assessment factor of 3 was shown to be warranted to be applied on the median HC_5. Following this approach an overall **RAC** for edge-of-field surface waters **of 1.13 µg a.s./L** can be derived.

Overall Conclusion

The fungicidal product BAS 516 07 F and the active substance pyraclostrobin, respectively, show toxicity to aquatic organisms and contamination of aquatic ecosystems must be avoided. However, the standard and refined risk assessment provided for pyraclostrobin and its major metabolites demonstrate that application of BAS 516 07 F in potatoes according to good agricultural practice is of low risk to aquatic ecosystems with no need for additional mitigation measures.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

The following fish acute toxicity study performed with the new representative formulation BAS 516 07 F (tested with the minor change formulation BAS 516 00 F) is provided in support of the assessment and has not been previously evaluated on the EU-level. The very similar composition of both products (BAS 516 07 F and BAS 516 00 F) is shown in Doc JCP 1.4.1.

Report:	CP 10.2.1/1 [redacted] 2000a BAS 516 00 F - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a static system (96 hours) 2000/1018726
Guidelines:	EPA 72-1, OECD 203, EEC 92/69 A V C 1
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a static acute toxicity laboratory study, juvenile rainbow trout were exposed to BAS 516 00 F nominal concentrations of 0.040, 0.063, 0.100, 0.160 and 0.250 mg BAS 516 00 F/L and a water control in groups of 10 animals in glass aquaria containing 50 L water. Fish were observed for survival and symptoms of toxicity within 1 h after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on the nominal concentrations. After 96 hours of exposure no mortality was observed in the control and at concentrations of up to and including 0.063 mg BAS 516 00 F/L, whereas 80% mortality was observed at 0.100 mg/L. At the two highest tested concentrations of 0.160 and 0.250 mg/L, 100% mortality was observed within the first day of the test.

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of BAS 516 00 F was 0.088 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 0.063 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F, batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal: 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal: 6.7%).

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1972), age: about 4 months, mean body length 5.4 cm (4.5 - 6.4 cm); mean body weight 1.4 g (0.8 - 2.1 g); raised in house from fertilized eggs supplied by "Trout Breeding, Erber Soehne", Eusserthal, Germany.

Test design: Static system (96 hours); 10 fish per aquarium (loading: about 0.3 g fish/L) and per concentration; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.

Test concentrations: Control, 0.040, 0.063, 0.100, 0.160 and 0.250 mg/L (nominal).

Test conditions: Glass aquaria with stainless steel frame (60 x 35 x 40 cm), test volume: 50 L, dechlorinated, filtered tap water; temperature: 12°C; pH 8.1 - 8.4; oxygen content: 8.0 mg/L - 10.5 mg/L; total hardness: about 2.5 mmol; acid capacity: about 5.5 mmol/L; photoperiod 16 h light : 8 h dark; no aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a RP-HPLC with UV-detection.

Statistics: Descriptive statistics; calculation of approximate LC_{50} as geometric mean of LC_0 and LC_{100}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning and, except for the highest concentration tested, at the end of the test. The analyzed contents of boscalid ranged from 93.6 to 99.7% of nominal at test initiation and from 93.1 to 94.7% of nominal at test termination. Measured concentrations of pyraclostrobin were between 87.8 and 104.8% of nominal in samples taken at test initiation and decreased to values between 32.1 and 76.2% of nominal at test termination. As initial measured concentrations confirmed correct application of the test substances, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure no mortality was observed in the control and at concentrations of up to and including 0.063 mg BAS 516 00 F/L, whereas 80% mortality was observed at 0.100 mg/L. At the two highest tested concentrations of 0.160 and 0.250 mg/L, 100% mortality was observed within the first day of the test. Sub-lethal effects (i.e. swimming near the bottom) were found at 0.100 mg BAS 516 00 F/L after 96 hours. The results are summarized in Table 10.2.1-1.

Table 10.2.1-1: Acute toxicity (96 h) of BAS 516 00 F on rainbow trout (*O. mykiss*)

Concentration [mg/L] (nominal)	**Control**	0.040	0.063	0.100	0.160	0.250
Mortality [%]	0	0	0	80	100	100
Symptoms [1)]	None	none	none	D	--	--
Endpoints [mg BAS 516 00 F/L] (nominal)						
LC_{50} (96 h)	0.088					
NOEC (96 h)	0.063					

[1)] Symptoms: D = swimming near the bottom
-- not observed / all animals dead

III. CONCLUSION

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of BAS 516 00 F was 0.088 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 0.063 mg/L (nominal).

The following *Daphnia* acute toxicity studies performed on BAS 516 07 F are provided in support of the assessment and have not been previously evaluated on the EU-level. The first study showed some shortcomings (i.e. measured concentrations of boscalid were > 120% of nominal concentrations at test initiation and test end, indicating an overdose over the whole study period). Therefore, and as the study results could not be based on mean measured concentrations (formulation contains two active substances with different recoveries) the study was repeated and the results of the second study are considered as relevant endpoints for the aquatic risk assessment. Nevertheless, the summary of the first study is provided below for completeness.

Report:	CP 10.2.1/2 Janson G.-M., 2007a Acute toxicity of BAS 516 07 F to Daphnia magna STRAUS in a 48 hour static test 2007/1008605
Guidelines:	OECD 202, EPA 850.1010
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 48-hour static acute toxicity laboratory study, water flea neonates were exposed to BAS 516 07 F at nominal concentrations of 0 (control), 0.030, 0.054, 0.097, 0.175, 0.315 and 0.567 mg BAS 516 07 F/L in 4 replicates per concentration, containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations. After 48 hours of exposure, no immobility was observed in the control and at the test item concentration of 0.054 mg/L, whereas 5, 50 and 80% immobility occurred at 0.030, 0.097 and 0.175 mg/L. At the two highest tested concentrations all daphnids were immobile after 48 hours of exposure. The effects after 48 hours observed at the four highest tested concentrations of exposure were statistically significantly different compared to the control. No other sublethal effects were observed during the test.

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} (48 h) of BAS 516 07 F was 0.120 mg/L based on nominal concentrations. The NOEC was determined to be 0.054 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 07 F, batch no. 1789, content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 26.7% (nominal: 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.8% (nominal: 6.7%).

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture (originally obtained from Institute National de Recherché Chimique Appliquée, France), >2 <24 hours old at test initiation.

Test design: Static system (48 hours), 6 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: Control, 0.030, 0.054, 0.097, 0.175, 0.315 and 0.567 mg BAS 516 07 F/L (nominal).

Test conditions: Glass vessels, test volume 50 mL, dilution water "M4" (Elendt medium); pH 7.92 - 8.12; oxygen content: 8.5 mg/L - 9.3 mg/L; total hardness: 2.42 mmol/L at test initiation; conductivity: 654µS/cm at test initiation; temperature: 19.9°C - 20.9°C; light intensity: 290 lux - 465 lux; photoperiod: 16 h light : 8 h dark; no feeding, no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; Fisher's Exact Test for determination of the NOEC ($\alpha = 0.05$), probit analysis for determination of the EC_{50} value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of boscalid ranged from 107.9 to 124.3% of nominal concentrations at test initiation and further increased to 111.9 to 129.1% of nominal at test termination. Measured concentrations of pyraclostrobin were between 102.2 and 112.1% of nominal in samples taken at test initiation and between 100.4 and 112.5% at test termination. The measured concentrations did not confirm the correct application of the test substance. However, since two compounds were measured with different recoveries a simple conversion to mean measured concentrations was not meaningful. Therefore, the following biological results are based on nominal concentrations.

Biological results: After 48 hours of exposure, no immobility was observed in the control and at the test item concentration of 0.054 mg/L, whereas 5, 50 and 80% immobility occurred at 0.030, 0.097 and 0.175 mg/L. At the two highest tested concentrations all daphnids were immobile after 48 hours of exposure. The effects after 48 hours observed at the four highest tested concentrations of exposure were statistically significantly different compared to the control (Fisher's exact test, $\alpha = 0.05$). No other sublethal effects were observed during the test. For results see Table 10.2.1-2.

Table 10.2.1-2: Effects of BAS 516 07 F on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	**Control**	**0.030**	**0.054**	**0.097**	**0.175**	**0.315**	**0.567**
Immobility (24 h) [%]	0	5	0	10	20	90 *	100 *
Immobility (48 h) [%]	0	5	0	50 *	80 *	100 *	100 *
Endpoints [mg BAS 516 07 F/L] (nominal)							
EC_{50} (48 h)	0.120 (95% confidence limits: 0.102 - 0.139)						
NOEC (48 h)	0.054						

* Statistically significantly different compared to the control (Fisher's exact test, $\alpha = 0.05$)

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna*, the EC_{50} (48 h) of BAS 516 07 F was 0.120 mg/L based on nominal concentrations. The NOEC was determined to be 0.054 mg/L (nominal).

Report:	CP 10.2.1/3 Janson G.-M., 2009a Acute toxicity of BAS 516 07 F to Daphnia magna STRAUS in a 48 hour static test 2009/1117877
Guidelines:	OECD 202, EPA 850.1010
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to BAS 516 07 F at nominal concentrations of 0 (control), 0.060, 0.096, 0.154, 0.246 and 0.393 mg BAS 516 07 F/L in 4 replicates per concentration, containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations. After 48 hours of exposure, no immobility was observed in the control, whereas 5, 10, 10 and 55% immobility occurred at 0.060, 0.096, 0.154 and 0.246 mg/L. At the highest tested concentration of 0.393 mg BAS 516 07 F/L all daphnids were immobile after 48 hours of exposure. After 48 hours of exposure statistically significant effects on mobility of daphnids was observed at the two highest concentrations of 0.246 and 0.393 mg BAS 516 07 F/L. No other sublethal effects were observed during the test.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of BAS 516 07 F was 0.210 mg/L based on nominal concentrations. The NOEC was determined to be 0.154 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 07 F, batch no. 11041, content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 26.9% (nominal: 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.5% (nominal: 6.7%).

B. STUDY DESIGN

Test species: Water flea (*Daphnia magna* STRAUS), neonates collected from in house culture (originally obtained from Institute National de Recherché Chimique Appliquée, France), >2 <24 hours old at test initiation.

Test design: Static system (48 hours), 5 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.

Endpoints: NOEC and EC_{50} based on immobility of daphnids.

Test concentrations: Control, 0.060, 0.096, 0.154, 0.246 and 0.393 mg BAS 516 07 F/L (nominal).

Test conditions: Glass vessels, test volume 50 mL, dilution water "M4" (Elendt medium); pH 7.98 - 8.04; oxygen content: 8.7 mg/L - 8.9 mg/L; total hardness: 2.37 mmol/L at test initiation; conductivity: 659µS/cm at test initiation; temperature: 21.6°C - 21.7°C; light intensity: 426 lux - 721 lux; photoperiod: 16 h light : 8 h dark; no feeding, no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with MS detection.

Statistics: Descriptive statistics; Fisher's Exact Test for determination of the NOEC, probit analysis for determination of the EC_{50} value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of boscalid ranged from 98.0 to 110.0% of nominal at test initiation and from 95.8 to 105.1% of nominal at test termination. Measured concentrations of pyraclostrobin were between 94.6 and 104.3% of nominal in samples taken at test initiation and between 89.6 and 99.8% at test termination. As measured concentrations confirmed correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: After 48 hours of exposure, no immobility was observed in the control, whereas 5, 10, 10 and 55% immobility occurred at 0.060, 0.096, 0.154 and 0.246 mg/L. At the highest tested concentration of 0.393 mg BAS 516 07 F/L all daphnids were immobile after 48 hours of exposure. After 48 hours of exposure statistically significant effects on mobility of daphnids was observed at the two highest concentrations of 0.246 and 0.393 mg BAS 516 07 F/L (Fisher's exact test, $\alpha = 0.05$). No other sublethal effects were observed during the test. For results see Table 10.2.1-3.

Table 10.2.1-3: Effects of BAS 516 07 F on *Daphnia magna* immobility

Concentration [mg/L] nominal	**Control**	**0.060**	**0.096**	**0.154**	**0.246**	**0.393**
Immobility (24 h) [%]	0	5	10	10	25	70 *
Immobility (48 h) [%]	0	5	10	10	55 *	100 *
Endpoints [mg BAS 516 07 F/L] nominal						
EC_{50} (48 h)	0.210					
NOEC (48 h)	0.154					

* Statistically significantly different compared to the control (Fisher's exact test, $\alpha = 0.05$)

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of BAS 516 07 F was 0.210 mg/L based on nominal concentrations. The NOEC was determined to be 0.154 mg/L (nominal).

The following algae toxicity study performed with BAS 516 07 F is provided in support of the assessment and has not been previously evaluated on the EU-level.

Report:	CP 10.2.1/4 Hoffmann F., 2007a Effect of BAS 516 07 F on the growth of the green alga Pseudokirchneriella subcapitata 2007/1005074
Guidelines:	OECD 201
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static acute toxicity laboratory study, the effect of BAS 516 07 F on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 1, 2, 4, 8, 16 and 32 mg BAS 516 07 F/L. Assessment of growth was conducted 0, 24, 48 and 72 h after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration from mean growth rates and yield based on number of cells.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control and all test item concentrations.

In a 72-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} for BAS 516 07 F was determined to be 10.8 mg/L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 07 F, batch no. 1789, content of a.s. boscalid (BAS 510 F, Reg. No. 300 355): 26.7% (nominal: 26.7%), pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.8% (nominal: 6.7%).

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81; stock obtained from the "Sammlung von Algenkulturen" Göttingen, Germany.

Test design: Static system; test duration 72 hours; 6 test concentrations, each with 5 replicates per treatment plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.

Test concentrations: Control, 1, 2, 4, 8, 16 and 32 mg BAS 516 06 F/L (nominal).

Test conditions: Erlenmeyer dimple flasks; test volume 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.78 - 7.91 at test termination; temperature: 22°C ± 1°C; initial cell densities: 1 x 10^4 cells/mL; continuous light at about 8000 lux; continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection.

Statistics: Descriptive statistics; probit analysis and logit analysis for determination of EC_x values for growth rate and yield, respectively.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of boscalid ranged from 100.3 to 117.5% of nominal at test initiation and from 100.2 to 105.1% of nominal at test termination. Measured concentrations of pyraclostrobin were between 100.0 and 110.4% of nominal at test initiation and between 89.8 and 93.4% of nominal at test termination. As measured concentrations confirmed correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control and all test item concentrations. The effects on algal growth rate and yield are summarized in Table 10.2.1-4.

Table 10.2.1-4: Effect of BAS 516 07 F on the growth of green alga *P. subcapitata*

Concentration [mg/L] nominal	**Control**	**1**	**2**	**4**	**8**	**16**	**32**
Inhibition in 72 h (growth rate) [%]	--	0.7	12.6	28.2	46.6	59.8	74.4
Inhibition in 72 h (yield) [%]	--	2.9	40.5	69.3	86.4	92.8	96.8
Endpoints [mg BAS 516 07 F/L] nominal							
E_rC_{50} (72 h)	**10.8** (95% confidence limits: 10.2 - 11.4)						
E_rC_{10} (72 h)	1.8 (95% confidence limits: 1.6 - 1.9)						
E_yC_{50} (72 h)	3.0 (95% confidence limits: 2.8 - 3.1)						
E_yC_{10} (72 h)	0.9 (95% confidence limits: 0.8 - 1.0)						

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} for BAS 516 07 F was determined to be 10.8 mg/L based on nominal concentrations.

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

Chronic toxicity to fish and aquatic invertebrates

The results obtained in the acute study on *O. mykiss* and *D. magna* with the formulated product BAS 516 07 F (partly tested with the minor change formulation BAS 516 00 F) are in good agreement with the results expected from the data with the active substances (see reasoning given above). Furthermore, the results obtained with the formulated product are in good agreement with the results expected from the data with the active substance pyraclostrobin, which basically determines the toxicity of the product to aquatic organisms whereas boscalid contributes only minor to the overall toxicity of the product. The data demonstrate that the formulation does not cause significant unexpected (additional) toxicity to fish and daphnids. Accordingly, there are no indications of synergism or additional toxicity from the formulation. Therefore, the studies conducted with the active substances can be used to assess the chronic risk resulting from BAS 516 07 F application. No further testing with the product is indicated.

CP 10.2.3 Further testing on aquatic organisms

Marine or estuarine organisms

Studies on marine or estuarine species are not required according to the relevant EU documents and no studies have been conducted with the formulation BAS 5160 07 F on marine or estuarine organisms. The contamination of estuarine and marine environments is considered to be minimal compared to freshwater habitats adjacent to agricultural land according to the use pattern, the potential route of contamination and the dissipation of the active substance. Thus, the risk to those habitats is covered by the risk assessment for freshwater ecosystems.

Microcosm or mesocosm study

No microcosm or mesocosm study has been performed with the formulated product BAS 516 07 F, however, a mesocosm study has been performed with pyraclostrobin (using the previous representative solo formulation BAS 500 00 F, which is of the same formulation type). The mesocosm study has been evaluated following the RIVM Guidance document (de Jong et al., 2008). Executive summaries of the originally submitted study and the evaluation according to RIVM are provided in M-CA 8.2.

The mesocosm study provided a clear NOEC of 8 µg a.s./L. The ecologically acceptable concentration (NOEAEC) of pyraclostrobin was determined to be between 8 µg a.s./L and 24 µg a.s./L. Based on the results of this study a **$RAC_{mesocosm}$ of 4 µg a.s./L** could be derived (by applying an assessment factor of 2 on NOEC), which was considered for a refined risk assessment (as described above).

Residue data in fish
A respective evaluation has been performed during the previous Annex I inclusion process. No new data are provided. The following gives a brief summary of the previous evaluation:
The log P_{ow} of the active substance pyraclostrobin was determined to be 3.99 (EU Review Report, SANCO/1420/2001-final, September 2004). Hence, a bioaccumulation study in fish has been performed with pyraclostrobin, which is (together with a new fish metabolism study, CA 6.2.5/2) discussed in detail in M-CA 6.2.5 (please refer to CA 6.2.5/1). An apparent steady state was reached after 2 - 4 days of exposure. The bioconcentration factors for whole fish were 379 - 507 (two labels). The half-life for elimination was 0.9 days. The time for elimination of 90% of the activity varied between 2.8 and 3.0 days. The nature of radioactivity in fish tissues after 28 days of exposure consisted of the parent substance (39 - 74%) and 4 metabolites (2 - 9%). Due to the limited bioaccumulation and the rapid metabolization and excretion of the active substance (and its metabolites), there is no risk of bioaccumulation. In addition, pyraclostrobin dissipates rapidly in water prohibiting continuous exposure.

Residues of pyraclostrobin in fish are of no concern and no accumulation in the food chain is to be expected.

Accumulation in aquatic non-target organisms
A respective evaluation has been performed during the previous Annex I inclusion process. No new data are provided. Bioaccumulation of the active substance pyraclostrobin under natural conditions is not expected to occur (see "Residue data in fish" above). Additional studies are not required or necessary to determine bioaccumulation in aquatic non-target organisms.

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Appendix

The Predicted Environmental Concentrations (PEC) of pyraclostrobin and its major metabolites in surface water and sediment used for the aquatic risk assessment are summarized in the tables below. No additional PEC values have been calculated for the formulated product, as there is no indication for any unexpected (higher) toxicity compared to the active substance - see the reasoning given below.

Table 10.2.3-1: Worst-case FOCUS Step 1 and 2 $PEC_{sw,\ max}$ and $PEC_{sed,\ max}$ values for pyraclostrobin and its major metabolites following application of BAS 516 07 F in potatoes

Test substance	$PEC_{sw,\ max}$ [µg/L]			$PEC_{sed,\ max}$ [µg/kg dry sediment]		
	Step 1	Step 2		Step 1	Step 2	
		EU North	EU South		EU North	EU South
pyraclostrobin	2.316	0.156	0.164	157.319	7.990	14.142
BF 500-3 #	-- #	-- #	-- #	2.724	1.805	1.805
BF 500-5 *	0.035	0.016	0.016	--*	--*	--*
BF 500-6 #	-- #	-- #	-- #	41.471	2.535	4.912
BF 500-7 #	-- #	-- #	-- #	24.885	1.569	2.989
BF 500-11 §	0.055	0.024	0.024	--*	--*	--*
BF 500-13 §	0.072	0.041	0.041	--*	--*	--*
BF 500-14 §	0.078	0.031	0.031	--*	--*	--*

§ Metabolites were mainly observed in irradiated water/sediment studies. Accordingly, only PEC values derived from irradiated water/sediment studies are reported.
# The metabolite was not detected in relevant amounts. Accordingly, no PEC_{sw} values were reported.
* The metabolites were not detected in relevant amounts. Accordingly, no PEC_{sed} values were reported.

Table 10.2.3-2: Worst-case FOCUS Step 3 $PEC_{sw,\ max}$ and $PEC_{sed,\ max}$ values for pyraclostrobin in different water bodies following application of BAS 516 07 F in potatoes

FOCUS Scenarios		FOCUS Step 3	
		$PEC_{sw,\ max}$ [µg/L]	PEC_{sed} [µg/kg dry sediment]
D3	ditch	0.088	0.071
D4	pond	0.005	0.057
	stream	0.069	0.005
D6 (1st)	ditch	0.088	0.080
D6 (2nd)	ditch	0.087	0.095
R1	pond	0.010	0.139
	stream	0.061	1.550
R2	stream	0.081	1.052
R3	stream	0.086	0.964

CP 10.3 Effects on arthropods

The new representative formulation BAS 516 07 F was not evaluated within a previous Annex I inclusion process. It is a water dispersible granule (WG) containing 67 g/kg pyraclostrobin and 267 g/kg boscalid.

CP 10.3.1 Effects on bees

The EU agreed endpoints for the active substance pyraclostrobin as described in the EU Review Report (SANCO/1420/2001-final, September 2004) plus endpoints from new studies with the active substance and the new representative formulation BAS 516 07 F are used for the risk assessment on honeybees (see Table 10.3.1-1).

Table 10.3.1-1: Ecotoxicological endpoints for honeybees and bumblebees

Test substance	EU agreed endpoints	Endpoints used in risk assessment
Studies on adult honeybees		
Pyraclostrobin	oral (48 h) LD_{50} > 73.1 µg a.s./bee contact (48 h) LD_{50} > 100.0 µg a.s./bee	oral (48 h) LD_{50} > 73.1 µg a.s./bee contact (48 h) LD_{50} > 100.0 µg a.s./bee
Pyraclostrobin [1)]	--	oral (48 h) LD_{50} > 110.0 µg a.s./bee contact (48 h) LD_{50} > 100.0 µg a.s./bee
BAS 516 07 F [2)]	--	oral (48 h) LD_{50} > 258.7 µg/bee contact (48 h) LD_{50} > 299.4 µg/bee
Studies on honeybee queens		
Pyraclostrobin (tested as Pristine) [3)]	--	No unacceptable effects in queen development, queen survival and adult worker bee health using 400 ppm Pristine treated pollen (measured concentration: 22 ppm pyraclostrobin)
Studies on adult bumblebees		
Pyraclostrobin	--	Oral (96 h) LD_{50} > 97.2 µg a.s./bumblebee Contact (96 h) LD_{50} > 100 µg a.s./bumblebee
Studies on residues		
Pyraclostrobin [4)]	--	Applied rate: 143 g pyraclostrobin/ha Highest residue: 16.6 mg pyraclostrobin/kg (90^{th} percentile, found in sunflower pollen)
Pyraclostrobin [4)]	--	Applied rate: 143 g pyraclostrobin/ha Highest residue: 6.7 mg pyraclostrobin/kg (90^{th} percentile, found in oilseed rape pollen)

1) New acute study has been conducted since the results for the reference substance were out of range in the original study; the results of the new study will be used in the risk assessments below.

2) Tests were carried out with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).

3) Peer-reviewed scientific study was carried out with Pristine, a formulation similar to BAS 516 07 and containing 25.2% boscalid and 12.8% pyraclostrobin (for details see study summary in M-CP 10.3.1.3 below). The study is presented as additional information.

4) Study was carried out with BAS 556 03 F as a surrogate for pyraclostrobin. BAS 556 03 F contains 130 g pyraclostrobin/L and 80 g metconazole/L. The study is presented as additional information.

Overall summary

Data on BAS 516 07 F and its potential effects on honeybees are evaluated and appropriate risk assessments are provided for the active substance and the formulation based on the already registered use pattern in potatoes (critical GAP: maximum 4 x 17 g pyraclostrobin per ha).

The endpoints and calculated HQs for acute oral and acute contact exposure of honeybees to pyraclostrobin and BAS 516 07 F (see Table 10.3.1-2) are below the trigger value of 50 demonstrating low acute risk to honeybees.

Tests with the formulation have been partly carried out with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).

Table 10.3.1-2: Toxicity data, maximum single application rates and HQ values for honeybees

Test substance	Use pattern	Exposure route	Endpoint [µg/bee]	Maximum single application rate	Hazard quotient (HQ)	HQ assessment trigger
Risk assessment on adult honeybees						
Pyraclostrobin	potatoes	acute oral	$LD_{50} > 110$	17 g a.s./ha	< 0.15	50
		acute contact	$LD_{50} > 100$		< 0.17	
BAS 516 07 F [1)]		acute oral	$LD_{50} > 258.7$	250 g/ha	< 0.97	
		acute contact	$LD_{50} > 299.4$		< 0.84	

1) Tests were carried out with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).

Two studies were conducted with pyraclostrobin containing products in two different crops, sunflower and oilseed rape (OSR) to determine the residues in the bee relevant matrices pollen and nectar. The highest exposure (90th percentile) was found in pollen, i.e. 16.6 mg/kg (sunflower) and 6.7 mg/kg (OSR).

Additionally, no unacceptable effects in queen development, queen survival and adult worker bee health were observed using pollen treated with the product Pristine (measured concentration: 22 ppm pyraclostrobin). As currently no risk assessment scheme exists, these results are presented as additional information.

Also for non-*Apis* bees currently no risk assessment scheme exists under Regulation (EC) No 1107/2009. Nevertheless, the potential acute toxicity (oral and contact) of pyraclostrobin to adult bumblebees was addressed in a laboratory study (BASF DocID 2016/1000530), which is described in M-CA 8.3.1.1).

Overall conclusion:
The proposed use of BAS 516 07 F in potatoes present a low risk to honeybees and will not adversely affect honeybees or honeybee colonies.

Toxicity

Table 10.3.1-3 presents the results of honeybee and bumblebee toxicity studies. For a summary of the studies with pyraclostrobin, please refer to M-CA 8.3. Further details regarding the tests with the formulation are provided in M-CP 10.3.1.1.

Table 10.3.1-3: Summary of endpoints of pyraclostrobin and BAS 516 07 F to honeybees and bumblebees

Substance	Endpoint	LD_{50} [µg/bee]	Reference (BASF DocID)
Studies on adult honeybees			
Pyraclostrobin	48 h oral LD_{50}	> 73.1	1999/11457
	48 h contact LD_{50}	> 100	
Pyraclostrobin [1)]	48 h oral LD_{50}	> 110	2013/1003210
	48 h contact LD_{50}	> 100	
BAS 516 07 F [2)]	48 h oral LD_{50}	> 258.7	2001/1000868
	48 h contact LD_{50}	> 299.4	
Studies on honeybee queens			
Pyraclostrobin (tested as Pristine) [3)]	No unacceptable effects in queen development, queen survival and adult worker bee health using 400 ppm Pristine treated (measured concentration: 22 ppm pyraclostrobin)		2013/1416303
Studies on adult bumblebees			
Pyraclostrobin	96 h oral LD_{50}	> 97.2 µg a.s./bee	2016/1000530
	96 h contact LD_{50}	> 100 µg a.s./bee	
Residue studies			
Pyraclostrobin [4)]	Applied rate: 143 g pyraclostrobin/ha Highest residue: 16.6 mg pyraclostrobin/kg (90th percentile, found in sunflower pollen)		2014/1000204
Pyraclostrobin [4)]	Applied rate: 143 g pyraclostrobin/ha Highest residue: 6.7 mg pyraclostrobin/kg (90th percentile, found in oilseed rape pollen)		2014/1000182

1) New acute study has been conducted since the results for the reference substance were out of range in the original study; the results of the new study will be used in the risk assessments below.
2) Tests were carried out with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).
3) Peer-reviewed scientific study was carried out with Pristine, a formulation similar to BAS 516 07 and containing 25.2% boscalid and 12.8% pyraclostrobin (for details see study summary in M-CP 10.3.1.3 below). The study is presented as additional information.
4) Study was carried out with BAS 556 03 F as a surrogate for pyraclostrobin. BAS 556 03 F contains 130 g pyraclostrobin/L and 80 g metconazole/L. The study is presented as additional information.

Exposure

Applications of pesticides can potentially result in exposure of honeybees either through direct over-spray, or by contact with residues on plants while bees are foraging for food. Potatoes might be of attractiveness to foraging bees during the time of application. In order to consider a worst-case scenario, the maximum application rate for pyraclostrobin as applied with the maximum recommended rate of BAS 516 07 F is used for the risk assessment.

Table 10.3.1-4: Proposed use pattern of BAS 516 07 F

Crop	Application time (BBCH code)	Number of applications	Interval [d]	Application rate per treatment		
				Boscalid [g a.s./ha]	Pyraclostrobin [g a.s./ha]	BAS 516 07 F [g/ha]
potatoes	51 – 89	4	10	67	17	250

Risk assessment for bees

The acute risk to honeybees from the use of BAS 516 07 F in potatoes was assessed using the maximum single application rate and the LD_{50} values to calculate hazard quotients [*EPPO/OEPP, 2003: Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(2)). Bulletin OEPP/EPPO Bulletin 33: 141-145*] as follows:

$$\text{Hazard Quotient (HQ)} = \frac{\text{Maximum application rate [g formulation/ha]}}{\text{Acute } LD_{50} \text{ [µg formulation/bee]}}$$

HQs for honeybees were calculated for oral exposure and contact exposure to BAS 516 07 F. A HQ < 50 indicates low risk to honeybees in the field.

Table 10.3.1-5: Risk to honeybees from exposure to pyraclostrobin and BAS 516 07 F considering the worst-case application rate

Test substance	Application rate [g/ha]	Endpoint	LD_{50} [µg/bee]	Hazard quotient HQ	Trigger
Risk assessment on adult honeybees					
Pyraclostrobin	17	48 h oral	> 110	< 0.15	50
		48 h contact	> 100	< 0.17	
BAS 516 07 F [1)]	250	48 h oral	> 258.7	< 0.97	
		48 h contact	> 299.4	< 0.84	

1) Tests were carried out with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).

The calculated HQs for acute oral and acute contact exposure of honeybees to pyraclostrobin and BAS 516 07 F are clearly below the trigger value of 50. Therefore, low risk to honeybees is expected from the application of BAS 516 07 F in potatoes.

Additionally, no unacceptable effects in queen development, queen survival and adult worker bee health were observed using 400 ppm Pristine treated pollen (which is according to analysis equivalent to 22 ± 2 ppm pyraclostrobin treated pollen). Pristine is a formulation similar to BAS 516 07 F (both WG formulations containing pyraclostrobin and boscalid). The study summary is shown in M-CP 10.3.1.3 below. As currently no risk assessment scheme exists, these results are presented as additional information.

In an acute toxicity study on adult bumblebees with pyraclostrobin, the oral LD_{50} after 96 h was ≥ 97.2 µg a.s./bumblebee, while the contact LD_{50} after 96 h was > 100 µg a.s./bumblebee. As currently no risk assessment scheme exists, these results are presented as additional information.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

Report:	CP 10.3.1.1.1/1 Kling A., 2001a Assessment of side effects of BAS 516 00 F to the honey bee, Apis mellifera L. in the laboratory 2001/1000868
Guidelines:	OECD 213, OECD 214
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

In a limit test, young adult worker bees (*Apis mellifera* L.) were exposed to BAS 516 00 F. The toxicity of the test product was determined in an oral test at a nominal concentration of 100 µg a.s./bee (based upon the sum of the active substances, this corresponds to a dose of 299.4 µg product/bee). Additionally, honeybees were treated with dimethoate as reference item at concentrations ranging from 0.08 to 0.25 µg a.s./bee or with aqueous syrup solution as a control. The test was conducted with 5 replicates per test concentration, each of the test cages contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

In the oral toxicity test the maximum test concentration (299.4 µg/bee) corresponded to an actual intake of 258.7 µg/per bee. At this concentration a corrected mortality of 22.0% was observed after 48 hours. No abnormal behavior like discoordinated movements and apathy were observed over the entire time of the experiment.

Toxicity of BAS 516 00 F was tested in an acute oral toxicity test on honeybees. The LD_{50} (48 h) was > 258.7 µg BAS 516 00 F/bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).

Test species: Honeybee (*Apis mellifera* L.); working bees; age: approx. 22 - 32 days, deriving from a healthy colony which descended from a breeding line of a beekeeper in Rheinland-Pfalz, Germany.

B. STUDY DESIGN

Test design: Limit test; acute oral toxicity test; duration 48 h; 5 replicates per test concentration, each consisting of 10 bees in one cage; assessment of mortality after 4, 24 and 48 hours.

Endpoint: LD_{50}.

Reference item: BAS 152 11 I (dimethoate 400 g/L).

Test concentrations: Control, 100 µg a.s./bee (based upon the sum of the active substances on the nominal contents, this corresponds to a dose of 299.4 µg product/bee); 4 concentrations of the reference item

Test conditions: Temperature: 24.0°C - 26.0°C; relative humidity: 50% - 66%; photoperiod: 24 h darkness.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

In the oral toxicity test the maximum test concentration (299.4 µg/bee) corresponded to an actual intake of 258.7 µg/per bee. At this concentration a corrected mortality of 22.0% was observed after 48 hours. The results are summarized in Table 10.3.1.1.1-1.

Table 10.3.1.1.1-1: Toxicity of BAS 516 00 F to honeybees (*Apis mellifera*) in an oral toxicity test

Treatment [µg /bee]	Uptake of test item [µg /bee]	Corrected mortality [1] [%]	
		24 h	48 h
299.4 [2]	258.7	20	22
		Endpoints [µg/bee]	
LD_{50} (48 h)		> 258.7	

1) According to Schneider-Orelli (1947).
2) Equivalent to 100 µg a.s./bee (sum of both active substances).

III. CONCLUSION

Toxicity of BAS 516 00 F was tested in an acute contact toxicity test on honeybees. The LD_{50} (48 h) was > 258.7 µg BAS 516 00 F/bee.

CP 10.3.1.1.2 Acute contact toxicity to bees

Report:	CP 10.3.1.1.2/1 Kling A., 2001a Assessment of side effects of BAS 516 00 F to the honey bee, Apis mellifera L. in the laboratory 2001/1000868
Guidelines:	OECD 213, OECD 214
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

In a limit test, young adult worker bees (*Apis mellifera* L.) were exposed to BAS 516 00 F. The toxicity of the test product was determined in a contact test at a concentration of 100 µg a.s./bee (based upon the sum of the active substances, this corresponds to a dose of 299.4 µg product/bee). Additionally, honeybees were treated with dimethoate as reference item at concentrations ranging from 0.11 to 0.27 µg a.s./bee or with tap water as a control. The test was conducted with 5 replicates per test concentration, each of the test cages contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

In the contact toxicity test no corrected mortality was observed at the concentration of 299.4 µg/bee after 48 hours. No abnormal behavior like discoordinated movements and apathy were observed over the entire time of the experiment.

Toxicity of BAS 516 00 F was tested in an acute contact toxicity test on honeybees. The LD_{50} (48 h) was > 299.4 µg BAS 516 00 F/bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).

Test species: Honeybee (*Apis mellifera* L.); working bees; age: approx. 22 - 32 days, deriving from a healthy colony which descended from a breeding line of a beekeeper in Rheinland-Pfalz, Germany.

B. STUDY DESIGN

Test design: Limit test; acute contact toxicity test; duration 48 h; 5 replicates per test concentration, each consisting of 10 bees in one cage; assessment of mortality after 4, 24 and 48 hours.

Endpoint: LD_{50}.

Reference item: BAS 152 11 I (dimethoate 400 g/L).

Test concentrations: Control, 100 µg a.s./bee (based upon the sum of the active substances on the nominal contents, this corresponds to a dose of 299.4 µg product/bee);4 concentrations of the reference item.

Test conditions: Temperature: 24.0°C - 26.0°C; relative humidity: 50% - 66%; photoperiod: 24 h darkness.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

In the contact toxicity test no corrected mortality was observed at the concentration of 299.4 µg/bee after 48 hours. The results are summarized in Table 10.3.1.1.2-1.

Table 10.3.1.1.2-1: Toxicity of BAS 516 00 F to honeybees (*Apis mellifera*) in a contact toxicity test

Treatment [µg /bee]	Corrected mortality [1] [%]	
	24 h	48 h
299.4 [2]	0	0
Endpoints [µg/bee]		
LD_{50} (48 h)	> 299.4	

[1] According to Schneider-Orelli (1947).
[2] Equivalent to 100 µg a.s./bee (sum of both active substances).

III. CONCLUSION

Toxicity of BAS 516 00 F was tested in an acute contact toxicity test on honeybees. The LD_{50} (48 h) was > 299.4 µg BAS 516 00 F/bee.

CP 10.3.1.2 Chronic toxicity to bees

According to SANCO/10606/2014 (May 16th, 2014) a study on chronic toxicity on honeybees is not required for AIR 3 substances submitted before January 1st, 2015. In addition, this data point is covered by M-CA 8.3.1.3

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

From the performed literature search, the following peer-reviewed scientific study on queen-rearing success in honeybees (*Apis mellifera* L.) was considered relevant and reliable (with restrictions; RI 2) for the terrestrial risk assessment of pyraclostrobin. Thus, it is presented as additional information for the terrestrial risk assessment and has not been evaluated previously.

Report:	CP 10.3.1.3/1 Johnson R.M., Percel E.G., 2013a Effect of a fungicide and spray adjuvant on queen-rearing success in honey bees (Hymenoptera: Apidae) 2013/1416303
Guidelines:	<none>
GLP:	no

Executive Summary

The aim of this study was to test the effects of the fungicide Pristine (12.8% pyraclostrobin and 25.2% boscalid) and adjuvant on honeybee (*Apis mellifera*) queen development and survival. A new bioassay was developed, in which queens are reared in closed swarm boxes for 4 d, until capping, with nurse bees fed exclusively on artificially contaminated pollen. Pollen was treated with four concentrations of formulated Pristine (0.4, 4, 40, and 400 ppm). Additionally, the bees were treated with diflubenzuron (100 ppm) as reference item and with water as control.

No effect of the test item treatment was observed in relation to changes in the weight of the pollen frame. Pesticide treatment appeared to have no effect on syrup removal.
No changes in nurse bee activity or behavior were noted in any of the treatments. Considering queen development, there was no statistically significant difference between the survival rates in the Pristine treatments compared to the control. The capping rate of grafted larvae was also not statistically significantly different between the test item treatments compared to the control. The mean thoracic width of the control was 4.54 mm compared to 4.47, 4.52, 4.49 and 4.54 mm in the test item treatments with concentrations of 0.4, 4, 40 and 400 ppm, respectively. No statistically significant differences were observed up to and including the highest concentration tested.
Chemical analysis of four pollen samples treated with 400 ppm Pristine taken from pollen frames after 4 d in the swarm box contained 22 ± 2 ppm pyraclostrobin and 47 ± 5 ppm boscalid.

In a toxicity laboratory study with pyraclostrobin (tested as Pristine containing 12.8% pyraclostrobin and 25.2% boscalid and being similar to BAS 516 07 F) on honeybees, no unacceptable effects in queen development, queen survival and adult worker bee health were observed up to and including 400 ppm Pristine treated pollen, the highest concentration tested (according to analysis equivalent to 22 ± 2 ppm pyraclostrobin treated pollen).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Formulated Pristine (25.2% boscalid and 12.8% pyraclostrobin; BASF, Florham Park, NJ). Some information on the fungicide (batch no.; density, etc.) is missing in the study report.

Adjuvant: Break-Thru (100% Polyether-Polymethylsiloxane-Copolymer; Evonik Goldschmidt, Hopewell, VA).

B. STUDY DESIGN

Test species: Honeybee (*Apis mellifera*), young worker larvae (<24 h) were grafted from a mix of Italian and Carniolan breeder colonies and checked for queen emergence. No detailed information is given on the source of the bees.

Test design: To determine the potential effects that the fungicide Pristine has on immature queen development and survival, queens of *Apis mellifera* were reared inside closed swarm boxes provisioned with treated pollen. In total, 1320 larvae were grafted into swarm boxes containing pollen treated with the following: Water control, 4 concentrations of the test item Pristine, one concentration of the adjuvant, one concentration of the adjuvant + Pristine and one concentration of the reference item. Approximately 2.27 kg of nurse bees, measured by equivalent volume, were placed in the prepared swarm boxes. Young worker larvae (<24 h) were grafted into wax queen cups affixed to wooden bars and fitted into special grafting frames holding a total of 60 larvae. Grafting frames were quickly transferred to the middle frame position in prepared swarm boxes. As many as 360 grafts were completed within 1 h by three expert grafters working side by side. After 4 d in the closed swarm boxes, grafting frames were carefully removed and nurse bees were brushed off. Capped queen cells were counted, and frames with queen cells were transferred to an incubator. Three queen cells from each frame were opened, and the royal jelly was removed for chemical analysis. Pollen and syrup consumption in each swarm box was determined by comparing the starting and ending weight of the pollen frame and division board feeder. Dead bees remaining in the swarm box were collected and weighed. After 7 d in the incubator, queen cells were cut from the grafting bars and placed inside individually labeled plastic hair roller queen cages. Caged queen cells were returned to the incubator and checked for queen emergence 4 d later. Emerged queens were counted, and examined for abnormalities. Unemerged queen cells were dissected, and the age at which the unemerged queen died was estimated based on morphological characteristics. Queen quality was assessed by measuring the width of the thorax with a digital caliper. Fresh royal jelly and treated pollen were both collected for chemical-residue testing on the day capped queen cells were removed from swarm boxes.

Quantification of pyraclostrobin and boscalid concentrations in samples exposed to Pristine was accomplished at the USDA-AMS-NSL and a modified version of the QuEChERS method was used to extract the pesticide residues.

Endpoints: Queen development and survival, adult worker bee health.

Reference item: Pond Care Dimilin (0.10% diflubenzuron; Aquarium Pharmaceuticals, Inc., Chalfont, PA).

Test concentrations: Water control; test item: 0.4, 4.0, 40 and 400 ppm Pristine in pollen; reference item: 100 ppm diflubenzuron; adjuvant in combination with test item: 200 ppm Break-Thru and 400 ppm Pristine.

Test conditions: Nurse bees: placed in a dark well-ventilated area in prepared swarm boxes.
Capped and caged queen cells: placed in an incubator (temperature: 34°C; relative humidity: 80%).
Royal jelly and treated pollen storage: temperature: -20°C in a glass vial for chemical analysis.

Statistics: Descriptive statistics; A x^2 goodness-of-fit test was used to determine treatment effects on the survival of queen larvae to capping and adult emergence. One-way analysis of variance (ANOVA) was used to determine treatment differences in food consumption, dead bee accumulation, and queen thoracic width. Tukey's honestly significant difference (HSD) test was used for pairwise comparisons between treatments.

II. RESULTS AND DISCUSSION

In this subsection the focus is on the effects of Pristine on *A. mellifera* larvae, queens and adults. The results of the adjuvant and Pristine in combination with the adjuvant are not summarized here.

Pollen and syrup consumption
No effect of the test item treatment was observed in relation to changes in the weight of the pollen frame. Pesticide treatment appeared to have no effect on syrup removal.

Adult worker bee health
No changes in nurse bee activity or behavior were noted in any of the treatments.

Queen development
There was no statistically significant difference between the survival rates in the Pristine treatments compared to the control. The capping rate of grafted larvae was also not statistically significantly different between the test item treatments compared to the control.

The mean thoracic width of the control was 4.54 mm compared to 4.47, 4.52, 4.49 and 4.54 mm in the test item treatments at concentrations of 0.4, 4, 40 and 400 ppm, respectively. No statistically significant differences were observed up to and including the highest concentration tested. The results are summarized in Table 10.3.1.3-1.

Chemical analysis
Based on the ratio of active substances in pollen treated with this formulated fungicide at the 400 ppm level would be expected to contain 101 ppm boscalid and 51 ppm pyraclostrobin. Chemical analysis of four pollen samples treated with 400 ppm Pristine taken from pollen frames after 4 d in the swarm box contained 47 ± 5 ppm boscalid and 22 ± 2 ppm pyraclostrobin, approximately half the expected concentration. Three of four royal jelly samples taken from 400 ppm Pristine treatments contained detectable levels of pyraclostrobin (47-52 ppb), but boscalid was not detected in any royal jelly sample.

Table 10.3.1.3-1: Comparisons of thoracic width of queens reared with Pristine treated pollen

Treatment	Concentration (ppm)	n	Thoracic width (mm)
Water control	--	159	4.54
Pristine	0.4	76	4.47
Pristine	4.0	71	4.52
Pristine	40	65	4.49
Pristine	400	168	4.54

The reference item diflubenzuron showed statistically significant differences in queen development and survival. No diflubenzuron-treated queens survived to emergence; on dissection, all were putrefied black larvae that appeared to have died soon after capping.

III. CONCLUSION

In a toxicity laboratory study with pyraclostrobin (tested as Pristine containing 25.2% boscalid and 12.8% pyraclostrobin and being similar to BAS 516 07 F) on honeybees, no unacceptable effects in queen development, queen survival and adult worker bee health were observed up to and including 400 ppm Pristine treated pollen, the highest concentration tested (according to analysis equivalent to 22 ± 2 ppm pyraclostrobin treated pollen).

CP 10.3.1.4 Sub-lethal effects

As BAS 516 07 F does not pose an unacceptable risk to honeybees, further tests are not necessary.

CP 10.3.1.5 Cage and tunnel tests

As BAS 516 07 F does not pose an unacceptable risk to honeybees, further tests are not necessary.

CP 10.3.1.6 Field tests with honeybees

As BAS 516 07 F does not pose an unacceptable risk to honeybees, further tests are not necessary.

CP 10.3.2 Effects on non-target arthropods other than bees

The new representative formulation was not evaluated within a previous Annex I inclusion process. Consequently, no EU agreed endpoints are available for non-target arthropods. Endpoints from new studies with the new representative formulation BAS 516 07 F are used for the risk assessments (see Table 10.3.2-1).

Table 10.3.2-1: Ecotoxicological endpoints for non-target arthropods

Test substance	Test species	EU agreed endpoints	Endpoints used in risk assessment
Tier I			
BAS 516 07 F [1)]	*Typhlodromus pyri*	--	LR_{50} > 5.4 kg/ha
	Aphidius rhopalosiphi	--	LR_{50} > 5.4 kg/ha
Tier II			
BAS 516 07 F [1)]	*Chrysoperla carnea* laboratory test	--	LR_{50} > 3.6 kg/ha No unacceptable effects on reproduction up to 3.6 kg/ha
	Chrysoperla carnea extended laboratory test	--	LR_{50} > 2 x 1.8 kg/ha No unacceptable effects on reproduction up to 2 x 1.8 kg /ha
	Poecilus cupreus laboratory test	--	LR_{50} > 3.6kg /ha No unacceptable effects on reproduction up to 3.6 kg /ha
	Aleochara bilineata laboratory test	--	No unacceptable effects on reproduction up to 3.6 kg/ha
	Pardosa spec laboratory test	--	LR_{50} > 3.6 kg/ha No unacceptable effects on reproduction up to 3.6 kg/ha
Higher Tier			
BAS 516 07 F [1)]	*Typhlodromus pyri* field studies	--	No unacceptable effects up to 5 x 0.75 kg/ha

[1)] The toxicity tests on non-target arthropods were carried out with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).

Overall summary

Data on BAS 516 07 F and its potential effects on non-target arthropods are evaluated and appropriate risk assessments are provided based on the already registered use pattern in potatoes (critical GAP: maximum 4 x 17 g pyraclostrobin per ha). Tests with the formulation have been carried out with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).

The endpoints of non-target arthropods to BAS 516 07 F are shown in Table 10.3.2-2.

Table 10.3.2-2: Effects on other arthropod species

Test substance [1)]	Worst-case use pattern	Species / Life stage	Test type	Endpoint [kg/ha]	PER in-field [kg/ha]	PER off-field [kg/ha]	HQ in-field	HQ off-field
Tier I								
BAS 516 07 F	4 x 0.25 kg/ha	*T. pyri* protonymphs	Laboratory test, artificial substrate, 2D	LR_{50} > 5.4	0.675	0.0012	< 0.13	< 0.002
		A. rhopalosiphi adults		LR_{50} > 5.4	0.675	0.0012	< 0.13	< 0.002
Tier II								
BAS 516 07 F	4 x 0.25 kg/ha	*C. carnea* larvae	Laboratory test, artificial substrate, 2D	LR_{50} > 3.6	0.675	0.0012 [2)]	Endpoint ≥ PER → acceptable risk	
		C. carnea larvae	Extended laboratory test, natural substrate, 2D	LR_{50} > 2 x 1.8 ER_{50} > 2 x 1.8	0.675	0.0012 [2)]	Endpoint ≥ PER → acceptable risk	
		P. cupreus adults	Laboratory test, artificial substrate, 2D	LR_{50} > 3.6 ER_{50} > 3.6	0.675	0.012 [2)]	Endpoint ≥ PER → acceptable risk	
		A. bilineata adults	Laboratory test, artificial substrate, 2D	ER_{50} > 3.6	0.675	0.0012 [2)]	Endpoint ≥ PER → acceptable risk	
		Pardosa spec. adults	Laboratory test, artificial substrate, 2D	LR_{50} > 3.6 ER_{50} > 3.6	0.675	0.012 [2)]	Endpoint ≥ PER → acceptable risk	
Higher Tier								
BAS 516 07 F	4 x 0.25 kg/ha	Predatory mites all live stages	Field tests, orchards, 3D (3 studies)	No unacceptable effects up to 5 x 0.75 kg/ha	Max. intended use = 4 x 0.25 kg/ha		Endpoint ≥ intended application rate → acceptable risk	

PER = predicted environmental rate, HQ = Hazard Quotient

1) The toxicity tests on non-target arthropods were carried out with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).

2) Standard 5-fold uncertainty (correction) factor should be included in the calculation to cover the inter-species variability in sensitivity of off-field non-target arthropod species. However, as additional species are tested, the uncertainty is reduced and no additional safety factor was applied.

The calculated hazard quotient of the first tier risk assessment indicated no in-field and no off-field risk for both tested species. However, in order to have additional information for the risk assessment, Tier II tests were carried out with *C. carnea*, *A. bilineata*, *P. cupreus* and *Pardosa* spec. For these species the Tier II risk assessment indicated no in-field and no off-field risk, too. Additionally, three field studies were carried out to observe effects on natural occurring populations of predatory mites. No unacceptable effects on population development of predatory mites were observed up to 5 x 0.75 kg/ha. Therefore, a potential in-field and off-field risk can be excluded for BAS 516 07 F applied to potatoes at 4 x 0.25 kg/ha.

Overall conclusion
Taking into account all available data, it can be concluded that low risk for non-target arthropods is expected from the use of BAS 516 07 F in potatoes at application rates of up to 4 x 0.25 kg/ha. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

Toxicity

The toxicity of BAS 516 07 F to non-target arthropods has been investigated. The testing and risk assessment strategy used follow the approach recommended in the ESCORT 2 guidance document [*Candolfi et al. (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' From the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000*], ESCORT 3 [*Alix, et al. (2012) 'Linking non-target arthropod testing and risk assessment with protection goals'*] and the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002).

The toxicity of BAS 516 07 F (tested as the minor change formulation BAS 516 00 F; the detailed composition of both products is given in Document JCP 1.4.1) to non-target arthropods has been investigated by carrying out Tier I tests on *Aphidius rhopalosiphi* and *Typhlodromus pyri* and Tier II tests on *Chrysoperla carnea*, *Poecilus cupreus*, *Aleochara bilineata* and *Pardosa* spec. These species are tested, in accordance with ESCORT 2 and 3, as representative non-target arthropods since they have been found to be particularly sensitive species, and therefore can be considered as indicators of potential effects to the most sensitive arthropods in the field. Furthermore, overall three field studies with predatory mites have been carried out with BAS 516 07 F (tested as the minor change formulation BAS 516 00 F; the detailed composition of both products is given in Document JCP 1.4.1). For convenience, the results of these studies are summarized in Table 10.3.2-3. Study summaries are provided below (see M-CP 10.3.2.1, M-CP 10.3.2.2 and M-CP 10.3.2.4).

Table 10.3.2-3: Summary of endpoints for BAS 516 07 F to non-target arthropods [1)]

Species	Exposed life stage	Study type	Application rate [kg/ha]	Corrected mortality [2)] [%]	Sublethal effects [%]	Reference (BASF DocID)
Tier I						
Typhlodromus pyri	Protonymphs	Laboratory test using artificial substrate	0.0667 0.200 0.600 1.80 5.40	1.1 -5.6 1.1 -7.8 5.6	n.d.	2001/1000880
			LR_{50} > 5.4 kg/ha			
Aphidius rhopalosiphi	Adults	Laboratory test using artificial substrate	0.450 0.900 1.80 3.60 5.40	-0.30 -5.57 -0.30 2.34 7.62	n.d.	2001/1001864
			LR_{50} > 5.4 kg/ha			
Tier II						
Chrysoperla carnea	Larvae	Laboratory test using artificial substrate	3.60	36.6	no effects	2001/1001860
			LR_{50} > 3.6 kg/ha			
Chrysoperla carnea	Larvae	Extended laboratory test using natural substrate	2 x 0.360 2 x 1.80	6.8 18.2	no effects no effects	2001/1001874
			LR_{50} > 2 x 1.8 kg/ha ER_{50} > 2 x 1.8 kg/ha			
Poecilus cupreus	Adults	Laboratory test using artificial substrate	3.60	0.0	2.7	2000/1012478
			LR_{50} > 3.6 kg/ha ER_{50} > 3.6 kg/ha			
Aleochara bilineata	Adults	Laboratory test using artificial substrate	3.60	n.d.	8.3	2001/1001863
			ER_{50} > 3.6 kg/ha			
Pardosa spec.	Adults	Laboratory test using artificial substrate	3.60	2.9	3.0	2001/1005964
			LR_{50} > 3.6 kg/ha ER_{50} > 3.6 kg/ha			

n.d. = not determined

1) The toxicity tests on non-target arthropods were carried out with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).

2) Negative values indicate an increase, positive a decrease relative to the control.

Table 10.3.2-4: BAS 516 07 F – effects on terrestrial arthropods in field tests

Species	Crop	Application rate [1] [kg BAS 516 07 F/ha]	Sampling time [2]	Effects [3] [%]	Reference (BASF DocID)
predatory mites, natural field population	stone fruit orchard in Germany	5 x 0.75	8 days after 3rd application 7 days before 4th application 9 days after 5th application 29 days after 5th application	15 20 19 -87	2001/1000905
predatory mites, natural field population	apple orchard in Germany	5 x 0.75	5 days after 1st application 6 days after 3rd application 2 days before 4th application 6 days after 5th application 4 weeks after 5th application	25.3 47.2 -12.5 32.6 -28.5	2001/1001866
predatory mites	stone fruit orchard in Switzerland	5 x 0.75	6 days after 5th application 26 days after 5th application	6.9 1.8	2001/1001870

1) The toxicity tests on non-target arthropods were carried out with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).
2) The effect on mite population according to Abbott (1925) was only calculated for dates in which mite density was higher than 0.1 mites / leaf in the control.
3) Effects calculated according to Abbott (1925). Negative values indicate a higher population development compared to the control.

Exposure

Table 10.3.2-5: Critical use pattern of BAS 516 07 F

Crop	Application time (BBCH code)	Number of applications	Interval [d]	Application rate per treatment		
				Boscalid [g a.s./ha]	Pyraclostrobin [g a.s./ha]	BAS 516 07 F [g/ha]
potatoes	51 - 89	4	10	67	17	250

In-field exposure

Non-target arthropods inhabiting the crop can be exposed to residues of BAS 516 07 F by direct contact, either as a result of overspray or through contact with residues on plants and soil or in food items. BAS 516 07 F is applied at a maximum rate of 4 x 0.25 kg/ha in potatoes.

The in-field exposure (predicted environmental rate, PER) is calculated according to ESCORT 2 using the following equation:

$PER_{in\text{-}field}$ = Application rate [kg/ha] x MAF

The MAF is a generic multiple application factor, which is used to take into account the potential build-up of applied substances between applications based on the application interval, DT_{50} value and number of applications. Default foliar and soil MAF values following multiple applications are given in the ESCORT 2 Guidance Document. For 4 applications, the default MAF of 2.7 (foliar) and 3.4 (soil) is considered, respectively. However, as a pre-emergence or early post-emergence application is not foreseen according to the proposed use pattern, the PER (soil) will not be used in the following risk assessment.

The maximum predicted environmental rate (PER) occurring within the field after application of BAS 516 07 F at the maximum application rate are presented in Table 10.3.2-6.

Table 10.3.2-6: In-field PER values for application of BAS 516 07 F

Substance	Worst-case application rate	$PER_{in\text{-}field}$ (foliar) [kg/ha]
BAS 516 07 F	4 x 0.25 kg/ha	0.675

Off-field exposure

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods living in off-field areas to BAS 516 07 F will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure *via* soil residues in off-field areas was not considered. Off-field foliar PER values were calculated from in-field foliar PERs in conjunction with drift values published by the BBA [*90th percentile drift according to BBA (2000): Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden*] as shown in the following equation:

$$PER_{off\text{-}field} = \frac{\text{maximum } PER_{in\text{-}field} \times (\%\ \text{drift}/100)}{\text{vegetation distribution factor}}$$

The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 10 is recommended by ESCORT 2. For 3-dimensional studies, i.e. where spray treatment is applied onto whole plants, the dilution factor of 10 is not used, as any dilution over the 3-dimensional vegetation surface is accounted for in the study design. The correction factor from the ESCORT 2 formula for off-field exposure calculation is considered for in the respective risk assessment.

The drift value for four applications at 1 m distance in field crops (i.e. potatoes) is 1.85% of the application rate (74^{h} percentile drift). The drift factor (% drift/100) is therefore 1.85/100 = 0.0185. The resulting PER off-field values are shown in Table 10.3.2-7.

Table 10.3.2-7: Off-field Predicted Environmental Rates (PER)

Study type	Maximum $PER_{in\text{-}field}$ [kg/ha]	drift factor [% drift/100]	Vegetation distribution factor	$PER_{off\text{-}field}$ [kg/ha]
2D exposure scenario	0.675	0.0185	10	0.0012
3D exposure scenario	0.675	0.0185	--	0.012

Risk assessment for other non-target arthropods

The risk to non-target arthropods is assessed using the approach recommended in the published ESCORT 2 document [*Candolfi et al. 2001*] and the EC Guidance Document on Terrestrial Ecotoxicology (SANCO/10329, 17 October 2002).

In-field risk assessment (Tier I)

The potential risk of BAS 516 07 F to in-field non-target arthropods was assessed by calculation of the hazard quotient (HQ) using the $PER_{in\text{-}field}$ and the lowest lethal rate (LR_{50}) values according to the following equation:

$$HQ_{in\text{-}field} = \frac{PER_{in\text{-}field}\ [kg/ha]}{LR_{50}\ [kg/ha]}$$

The HQ trigger for Tier I laboratory studies is 2. The resulting $HQ_{in\text{-}field}$ values are presented in Table 10.3.2-8.

Table 10.3.2-8: In-field HQs for non-target arthropods

Species	LR_{50} [kg/ha]	In-field		Trigger value
		PER [kg/ha]	HQ	
T. pyri Tier I, 2D exposure scenario	> 5.4	0.675	< 0.13	2
A. rhopasoliphi Tier I, 2D exposure scenario	> 5.4	0.675	< 0.13	

The in-field HQ values for the representative test species *T. pyri* and *A. rhopalosiphi* are below the ESCORT 2 trigger value of 2. Thus, the potential in-field risk to non-target arthropods exposed to BAS 516 07 F is considered to be low at this level of testing.

In-field risk assessment (Tier II)

Both $HQ_{in\text{-}field}$ for *T. pyri* and *A. rhopalosiphi* were below the trigger value of 2, therefore no further assessment is necessary. However, a summary of a Tier II in-field evaluation is presented in Table 10.3.2-9.

Table 10.3.2-9: In-field Tier II and higher tier risk assessment for non-target arthropods

Species	Endpoints [kg/ha]	$PER_{in\text{-}field}$ [kg/ha]	Trigger
Tier II			
C. carnea, laboratory test, artificial substrate, 2D exposure scenario	LR_{50} > 3.6	0.675	Endpoint ≥ PER → acceptable risk
C. carnea, extended laboratory test, natural substrate, 2D exposure scenario	LR_{50} > 2 x 1.8 ER_{50} > 2 x 1.8	0.675	Endpoint ≥ PER → acceptable risk
P. cupreus, laboratory test, artificial substrate, 2D exposure scenario	LR_{50} > 3.6 ER_{50} > 3.6	0.675	Endpoint ≥ PER → acceptable risk
A. bilineata, laboratory test, artificial substrate, 2D exposure scenario	ER_{50} > 3.6	0.675	Endpoint ≥ PER → acceptable risk
Pardosa spec., extended laboratory test, natural substrate, 2D exposure scenario	LR_{50} > 3.6 ER_{50} > 3.6	0.675	Endpoint ≥ PER → acceptable risk
Higher Tier			
Predatory mites, 3D exposure scenario, overall 3 field studies	No unacceptable effects up to 5 x 0.75 kg/ha	Max. intended use = 4 x 0.25 kg/ha	Endpoint ≥ intended application rate → acceptable risk

Chrysoperla carnea:
The rate of 3.6 kg BAS 516 00 F/ha, at which no unacceptable effects on survival were observed in a worst-case laboratory, exceeded the in-field predicted environmental rate (PER) of 0.675 kg/ha. Additionally, the rate of 2 x 1.8 kg/ha, at which no unacceptable effects on survival and reproduction were observed in an extended laboratory test, exceeded the in-field predicted environmental rate (PER) of 0.675 kg/ha, too. Therefore, no risk or unacceptable effects are expected for *Chrysoperla carnea* resulting from the intended use of BAS 516 07 F considering in-field habitats.

Poecilus cupreus:
The rate of 3.6 kg BAS 516 00 F/ha, at which no unacceptable effects on survival and feeding capacity were observed, exceeded the in-field predicted environmental rate (PER) of 0.675 kg/ha. Therefore, no risk or unacceptable effects are expected for *Poecilus cupreus* resulting from the intended use of BAS 516 07 F considering in-field habitats.

Aleochara bilineata:
The rate of 3.6 kg BAS 516 00 F/ha, at which no unacceptable effects on reproduction were observed, exceeded the in-field predicted environmental rate (PER) of 0.675 kg/ha. Therefore, no risk or unacceptable effects are expected for *A. bilineata* resulting from the intended use of BAS 516 07 F considering in-field habitats.

Pardosa spec.:
The rate of 3.6 kg BAS 516 00 F/ha, at which no unacceptable effects on survival and feeding capacity were observed, exceeded the in-field predicted environmental rate (PER) of 0.675 kg/ha. Therefore, no risk or unacceptable effects are expected for *Pardosa* spec. resulting from the intended use of BAS 516 07 F considering in-field habitats.

Predatory mites:
To investigate short- and long-term effects of BAS 516 07 F under normal agricultural conditions, three field studies were carried out with the minor change formulation BAS 516 00 F in order to observe effects on natural occurring populations of predatory mites under field conditions.

The field studies were conducted in orchards at different locations in Germany and in Switzerland. BAS 516 00 F was applied at 5 x 0.75 kg/ha, resulting in a total amount of 3.75 kg/ha BAS 516 07 F.

The population density of predatory mites was determined by counting the total number of mites on leaf or flower bud samples. No unacceptable effects on predatory mite populations occurred during the studies and 4 weeks after the last application. This clearly indicates that no unacceptable effects on natural populations occurred at a rate up to and including 5 x 0.75 kg/ha BAS 516 00 F. This rate exceeds the maximum intended application rate of 4 x 0.25 kg/ha BAS 516 07 F. Therefore, no risk or unacceptable effects are expected for predatory mites resulting from the intended use of BAS 516 07 F considering in-field habitats, too.

Off-field risk assessment (Tier I)

In order to assess the potential risk of BAS 516 07 F to off-field non-target arthropods, the predicted environmental rate (Table 10.3.2-7) is compared with the toxicity endpoints according to the following formula:

$$HQ_{off\text{-}field} = \frac{PER_{off\text{-}field}\ [kg/ha]}{LR_{50}\ [kg/ha]} \times \text{Correction factor}$$

The HQ trigger value for Tier I laboratory studies is 2. A correction factor of 10 for Tier I data is recommended by ESCORT 2 to account for extrapolation from testing of only 2 representative species to the expected higher species diversity in off-crop areas. The results are presented in Table 10.3.2-10.

Table 10.3.2-10: Off-field HQ values for non-target arthropods

Species	LR_{50} [kg/ha]	Off-field PER [kg/ha]	Correction factor	Off-field HQ	Trigger value
T. pyri Tier I, 2D exposure scenario	> 5.4	0.0012	10	< 0.002	2
A. rhopasoliphi Tier I, 2D exposure scenario	> 5.4	0.0012		< 0.002	

The off-field HQ values for *T. pyri* and *A. rhopalosiphi* are considerably below the trigger value of 2, indicating that BAS 516 07 F does not pose an unacceptable risk to non-target arthropods in off-field areas.

Off-field risk assessment (Tier II)

Both $HQ_{off\text{-}field}$ for the standard indicator species *T. pyri* and *A. rhopalosiphi* were clearly below the trigger value of 2. In addition, all endpoint from extended lab testing provide sufficient margin of safety to the anticipated off-field PER-values. Therefore, no further assessment is considered necessary.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Report:	CP 10.3.2.1/1 Buehler A., 2000a Effect of BAS 516 00 F on the ground dwelling predator Poecilus cupreus (Coleoptera, Carabidae) in a laboratory trial 2000/1012478
Guidelines:	BBA VI 23-2.1.8, Heimbach U. (1991) Auswirkungen von Pflanzenschutzmitteln auf Imagines von Poecilus cupreus L. als Vertreter der Familie Carabidae (= Laufkaefer) im Laboratorium
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a worst-case laboratory study, adults of *Poecilus cupreus* L (carabid beetle) were exposed to direct application of BAS 516 00 F at a rate of 3600 g/ha on quartz sand. Mortality and behavior were assessed 1 hour, 1, 2, 4, 7, 10 and 14 days after application. Food consumption was recorded 1, 2, 4, 7, 10 and 14 days after application.

BAS 516 00 F caused a mortality of 3.3% after 14 days of exposure. The mean mortality in the water control was 3.3%. So a corrected mortality of 0% was calculated for the test item treatment.

The mean number of consumed pupae per beetle from day 0 - 14 reached 5.67 in the test item and 5.83 in the control, respectively. This results in 97.3% food consumption in the test item relative to the control (effect on food consumption: 2.7%). No statistically significant differences between control and test item concerning food consumption could be shown.

Under worst-case laboratory conditions, no unacceptable effects on survival or feeding rate of *Poecilus cupreus* occurred at an application rate of 3600 g/ha BAS 516 00 F in 400 L water/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).
Test species:	*Poecilus cupreus* L. (carabid beetle), age: 7 weeks old; source: Bio Test Labor GmbH, Sagerheide, Germany.

B. STUDY DESIGN

Test design:	The beetles were exposed to direct application on moist quartz sand. Three treatments (test item, water treated control, reference item) were set up, with 5 test units each and 6 individuals (3 males and 3 females) per test unit. Assessment of mortality and behavior 1 hour, 1, 2, 4, 7, 10 and 14 days after application. Food consumption was recorded 1, 2, 4, 7, 10 and 14 days after application.
Endpoints:	Mortality, behavior and feeding rate of exposed beetles.
Reference item:	BAS 152 11 I (dimethoate, 400 g/L).
Test rates:	3600 g/ha BAS 516 00 F. The reference item was applied equivalent to 1.0 L/ha. The substances were applied in 400 L water/ha. The substances were sprayed upon the substrate and the beetles via laboratory spray applicator.
Test conditions:	Temperature: 18.5°C – 21.5°C; relative humidity: 67% – 85%; photoperiod: 16 h light : 8 h dark; light intensity: 660 lux – 1020 lux; food: fly pupae (*Delia antiqua*).
Statistics:	Descriptive statistics, Wilcoxon's Rank Sum Test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

BAS 516 00 F caused a mortality of 3.3% after 14 days of exposure. The mean mortality in the water control was 3.3%. So a corrected mortality of 0% was calculated for the test item treatment.

The mean number of consumed pupae per beetle from day 0 - 14 reached 5.67 in the test item and 5.83 in the control, respectively. This results in 97.3% food consumption in the test item relative to the control (effect on food consumption: 2.7%). No statistically significant differences between control and test item concerning food consumption could be shown (Wilcoxon's Rank Sum Test; $\alpha = 0.05$) (see Table 10.3.2.1-1).

Table 10.3.2.1-1: Effects on the carabid beetle (Poecilus cupreus) exposed to BAS 516 00 F in a laboratory trial

Treatment	Rate [1] [g/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Pupae consumed per beetle [4]	Effect on feeding behavior [%]
Control	--	3.3	--	5.83	--
BAS 516 00 F	3600	3.3	0	5.67 n s	2.7
Endpoint [g/ha]					
LR_{50}	> 3600				
Effects on feeding rate	> 3600				

1) Application rate in 400 L water/ha.
2) Mortality: mean of 5 replicates and 6 beetles/replicate, i.e. 30 beetles in total.
3) Corrected mortality according to Schneider-Orelli (1947)
4) mean number of eaten pupae over the entire observation period.
n.s.: not statistically significant compared to the control (Wilcoxon's Rank Sum Test; $\alpha = 0.05$).

The mean mortality in the reference item was 96.7%. This results in a corrected mortality of 96.6%. In the reference item 0.51 pupae per beetle were consumed. The food uptake was reduced by 91.3% when compared to the control.

III. CONCLUSION

Under worst-case laboratory conditions, no unacceptable effects on survival or feeding rate of *Poecilus cupreus* occurred at an application rate of 3600 g/ha BAS 516 00 F in 400 L water/ha.

Report:	CP 10.3.2.1/2 Adelberger I., 2001a BAS 516 00 F: Toxicity to the predatory mite, Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) in the laboratory 2001/1000880
Guidelines:	Louis F. Ufer A. (1995) Methodical improvements of standard laboratory tests for determining the side-effects of agrochemicals in predatory mites (Acari: Phytoseiidae) Anz. Schaedlingskde. Pflanzenschutz Umweltschutz 68 pp. 154-154, Overmeer W.P.J. (1988) Laboratory method for testing side-effects of pesticides on the predacious mites Typhlodromus pyri and Amblyseius potentillae (Acari: Phytoseiidae), Bluemel et al. (2000)
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

In a worst-case laboratory study, *Typhlodromus pyri* Scheuten (Acarina: Phytoseiidae) was exposed to dried residues of BAS 516 00 F on glass plates. Mortality was assessed 3 and 7 days after treatment. For the reproduction assessment, surviving mites from the control and from any treatment group displaying less than 50% corrected mortality were sexed and the number of eggs per females was recorded on day 10, 13 and 14.

The corrected mortality in the different treatment groups ranged between -7.8 and 5.6%. Therefore, the LR_{50} was determined to be > 5400 g/ha. The mean cumulative number of offspring per female was 7.5 in the control and ranged between 7.1 and 8.7 in the test item treatments. The differences between control and test item treatments were not statistically different. No significant effects on the survival and reproduction of *T. pyri* were observed in any of the BAS 516 00 F treatments.

The LR50 obtained under worst-case laboratory conditions on *Typhlodromus pyri* was determined to be > 5400 g/ha BAS 516 00 F in 200 L water/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).

Test species: *Typhlodromus pyri* (predatory mite), protonymphs approximately 1 day old; source: GAB Biotechnologie GmbH, Germany.

B. STUDY DESIGN

Test design: Exposure of the predatory mites was reached via air-dried residues on treated glass plates. Initial evaluation of the test item was done in a range finding test. Based on these results, a main test was designed. Seven treatments (five rates of test item, water control, reference item) were set up with 5 replicates each and each replicate containing 20 mites. The mortality was assessed 3 and 7 days after treatment. For the reproduction assessment, surviving mites from the control and from any treatment group displaying less than 50% corrected mortality were sexed and the number of eggs per females was recorded on day 10, 13 and 14.

Endpoints: LR_{50} (50% mortality of mites after exposure over 7 days), additionally reproduction capacity for three treatment groups displaying less than 50% corrected mortality.

Reference item: BAS 152 11 I (Dimethoate, 400 g/L).

Test rates:

Treatment	BAS 516 00 F [g/ha]
1	66.7
2	200
3	600
4	1800
5	5400

The reference item was applied at an application rate of 45 mL/ha. All substances were applied in 200 L water/ha. The substances were sprayed onto glass plates via laboratory spraying equipment and air dried afterwards.

Test conditions: Exposure: temperature: 23°C - 27°C; relative humidity: 55% - 88%; photoperiod: 16 h light : 8 h dark; light intensity: 3000 - 6000 lux; food: pollen of birch (*Betula pendula*) and bean (*Vicia faba*), mixture of tap water and deionized water (1:2).

Statistics: Descriptive statistics; ANOVA followed by Dunnett's test for mortality and reproduction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

The LR_{50}-value was determined to be > 5 400 g/ha BAS 516 00 F, the highest rate tested.
The mean cumulative number of offspring per female was 7.5 in the control and ranged between 7.1 and 8.7 in the test item treatments. The differences between control and test item treatments were not statistically different. No significant effects on the survival and reproduction of *T. pyri* were observed in any of the BAS 516 00 F treatments (Dunnett's test, $\alpha = 0.05$) (see Table 10.3.2.1-2).

Table 10.3.2.1-2: Effects on predatory mites (*Typhlodromus pyri*) exposed to BAS 516 00 F in a laboratory trial

Treatment	Rate[1)] [g/ha]	Mortality[2)] [%]	Corrected mortality[3)] [%]	Reproduction[4)] [eggs/female]	Effects on reproduction[5)] [%]
Control	--	10.0	--	7.5	--
BAS 516 00 F	66.7	11.0 n s	1.1	8.0 n s	-8
BAS 516 00 F	200.0	5.0 n s	-5.6	7.3 n s	3
BAS 516 00 F	600.0	11.0 n s	1.1	8.0 n s	-7
BAS 516 00 F	1800.0	3.0 n s	-7.8	7.1 n s	4
BAS 516 00 F	5400.0	15.0 n s	5.6	8.7 n s	-16
Endpoint [g/ha]					
LR_{50}	> 5400				

1) Application rate in 200 L water/ha
2) Mortality: dead and missing mites after 7 days of exposure to BAS 516 00 F on glass surface.
3) Corrected mortality according to Abbott.
4) Reproduction: mean cumulative number of offspring per female.
5) Negative values indicate an increase compared to the control.
n.s. = not statistically significant when compared to the control (Dunnett's test, $\alpha = 0.05$).

The reference item produced 89% mortality of exposed mites after 7 days.

III. CONCLUSION

The LR_{50} obtained under worst-case laboratory conditions on *Typhlodromus pyri* was determined to be > 5400 g/ha BAS 516 00 F in 200 L water/ha.

Report:	CP 10.3.2.1/3 Ufer A., 2001a Effect of BAS 516 00 F on the green lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) in a laboratory trial 2001/1001860
Guidelines:	Hassan S.A. Standard methods to test the side-effects of pesticides on natural enemies of insects and mites. Bull. OEPP 15 pp. 214-255 (1985), Vogt H. et al. (Draft version 02/2000) Laboratory method to test effects of plant protection products on larvae of Chrysoperla carnea (Neuroptera: Chrysopidae)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a worst-case laboratory study, larvae of *Chrysoperla carnea* (green lacewing) were exposed to dried residues of BAS 516 00 F on glass plates. Test organisms were exposed until pupae were transferred to preserving jars for development of adults. Mortality checks were carried out regularly until hatching of adult lacewings. In addition, for the control and the test item treatment, the reproduction, i.e. egg deposition and hatching rate, was determined over a 7 days period (2 assessments, 24 hours period each).

24 out of 50 juveniles (48%) died in the 3600 g/ha BAS 516 00 F treatment, compared to a mortality of 18% in the control. This resulted in a corrected mortality of 36.6%. A significant difference in mortality compared to the control was calculated for the test item group. In the 3600 g/ha BAS 516 00 F treatment, 37.4 eggs/female and day were produced and a hatching rate of 84.5% was observed. Slightly less eggs (i.e. 32.7 eggs/female and day) were produced in the water control, where a hatching rate of 93.2% was observed. As all values exceeded the mean number of 15 eggs/female, no treatment related effects on reproduction did occur. No effects on the hatching rate of the F1-generation could be observed, as all hatching rates were well above a mean value of 70%.

Under worst-case laboratory conditions, the LR_{50} for the foliage-dwelling predator *Chrysoperla carnea* can be estimated to be LR_{50} > 3600 g/ha BAS 516 00 F in 200 L water/ha. BAS 516 00 F caused no unacceptable effects on reproduction at an application rate of 3600 g/ha BAS 516 00 F in 200 L water/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).

Test species: *Chrysoperla carnea* (green lacewing), larvae (2-3 days old); source: BASF Agricultural Centre, Limburgerhof, Germany.

B. STUDY DESIGN

Test design: Exposure of the larvae was reached via air-dried residues on treated glass plates. Three treatments (test item, water control, reference item) were set up; each treatment including 50 individuals (= replicates). Test organisms were exposed until pupae were transferred to preserving jars for development of adults. Mortality checks were carried out regularly until hatching of adult lacewings. In addition, for the control and the test item treatment, the reproduction, i.e. egg deposition and hatching rate, was determined over a 7 days period (2 assessments, 24 hours period each).

Endpoints: Pre-imaginal mortality, number of produced eggs/female/day and hatching rate.

Reference item: BAS 152 11 I (dimethoate, 400 g/L).

Test rates: 3600 g/ha BAS 516 00 F. The reference item was applied at a rate of 40 mL/ha. All substances were applied in 200 L water/ha. The substances were sprayed via laboratory spray applicator on glass plates and air dried afterwards.

Test conditions: Temperature: 23.3°C - 26.6°C; relative humidity: 56.0% - 94.2%; photoperiod: 16 h light : 8 h dark; light intensity: larvae: 2100 lux - 4500 lux, pupae: 2100 lux – 6600 lux; food: larvae: fresh *Sitotroga cerealella* eggs, adults: artificial diet.

Statistics: Descriptive statistics; Fisher's Exact test for mortality($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

24 out of 50 juveniles (48%) died during their development to adults in the 3600 g/ha BAS 516 00 F treatment, compared to a mortality of 18% in the control. This resulted in a corrected mortality of 36.6%. A significant difference in mortality compared to the control was calculated for the test item group (Fisher's Exact test; $\alpha = 0.05$).
In the 3600 g/ha BAS 516 00 F treatment, 37.4 eggs/female and day were produced and a hatching rate of 84.5% was observed. Slightly less eggs (i.e. 32.7 eggs/female and day) were produced in the water control, where a hatching rate of 93.2% was observed. As all values exceeded the mean number of 15 eggs/female, no treatment related effects on reproduction did occur. No effects on the hatching rate of the F1-generation could be observed, as all hatching rates were well above a mean value of 70% (see Table 10.3.2.1-3).

Table 10.3.2.1-3: Effects on green lacewing (*Chrysoperla carnea*) exposed to BAS 516 00 F in a laboratory trial

Treatment	Rate[1)] [g/ha]	Mortality[2)] [%]	Corrected mortality[3)] [%]	Reproduction [eggs/female/day]	Hatching rate [%]
Control	--	18	--	32.7	93.2
BAS 516 00 F	3600	48 *	36.6	37.4	84.5
Endpoint [g/ha]					
LR_{50}	> 3600				

1) Application rate in 200 L deionized water/ha.
2) Mortality: percentage of individuals, which did not reach maturity.
3) Corrected mortality according to Schneider-Orelli (1947).
* Statistically significant differences compared to the control (Fisher`s Exact test, $\alpha = 0.05$).

The reference item BAS 152 11 I produced 72% mortality of exposed lacewings. This results in a corrected mortality of 65.9%.

III. CONCLUSION

Under worst-case laboratory conditions, the LR_{50} for the foliage-dwelling predator *Chrysoperla carnea* can be estimated to be LR_{50} > 3600 g/ha BAS 516 00 F in 200 L water/ha. BAS 516 00 F caused no unacceptable effects on reproduction at an application rate of 3600 g/ha BAS 516 00 F in 200 L water/ha.

Report:	CP 10.3.2.1/4 Schuld M., 2001a BAS 516 00 F: Toxicity to the aphid parasitoid, Aphidius rhopalosiphi (Hymenoptera, Braconidae) DESTEFANI-PEREZ in the laboratory 2001/1001864
Guidelines:	Polgar L. (1988) Guideline for testing the effect of pesticides on Aphidius rhopalosiphi Hal. Hym. Aphidiidae. IOBC/WPRS Bulletin XI/4. Meeting Working Group Pesticides and Beneficial Organisms pp. 29-34, Mead-Briggs M. (1992) A laboratory method for evaluating the side-effects of pesticides on the cereal aphid parasitoid Aphidius rhopalosiphi (Destefani-Perez). Aspects of Applied Biology 31 pp. 179-189, Mead-Briggs M. (2000) Draft guideline of the ring testing group
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

In a worst-case laboratory study, adults of *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) were exposed to dried residues of BAS 516 00 F on glass plates. Assessment of mortality was done approximately 30 min, 2, 24 and 48 hours after test initiation. For the reproduction assessment, 15 females from the control and from any treatment group displaying less than 50% corrected mortality (at maximum 3 rates) were individually confined over pots of untreated, aphid-infested barley plants for 24 h and then removed. The number of parasitized aphid mummies was recorded after 10 days.

The corrected mortality in the different treatment groups ranged between -5.57 and 7.62%. Therefore, the LR_{50} was determined to be > 5400 g/ha. Reproduction of *A. rhopalosiphi* was assessed in the 3 highest test item rates and in the control group. The mean number of mummies was 14.93 in the group treated with 1800 g/ha, 13.07 in the group treated with 3600 g/ha and 10.67 in the group treated with 5400 g/ha, compared to 20.67 in the control group, resulting in effects of 27.8, 36.8 and 48.4%, respectively.

The LR_{50} obtained under worst-case laboratory conditions on the parasitic wasp *Aphidius rhopalosiphi* was determined to be > 5400 g/ha BAS 516 00 F in 200 L water/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).

Test species: *Aphidius rhopalosiphi* (parasitoid), adults less than two days old; source: PK Nützlingszuchten, Welzheim, Germany.

B. STUDY DESIGN

Test design: Exposure of the parasitoids was reached via air-dried residues on treated glass plates. Initial evaluation of the test item was done in a range finding test. Based on these results, a main test was designed. Seven treatments (five rates of the test item, water control, reference item) were set up, with 4 replicates per treatment and each replicate containing 10 wasps. Assessment of mortality was done approximately 30 min, 2, 24 and 48 hours after test initiation. For the reproduction assessment, 15 females from the control and from any treatment group displaying less than 50% corrected mortality (at maximum 3 rates) were individually confined over pots of untreated, aphid-infested barley plants for 24 h and then removed. The number of parasitized aphid mummies was recorded after 10 days. Host: cereal aphids (*Rhopalosiphum padi*).

Endpoints: LR_{50} (50% mortality of wasps after exposure over 48 h), additionally reproduction capacity.

Reference item: BAS 152 11 I (dimethoate, 400 g/L).

Test rates:

Treatment	BAS 516 00 F [g/ha]
1	450
2	900
3	1800
4	3600
5	5400

The reference item was applied at an application rate of 0.85 mL/ha. All substances were applied in 200 L water/ha. The substances were sprayed onto glass plates via laboratory spraying equipment and air dried afterwards.

Test conditions: Exposure: temperature: 20.5°C – 23.5°C, relative humidity: 60% - 77%; continuous light; light intensity: approximately 1400 lux.

Reproduction: temperature: 19.0°C - 22.0°C, relative humidity: 61% - 70.5%; photoperiod: 16 hours light : 8 hours dark; light intensity: 2000 lux - 6000 lux.

Statistics: Descriptive statistics; ANOVA followed by Dunnett's test for mortality; Kruskal-Wallis Test followed by Dunn Test for reproduction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

The LR_{50}-value was determined to be > 5400 g BAS 516 00 F/ha, the highest rate tested.
Reproduction of *Aphidius rhopalosiphi* was assessed in the 3 highest test item rates and in the control group. The mean number of mummies was 14.93 in the group treated with 1800 g/ha, 13.07 in the group treated with 3600 g/ha and 10.67 in the group treated with 5400 g/ha, compared to 20.67 in the control group, resulting in effects of 27.8, 36.8 and 48.4%, respectively (see Table 10.3.2.1-4).

Table 10.3.2.1-4: Effects on parasitoids (*Aphidius rhopalosiphi)* exposed to BAS 516 00 F in a laboratory trial

Treatment	Rate[1)] [g/ha]	Mortality[2)] [%]	Corrected mortality[3)] [%]	Reproduction[4)] [mummies/female]	Effects on reproduction [%]
Control	--	5.28	--	20.67	--
BAS 516 00 F	450	5.00	-0.30	n. a.	n. a.
BAS 516 00 F	900	0.00	-5.57	n. a.	n. a.
BAS 516 00 F	1800	5.00	-0.30	14.93	27.8
BAS 516 00 F	3600	7.50	2.34	13.07	36.8
BAS 516 00 F	5400	12.50	7.62	10.67 *	48.4
Endpoint [g/ha]					
LR_{50}	> 5400				

1) Application rate in 200 L water/ha.
2) Mortality: after 48 hours of exposure to BAS 516 00 F.
3) Corrected mortality according to Schneider-Orelli (1947).
4) Reproduction: mean number of parasitized aphids/female.
* Statistically significant differences compared to the control (Dunnett's test for mortality; Kruskal-Wallis Test for reproduction, $\alpha = 0.05$).

The reference item caused 100% mortality of exposed wasps after 48 hours.

III. CONCLUSION

The LR_{50} obtained under worst-case laboratory conditions on the parasitic wasp *Aphidius rhopalosiphi* was determined to be > 5400 g/ha BAS 516 00 F in 200 L water/ha.

Report:	CP 10.3.2.1/5 Hermann P., 2001a BAS 516 00 F: Toxicity to the staphylinid beetle, Aleochara bilineata GYLL. (Coleoptera, Staphylinidae) in the laboratory 2001/1001863
Guidelines:	Moreth L. Naton E. (1992) Richtlinie zur Pruefung der Nebenwirkung von Pflanzenschutzmitteln auf Aleochara bilineata Gyll. (Col. - Staphylinidae) (erweiterter Laborversuch) IOBC/WPRS Bulletin 1992
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

In a worst-case laboratory study, adults of *Aleochara bilineata* (rove beetle) were exposed to quartz sand treated at a rate of 3600 g BAS 516 00 F/ha. Assessment of reproduction was carried out by counting the number of beetles emerged from the parasitized fly pupae starting approximately 4 weeks after application.

The mean number of emerged beetles in the test item treatment was 741. In the water control a mean of 808 beetles emerged. This difference proved to be statistically not significant

BAS 516 00 F caused no effects on the reproduction of the rove beetle *Aleochara bilineata* if applied at a rate of 3600 g/ha BAS 516 00 F in 400 L water /ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).

Test species: *Aleochara bilineata* (rove beetle), adults 2-5 days old; source: De groene Vlieg; NL- 3244 LG - Nieuwe Tonge.

B. STUDY DESIGN

Test design: Exposure of the beetles was reached via treated quartz sand; beetles were introduced into the test units immediately after treatment. Three treatments (test item, water control, and reference item) were set up with 4 replicates per treatment and 10 pairs (male + female) of beetles per replicate. Assessment of reproduction was carried out by counting the number of beetles emerged from the parasitized fly pupae starting approximately 4 weeks after application.

Endpoints: Reproduction of *Aleochara bilineata*.

Reference item: Dursban 480 (chlorpyrifos, 480 g/L).

Test rates: Control, 3600 g/ha BAS 516 00 F. The reference item was applied at a rate of 1.0 L/ha. The substances were applied in 400 L water/ha. The substances were sprayed via laboratory spray applicator on the sand surface.

Test conditions: Temperature: exposure: 18°C - 22°C, emergence of adults: 17.5°C - 22°C; photoperiod: 16 h light : 8 h dark; host organism: onion fly pupae (*Delia antiqua*).

Statistics: Descriptive statistics; ANOVA followed by Dunnett's test for mortality and reproduction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

The mean number of emerged beetles in the test item treatment was 741. In the water control in the mean 808 beetles emerged. This difference proved to be statistically not significant (Dunnett's test, $\alpha = 0.05$) (see Table 10.3.2.1-5).

Table 10.3.2.1-5: Effects on reproduction efficiency of rove beetles (*Aleochara bilineata*) exposed to BAS 516 00 F in a laboratory trial

Treatment	Rate[1)] [g/ha]	Reproduction [mean number of emerged beetles ± SD]	Effect on reproduction [%]
Control	--	808 ± 77.5	--
BAS 516 00 F	3600	741 ± 37.5	8.3
	Endpoint [g/ha]		
Effects on reproduction	> 3600		

1) Application rate in 400 L water/ha.

The reference item produced a reduction of reproduction of 99.9% compared to the control.

III. CONCLUSION

Under worst-case laboratory conditions BAS 516 00 F caused no unacceptable effects on the reproduction of the ground dwelling predator *Aleochara bilineata* at a rate of 3600 g/ha BAS 516 00 F in 400 L water /ha.

Report:	CP 10.3.2.1/6 Schmitzer S., 2001a Effects of BAS 516 00 F on the wolf spider Pardosa spec. in the laboratory 2001/1005964
Guidelines:	BBA draft guideline 1994, Heimbach U. et al. (2000 Draft) Method for testing effects of plant protection agents on spiders of the genus Pardosa (Araneae Lycosidae) under laboratory conditions
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

Executive Summary

In a worst-case laboratory study spiders of the genus *Pardosa* (wolf spiders: *Pardosa amentata* 93%, *P. proximata* 3%) were exposed to direct application of BAS 516 00 F at 3600 g/ha on quartz sand. After 2 hours and on day 1, 2, 3, 4, 7, 8, 10, 11 and 14 the number of dead or damaged individuals was assessed. The food consumption was assessed on day 1, 2, 3, 4, 8 and 11.
BAS 516 00 F had no effect on survival and food consumption of *Pardosa sp.* No test item related behavioral abnormalities were observed.

Under worst-case laboratory conditions, no unacceptable effects on survival or feeding rate of *Pardosa* spp. occurred at an application rate of 3600 g/ha BAS 516 00 F in 400 L water/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).
Test species:	Spider of the genus *Pardosa* (wolf spiders: *Pardosa amentata* 93%, *P. proximata* 3%), source: outdoor collected near Münster, Westfalen, Germany.

B. STUDY DESIGN

Test design:	The spiders were exposed to direct application on inert quartz sand. Three treatments (test item, water treated control, reference item) were set up with 34 replicates per treatment with 17 replicates containing 1 adult male and 17 replicates containing 1 adult female. After 2 hours and on day 1, 2, 3, 4, 7, 8, 10, 11 and 14 the number of dead or damaged individuals was assessed. The food consumption was assessed on day 1, 2, 3, 4, 8 and 11.
Endpoints:	Mortality, behavior and feeding rate of exposed spiders.
Reference item:	Perfekthion EC (dimethoate, 400 g/L).
Test rates:	Control, 3600 g/ha BAS 516 00 F. The reference item was applied equivalent to 600 g a.s./ha. The substances were applied in 400 L water/ha. The substances were sprayed on the substrate and the spiders via laboratory spray applicator.
Test conditions:	Temperature: 20°C - 22°C; relative humidity: 60% - 90%; photoperiod: 16 h light : 8 h dark, light intensity: 686 lux - 1326 lux; food: *Drosophila spec.*
Statistics:	Descriptive statistics; Fisher's Exact test for mortality, ANOVA followed by Dunnett's test for food consumption ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

BAS 516 00 F had no effect on survival and food consumption of *Pardosa sp.* No test item related behavioral abnormalities were observed (see Table 10.3.2.1-6).

Table 10.3.2.1-6: Effects on wolf spiders (*Pardosa spec.*) exposed to BAS 516 00 F in a laboratory trial

Treatment	Rate [1] [g/ha]	Mortality [2] [%]	Corrected mortality[3] [%]	Flies consumed [per spider/day]	Effect on feeding capacity [%]
Control	--	0.0	--	3.4	--
BAS 516 00 F	3600	2.9 n s	2.9	3.3 n s	3
	Endpoint [g/ha]				
LR_{50}	> 3600				
Effects on feeding rate	> 3600				

1) Application rate in 400 L water/ha.
2) Mortality: mean of 17 replicates per gender and treatment, i.e. 34 spiders/treatment.
3) Corrected according to Abbott (1925).
n.s. = No statistically significant differences compared to the control (Fisher's Exact test for mortality, Dunnett's test for food consumption, ($\alpha = 0.05$).

The reference item caused a corrected mortality of 79.4%.

III. CONCLUSION

Under worst-case laboratory conditions, no unacceptable effects on survival or feeding rate of *Pardosa* spp. occurred at an application rate of 3600 g/ha BAS 516 00 F in 400 L water/ha.

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Report:	CP 10.3.2.2/1 Ufer A., 2001b Effect of BAS 516 00 F on the green lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) in an extended laboratory trial 2001/1001874
Guidelines:	Hassan S.A. Standard methods to test the side-effects of pesticides on natural enemies of insects and mites. Bull. OEPP 15 pp. 214-255 (1985), Vogt H. et al. (Draft version 02/2000) Laboratory method to test effects of plant protection products on larvae of Chrysoperla carnea (Neuroptera: Chrysopidae)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an extended laboratory study, larvae of *Chrysoperla carnea* (green lacewing) were exposed to dried residues of BAS 516 00 F on detached bean leaves. The mortality of exposed individuals was assessed regularly until hatching of adult lacewings. For the reproduction assessment in the control and the test item treatment groups the reproduction performance, i.e. egg deposition and hatching rate, was determined over a 7 days period (2 assessments, 24 hours period each).

9 out of 50 juveniles (18%) died in the 2 x 360 g/ha BAS 516 00 F treatment and 14 out of 50 juveniles (28%) died in the 2 x 1800 g/ha BAS 516 00 F treatment. In the control, 6 out of 50 individuals (12%) did not reach maturity. This results in a corrected mortality of 6.8% in the 2 x 360 g/ha treatment and 18.2% in the 2 x 1800 g/ha treatment. The difference between the control and the 2 x 1800 g/ha treatment was statistically significant.
39.8 eggs/female and day were produced in the 2 x 360 g/ha treatment and 36.0 eggs/female and day were counted in the 2 x 1800 g/ha BAS 516 00 F treatment. 41.1 eggs/female and day were produced in the water treated control. As all treatments exceeded the mean number of 15 eggs/female/day, no treatment related effects on reproduction could be shown. No effects on the hatching rate of the F1-generation could be observed, as all hatching rates are well above a mean value of 70%.

Under extended laboratory conditions, the LR_{50} of the foliage-dwelling predator *Chrysoperla carnea* was > 2 x 1800 g/ha BAS 516 00 F. No unacceptable effects on reproduction were observed up to an application rate of 2 x 1800 g/ha BAS 516 00 F in 400 L water/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).

Test species: *Chrysoperla carnea* (green lacewing), larvae (2-3 days old); source: BASF Agricultural Centre, Limburgerhof, Germany.

B. STUDY DESIGN

Test design: Exposure of the larvae was reached via air-dried residues on detached bean leaves (*Phaseolus vulgaris*, variety: St. Andreas). Four treatments were set up (two rates of the test item, water control, reference item) with 50 individuals (replicates)/treatment. Each test item treatment was applied two times with a spray interval of 5 days; for the second application the newly grown leaves were cut. After the test substances had dried, leaves were detached and placed in a plexiglass tray on a wet layer of cotton. The mortality of exposed individuals was assessed regularly until hatching of adult lacewings. For the reproduction assessment in the control and the test item treatment groups the reproduction performance, i.e. egg deposition and hatching rate, was determined over a 7 days period (2 assessments, 24 hours period each).

Endpoints: Preimaginal mortality, additionally reproduction capacity.

Reference item: BAS 152 11 I (dimethoate, 400 g/L).

Test rates: Control, 2 x 360 g/ha BAS 516 00 F and 2 x 1800 g/ha BAS 516 00 F. The reference item was applied corresponding to 1 x 0.1 L/ha. All substances were applied in 400 L water/ha. The substances were sprayed on bean plants via laboratory spray track and air dried afterwards.

Test conditions: Temperature: 23.1°C - 26.7°C; relative humidity: 48.7% - 85.7%; photoperiod: 16 h light : 8 h dark; light intensity: larvae: 1800 lux - 4200 lux, pupae 3100 lux - 6300 lux; food: larvae: fresh *Sitotroga cerealella* eggs, adults: artificial diet.

Statistics: Descriptive statistics, Fisher's Exact test for mortality ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

9 out of 50 juveniles (18%) died during their development to adults in the 2 x 360 g/ha BAS 516 00 F treatment and 14 out of 50 juveniles (28%) died in the 2 x 1800 g/ha BAS 516 00 F treatment. In the control, 6 out of 50 individuals (12%) did not reach maturity. This results in a corrected mortality of 6.8% in the 2 x 360 g/ha treatment and 18.2% in the 2 x 1800 g/ha treatment. The difference between the control and the 2 x 1800 g/ha treatment was statistically significant (Fischer's Exact test; $\alpha = 0.05$).
39.8 eggs/female and day were produced in the 2 x 360 g/ha treatment and 36.0 eggs/female and day were counted in the 2 x 1800 g/ha BAS 516 00 F treatment. 41.1 eggs/female and day were produced in the water control. As all treatments exceeded the mean number of 15 eggs/female/day, no treatment related effects on reproduction could be shown. No effects on the hatching rate of the F1-generation could be observed, as all hatching rates are well above a mean value of 70% (see Table 10.3.2.2-1).

Table 10.3.2.2-1: Effects on green lacewing (*Chrysoperla carnea*), exposed to BAS 516 00 F in an extended laboratory trial

Treatment	Rate[1)] [g/ha]	Mortality[2)] [%]	Corrected mortality[3)] [%]	Reproduction [eggs/female/day]	Hatching rate [%]
Control	--	12	--	41.1	87.8
BAS 516 00 F	2 x 360	18	6.8	39.8	82.6
BAS 516 00 F	2 x 1800	28 *	18.2	36.0	87.9
	Endpoint [g/ha]				
LR_{50}	> 2 x 1800				
Effects on reproduction	> 2 x 1800				

1) Application rate in 400 L water/ha.
2) Mortality: after 7 days of exposure to BAS 516 00 F on bean leaves.
3) Corrected mortality according to Schneider Orelli (1947).
* Statistically significant difference compared to the control (Fischer's Exact test, $\alpha = 0.05$).

The reference item BAS 152 11 I produced a mortality of 80% of exposed green lacewings. This resulted in a corrected mortality of 77.3%.

III. CONCLUSION

Under extended laboratory conditions, the LR_{50} of the foliage-dwelling predator *Chrysoperla carnea* was > 2 x 1800 g/ha BAS 516 00 F. No unacceptable effects on reproduction were observed up to an application rate of 2 x 1800 g/ha BAS 516 00 F in 400 L water/ha.

CP 10.3.2.3 Semi-field studies with non-target arthropods

As BAS 516 07 F does not pose an unacceptable risk to other non-target arthropods, further tests are not necessary.

CP 10.3.2.4 Field studies with non-target arthropods

Report:	CP 10.3.2.4/1 Muether J., 2001a A field study to evaluate the effects of BAS 516 00 F against predatory mites in damson plum 2001/1000905
Guidelines:	BBA VI 23-2.3.4, Bluemel et al. (2000)
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

A field trial in damson plum was carried out to determine the effects of the fungicide BAS 516 00 F on the population development of predatory mites (Acari: Phytoseiidae). The trial included four treatment groups, two test item treatments with BAS 516 00 F, a water treated control and a reference item treatment. BAS 516 00 F was applied 5 times between mid of April and end of June at a rate of 750 g/ha (treatment T1) in 1500 L water/ha and at a rate of 150 g/ha (treatment T2) in 1500 L water/ha, both calculated for a crown height of 3 m. Three applications were done early in the season during flowering with an spray interval of 6 to 8 days, two applications were done later in the season at BBCH 77 and BBCH 78. The reference item (Antracol WG) was applied 7 times in regularly intervals over the whole season. The population development of predatory mites was assessed in all treatment groups by determining the number of mites on samples consisting of short shoots with flower buds and flowers (early season) and Ieaf samples (Iate season), using the washing method (BOLLER, 1984).

The mean number of mites per short shoots and flower buds was between 3.0 and 15.4 in the control treatment, between 4.2 and 9.8 in the test item treatment (T1), between 2.4 and 14.2 in the test item treatment (T2) and between 3.0 and 12.0 in the reference item treatment. The mean number of mites per leaf was between 0.37 and 3.02 in the control treatment, between 0.31 and 3.66 in the test item treatment (T1), between 0.28 and 2.11 in the test item treatment (T2) and between 0.24 and 0.80 in the reference item treatment. The effect of the test item BAS 516 00 F on the predatory mite populations ranged between -87 and 20% according to ABBOTT (1925) in treatment T1 and ranged between -8 and 32% in the treatment T2. An effect >50% in the reference item treatment was achieved after the 3rd application and the toxic reference produced a maximum effect on mite density of 86%.

BAS 516 00 F caused no unacceptable effects on population development of predatory mites under field conditions in stone fruit (damson plum) if used as tested, with 5 applications and up to an application rate of 750 g/ha in 1500 L water/ha, calculated for a crown height of 3 m.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).

Test species: Predatory mites (52% *Typhlodromus pyri;* 45% *Euseius stipulatus)*, naturally established field population, all stages.

B. STUDY DESIGN

Test plots: The field study was carried out in Freinsheim, south-west Germany. The test site was located in a region typical for stone fruit production. The agricultural practices and stone fruit varieties (damson plum, variety: St. Hubertus) were in accordance with the local farming practices. Height of the trees: 3 m; 500 trees per ha; age of the trees: 11 years.

Test design: Damson plum harboring populations of predatory mites were exposed to spray treatment of two rates of the test item, water for a control, group and a reference item with 5 replicates per treatment and 3 trees per replicate (unsampled margin of 2 meters at both ends of each plot). Test plots were treated with 5 applications, starting with 3 applications in the early season during flowering (target: blossom blight) using a spray interval of 6 - 8 days and two applications in the later season at BBCH 77 - 78 (target: brown rot). The reference item was applied 7 times in regular intervals over the whole season according to the normal use of this product. Applications were carried out using a motorized knapsack atomizer (model SOLO type 423). Assessments: from every plot samples were taken consisting of 75 short shoots with flower buds and flowers (early season) and of at least 40 leaves (late season) to determine mite densities using the washing technique at 1 day before treatment, 6 days after the 1st application, 8 days after the 3rd application, 7 days before the 4th application and 9 days as well as 1 month after the last (5th) application. Because of the very low mite number at the sampling during the period of flowering (75 short shoots with flower buds and flowers), these numbers have not been used for the calculation of effect values.

Endpoints: Population development compared to the control

Reference item: Antracol WG (propineb, 705 g/kg).

Test rates: Control, 5 x 750 g/ha BAS 516 00 F and 5 x 150 g/ha BAS 516 00 F in 1500 L water/ha, both calculated for a crown height of 3 m (500 L water/ha per m crown height). The reference item was applied corresponding to 2250 g/ha in 1500 L water/ha, also calculated for a crown height of 3 m.

Statistics: Descriptive statistics, ANOVA followed by Dunnett's test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

The predatory mite numbers reached a level of 3 mites per leaf in the control plots, demonstrating the suitability of the test site. The predatory mite populations in the plots treated with BAS 516 00 F applied at 750 g/ha rate did not show a statistically significant reduction compared to the control during the whole study. The predatory mite numbers in the plots treated with BAS 516 00 F at the 150 g/ha rate showed a significant reduction of 32%. The statistically significant effect of 32% in the 150 g/ha treatment is considered to be due to natural variability, because this trend was not confirmed by the high rate of 750 g/ha. Besides of that the statistically significant difference of -87% in the 750 g/ha treatment gives additional indication for the variability in natural populations of predatory mites, if only single assessments are considered. Thus, no unacceptable effect on predatory mites was observed in both test item treatments (see Table 10.3.2.4-1 and Table 10.3.2.4-2).

Table 10.3.2.4-1: Mean number of predatory mites exposed to BAS 516 00 F in a field trial

Assessment	Date	Mean number of mites per 75 short shoots with 5-6 flower buds each [1)]			
		Control	BAS 516 00 F 750 g/ha	BAS 516 00 F 150 g/ha	Antracol WG 2250 g/ha / no. of applications performed
Pre-assessment	03.04.00	3.00	4.20	2.40	3.00
6 days after 1st application	10.04.00	15.40	9.80	14.20	12.00 / 1
		Mean number of mites per leaf [1, 2)]			
8 days after 3rd application	26.04.00	0.37	0.31	0.28	0.24 / 3
7 days before 4th application (50 days after 3rd application)	07.06.00	1.60	1.28	1.08	0.60 * / 5
9 days after 5th application	06.07.00	3.02	2.46	2.06*	0.42 * / 7
29 days after 5th application	26.07.00	1.96	3.66 *	2.11	0.80 / 7

1) Including all mobile stages
2) Assessing at least 40 leaves per plot
* Statistically significant different compared to the control (Dunnett's test, $\alpha = 0.05$)

Table 10.3.2.4-2: Effects of BAS 516 00 F and the reference item on populations of predatory mites in a field trial

Assessment	Date	Effects [%] [1]		
		BAS 516 00 F [2] 750 g/ha	BAS 516 00 F [2] 150 g/ha	Antracol WG 2250 g/ha / no. of applications performed
8 days after 3rd application	26.04.00	15	24	34 / 3
7 days before 4th application (50 days after 3rd application)	07.06.00	20	32	62 * / 5
9 days after 5th application	06.07.00	19	32 *	86 * / 7
29 days after 5th application	26.07.00	-87 *	-8	59 / 7

[1] Effects = % reduction compared to the control; calculated according to Abbott (1925).
[2] - = increase compared to the control.
* Statistically significant different compared to the control (Dunnett's test, $\alpha = 0.05$)

For the reference item Antracol WG a maximum reduction of 86% compared to the control at the assessment 9 days after the last application (7 applications of Antracol WG) was observed.

III. CONCLUSION

BAS 516 00 F caused no unacceptable effects on population development of predatory mites under field conditions in stone fruit (damson plum) if used as tested, with 5 applications and up to an application rate of 750 g/ha in 1500 L water/ha, calculated for a crown height of 3 m.

Report:	CP 10.3.2.4/2 Gossmann A., 2001a Effects of BAS 516 00 F on predatory mites Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) in apple orchards (field experiments) 2001/1001866
Guidelines:	BBA VI 23-2.3.4, Bluemel et al. (1999) Current improvements by the ring-test group
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

Executive Summary

A field trial in an apple orchard was carried out to determine the effects of the fungicide BAS 516 00 F on the population development of predatory mites (Acari: Phytoseiidae). The trial included four treatment groups, two test item treatments with BAS 516 00 F, a water treated control and a reference item treatment. BAS 516 00 F was applied 5 times between end of April and begin of July at a rate of 750 g/ha (treatment T1) in 1500 L water/ha and at a rate of 150 g/ha (treatment T2) in 1500 L water/ha, both calculated for a crown height of 3 m. Three applications were done early in the season during flowering with an spray interval of 6 to 8 days, two applications were done later in the season at BBCH 75. The reference item (Antracol WG) was applied 5 times at the same dates. The population development of predatory mites was assessed in all treatment groups by determining the number of mites on Ieaf samples, using the washing method (BOLLER, 1984).

The mean number of mites per leaf was between 0.23 and 1.35 in the control treatment, between 0.21 and 1.39 in the test item treatment (T1), between 0.23 and 1.17 in the test item treatment (T2) and between 0.10 and 0.82 in the reference item treatment. The effect of the test item BAS 516 00 F on the predatory mite populations ranged between -28.5 and 47.2% according to ABBOTT (1925) in treatment T1 and ranged between -8 and 39.9% in the treatment T2. An effect >50% in the reference item treatment was achieved after the 3rd application and the toxic reference produced a maximum effect on mite density of 88.4%.

BAS 516 00 F caused no unacceptable effects on population development of predatory mites under field conditions in apple trees if used as tested, with 5 applications and up to an application rate of 750 g/ha in 1500 L water/ha calculated for a crown height of 3 m.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).

Test species: Predatory mites (98.6% *Typhlodromus pyri)*, naturally established field population, all stages.

B. STUDY DESIGN

Test plots: The field study was carried out in Modautal, south Hessen Germany. The test site was a commercially managed orchard (apple, variety: Jona Gold) with a size of 5 ha. Height of the trees: 2.2 m; 1200 trees per ha; age of the trees: 23 years.

Test design: Apple orchards harboring populations of predatory mites were exposed to spray treatment of two rates of the test item, water for a control group and a reference item; each treatment consisted of 5 replicates with 8 apple trees / replicate; samples were taken from 4 trees in the middle of each plot. The test item was applied 5 times: early in the season with a spray interval of 6 - 8 days (target: blossom blight) and a set of 2 applications later in the season with a 2 weeks interval (target: brown rot). Applications were carried out at approximately BBCH stage 62-63 (start of flowering), 65-66 (full flowering), 67-68 (end of flowering), 6 weeks after 3rd application (BBCH 75) and 2 weeks after 4th application (BBCH 75). Applications were carried out using a back pack air blast sprayer (model Stihl SR 400 II). Assessments: from every plot samples of 100 leaves were taken to determine mite densities using the washing technique 1 day before treatment. Further samplings were performed 5 days after 1st application, 6 days after 3rd application, 2 days before the 4th application, 6 days after 5th application and 4 weeks after 5th application.

Endpoints: Population development compared to the control.

Reference item: Antracol WG (propineb, 705 g/kg).

Test rates: Control, 5 x 750 g/ha BAS 516 00 F and 5 x 150 g/ha BAS 516 00 F in 1500 L water/ha, both calculated for a crown height of 3 m (500 L water/ha per m crown height). The reference item was applied corresponding to 2250 g/ha in 1500 L water/ha, also calculated for a crown height of 3 m.

Statistics: Descriptive statistics, ANOVA followed by Dunnett's test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Very low numbers of mites were found in the early season, because population development just started. Values were far below 1 mite per leaf, therefore care has to be taken with the interpretation of the early assessments. Five days after the 1st application of BAS 516 00 F, no statistically significant effect in mite abundance was observed in either the low rate or the high rate tested in this experiment (Dunnett's test, two sided, $\alpha = 0.05$). Six days after 3rd application, a significant reduction (Dunnett's test, two sided, $\alpha = 0.05$) of mite abundance was observed in the higher rate. The effect on mite abundance was calculated to be 47.2%. Two days before the 4th application (39 days after 3rd application) the mite abundance in the high rate was fully recovered.
Four weeks after the 5th and the last application the mite abundance in both test item treatment groups was comparable to those in the control (see Table 10.3.2.4-3 and Table 10.3.2.4-4).

Table 10.3.2.4-3: Mean number of predatory mites exposed to BAS 516 00 F in a field trial

Assessment	Date	Mean number of mites per leaf [1, 2)]			
		Control	BAS 516 00 F 750 g/ha	BAS 516 00 F 150 g/ha	Antracol WG 2250 g/ha
Pre-assessment	24.04.00	0.23	0.21	0.23	0.25
5 days after 1st application	02.05.00	0.34	0.26	0.26	0.20
6 days after 3rd application	17.05.00	0.88	0.46 *	0.69	0.10 *
2 days before 4th application	19.06.00	1.05	1.18	0.73	0.56 *
6 days after 5th application	12.07.00	1.35	0.91	0.81	0.68
4 weeks after 5th application	03.08.00	1.08	1.39	1.17	0.82

1) Including all mobile stages, mean from 5 replicates.
2) Assessing about 100 leaves per sample.
* Statistically significant different compared to the control (Dunnett's test, $\alpha = 0.05$).

Table 10.3.2.4-4: Effects of BAS 516 00 F and the reference item on predatory mites in a field trial

Assessment	Date	Effects[1] [%]		
		BAS 516 00 F [2] 750 g/ha	BAS 516 00 F [2] 150 g/ha	Antracol WG 2250 g/ha
Pre-assessment	24.04.00	--	--	--
5 days after 1st application	02.05.00	25.3	22.9	41.2
6 days after 3rd application	17.05.00	47.2	21.2	88.4
2 days before 4th application	19.06.00	-12.5	30.4	46.4
6 days after 5th application	12.07.00	32.6	39.9	49.9
4 weeks after 5th application	03.08.00	-28.5	-8.0	23.9

1) Effects = % reduction compared to the control; calculated according to Abbott (1925)
2) - = increase compared to the control
* Statistically significant different compared to the control (Dunnett's test, $\alpha = 0.05$)

The reference item showed effects from more than 40% after the first application until 6 days after the 5th application. Six days after the 3rd application, the effect was by 88.4% and statistically significant. Two days before the 4th application the effect (46.4%) was also statistically significant. Four weeks after the 5th application no effect in the toxic standard group was observable any more.

III. CONCLUSION

BAS 516 00 F caused no unacceptable effects on population development of predatory mites under field conditions in apple trees if used as tested, with 5 applications and up to an application rate of 750 g/ha in 1500 L water/ha calculated for a crown height of 3 m.

Report:	CP 10.3.2.4/3 Nienstedt K.M., 2001a A field test to determine the effect of BAS 516 00 F on predatory mite populations (Acari: Phytoseiidae) on stone fruit trees (cherry, Prunus avium) 2001/1001870
Guidelines:	BBA VI 23-2.3.4, Bluemel et al. (2000)
GLP:	yes (certified by Eidgenoessisches Departement fuer Umwelt, Verkehr, Energie und Kommunikation, Bern, Schweiz)

Executive Summary

A field trial in stone fruit trees (cherry) was carried out to determine the effects of the fungicide BAS 516 00 F on the population development of predatory mites (Acari: Phytoseiidae). The trial included four treatment groups, two test item treatments with BAS 516 00 F, a water treated control and a reference item treatment. BAS 516 00 F was applied 5 times between Mid-April and end of June at a rate of 750 g/ha (treatment T1) in 1500 L water/ha and at a rate of 150 g/ha (treatment T2) in 1500 L water/ha, both calculated for a crown height of 3 m. Three applications were done early in the season during flowering with an spray interval of 6-7 days, two applications were done later in the season at BBCH 75 to BBCH 85 with an spray interval of 8 days. The reference item (Antracol WG) was applied 7 times in regularly intervals over the whole season. The population development of predatory mites was assessed in all treatment groups by determining the number of mites on vegetal material (buds and leaves), using the washing method (BOLLER, 1984).

The mean number of mites per leaf was between 0.01 and 0.54 in the control treatment, between 0.02 and 0.53 in the test item treatment (T1), between 0.02 and 0.63 in the test item treatment (T2) and between 0.0 and 0.01 in the reference item treatment. The effect of the test item BAS 516 00 F on the predatory mite (only calculated for dates on which mite density was higher than 0.1 mites/leaf in the control) populations ranged between 1.8 and 6.9% according to ABBOTT (1925) in treatment T1 and ranged between -18.0 and -6.4% in the treatment T2. The toxic reference produced a maximum effect on mite density of 99.6%.

BAS 516 00 F caused no unacceptable effects on population development of predatory mites under field conditions in stone fruit (cherry orchards) if used as tested, with 5 applications and up to an application rate of 750 g/ha in 1500 L water/ha calculated for a crown height of 3 m.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).

Test species: Predatory mites (100% *Euseius finlandicus)*, naturally established field population, all stages.

B. STUDY DESIGN

Test plots: The field study was carried out in Bischoffszell, Switzerland. The test site was a homogeneous cherry orchard (*Prunus avium*, variety: Oktavia). Height of the trees: 2.8 m; age of the trees: 3rd vegetative year after planting.

Test design: Stone fruit harboring populations of predatory mites were exposed to spray treatment of two rates of the test item, water for a control group and a reference item; 5 replicates / treatment and 8 trees / replicate. 2 trees left untreated between replicates. Test plots were treated with 5 applications, starting with 3 applications in the early season (BBCH 60-67) using a spray interval of 6 - 7 days (target: blossom blight) and two applications in the later season at BBCH 75 – 85 (target: brown rot) with an interval of 8 days. The reference item was applied 7 times in regular intervals over the whole season according to the normal use of this product. Applications were carried out using a back-pack sprayer (Birchmeier M125). Assessments: from every plot samples were taken consisting of about 100 short shoots with flower buds and flowers (early season) and of about 100 leaf samples (late season) to determine mite densities using the washing technique at 6 days before treatment, 5 days after the 1st application, 6 after the 3rd application, 30 days after the 3rd application (6 days before the 4th application) and 6 and 26 days after the last application. Because of the very low mite number at the sampling during the period of flowering (about 100 flower buds), these numbers have not been used for the calculation of effect values.

Endpoints: Population development compared to the control.

Reference item: Antracol WG (propineb, 705 g/kg).

Test rates: Control, 5 x 750 g /ha BAS 516 00 F and 5 x 150 g/ha BAS 516 00 F in 1500 L water/ha, both calculated for a crown height of 3 m (500 L water/ha per m crown height). The reference item was applied corresponding to 3000 g/ha in 1500 L water/ha (0.2%) also calculated for a crown height of 3 m.

Statistics: Descriptive statistics, Repeated Measurement ANOVA.

II. RESULTS AND DISCUSSION

Very low numbers of mites were found in the early season because population development just started. Values were far below 1 mite per leaf; therefore, care has to be taken with the interpretation of the early assessments. At the first sampling date (14.04.00), predatory mite density was 0.01 to 0.02 mites/bud in all treatments. The mite populations increased slowly during the next 2 sampling dates. Although remaining at levels of the same magnitude, which makes an interpretation of the data difficult. However, from the 4th sampling onwards, mite density was above 0.1 in the control and the test item treatments, increasing continuously during the remaining sampling period until a maximum level of 0.5 to 0.6 mites/leaf on the last sampling date (18.07.00). The mite population development was similar in the control and the test item treatments. No statistically significant differences were observed between the control and the test item treatments during the testing period (Repeated measurement ANOVA: α = 0.05).

Effects on mite population according to Abbott (1925) were calculated for the 2 last sampling dates, i.e. 6 and 26 days after the last test item application, since only on this dates the mite density was above 0.1 mites/leaf. For the 150 g/ha test item treatment, no effects on mite population were observed, since negative Abbott values were calculated for the 2 last sampling dates. For the 750 g/ha test item treatment the effects observed were 6.9% and 1.8%, 6 and 26 days after the last application, respectively (see Table 10.3.2.4-5 and Table 10.3.2.4-6).

Table 10.3.2.4-5: Mean number of predatory mites exposed to BAS 516 00 F in a field trial

Assessment	Date	Mean number of mites per flower bud or per leaf [1, 2)]			
		Control	BAS 516 00 F 750 g/ha	BAS 516 00 F 150 g/ha	Antracol WG 3000 g/ha / no. of applications performed
Pre-assessment	14.04.00	0.01	0.02	0.02	0.01
5 days after 1st application	25.04.00	0.04	0.04	0.04	0.01 * / 1
6 days after 3rd application	09.05.00	0.05	0.03	0.04	0 * / 3
6 days before 4th application (30 days after 3rd application)	08.06.00	0.12	0.10	0.16	0 * / 5
6 days after 5th application	28.06.00	0.43	0.40	0.46	0.004 * / 7
26 days after 5th application	18.07.00	0.54	0.53	0.63	0.002 * / 7

1) Including all mobile stages.
2) Assessing at least 100 flower buds or leaves per plot.
* Statistically significant different compared to the control (Repeated Measurement ANOVA, $\alpha = 0.05$).

Table 10.3.2.4-6: Effects of BAS 516 00 F and the reference item on predatory mites in a field trial

Assessment	Date	Effects [1) [%]]		
		BAS 516 00 F 750 g/ha	BAS 516 00 F 150 g/ha [2)]	Antracol WG 3000 g/ha
6 days after 5th application	28.06.00	6.9	-6.4	99.1 [3)]
26 days after 5th application	18.07.00	1.8	-18.0	99.6 [3)]

1) The effect on mite population according to Abbott (1925) was only calculated for dates in which mite density was higher than 0.1 mites / leaf in the control.
2) - = increase compared to the control
3) 7 applications performed.

For the reference item Antracol WG a maximum reduction of 99.6% compared to the control at the assessment 26 days after the last application (7 applications of Antracol WG) was observed.

III. CONCLUSION

BAS 516 00 F caused no unacceptable effects on population development of predatory mites under field conditions in stone fruit (cherry orchards) if used as tested, with 5 applications and up to an application rate of 750 g/ha in 1500 L water/ha calculated for a crown height of 3 m.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

As BAS 516 07 F does not pose an unacceptable risk to other non-target arthropods, further tests are not necessary.

CP 10.4 Effects on non-target soil meso- and macrofauna

The new representative formulation BAS 516 07 F was not evaluated within a previous Annex I inclusion process. It is a water dispersible granule (WG) containing 67 g/kg pyraclostrobin and 267 g/kg boscalid.

Endpoints from new studies with the active substance, its metabolites and the new representative formulation BAS 516 07 F are used for the risk assessment on earthworms and other non-target soil meso- and macrofauna (see Table 10.4-1).

Table 10.4-1: Ecotoxicological endpoints for earthworms and other non-target soil meso- and macrofauna

Test substance	Test species	EU agreed endpoints	Endpoints used in risk assessment
Acute			
Pyraclostrobin	*Eisenia fetida*	LC_{50} = 567 mg a.s./kg dry soil LC_{50} = 283 mg a.s./kg dry soil [1)]	--
BF 500-6		LC_{50} > 1000 mg/kg dry soil LC_{50} > 500 mg/kg dry soil [1)]	--
BF 500-7		LC_{50} > 1000 mg/kg dry soil LC_{50} > 500 mg/kg dry soil [1)]	--
BAS 516 07 F		LC_{50} > 1000 mg/kg dry soil (corresponding to 67 mg pyraclostrobin/kg dry soil and 334 mg total a.s./kg dry soil) LC_{50} > 33.5 mg pyraclostrobin and 167 mg total a.s./kg dry soil[1)]	--
Chronic			
Pyraclostrobin *	*Eisenia fetida*	--	$NOEC_{CORR}$ = 11.6 mg/kg dry soil [1)]
BF 500-6 *		--	$NOEC_{CORR}$ ≥ 160 mg/kg dry soil [1)]
BF 500-7 *		--	$NOEC_{CORR}$ ≥ 160 mg/kg dry soil [1)]
BAS 516 07 F			NOEC = 40 mg/kg dry soil (corresponding to 2.7 mg pyraclostrobin/kg dry soil and 13.4 mg total a.s./kg dry soil)
BF 500-6 *	*Folsomia candida*	--	NOEC ≥ 1000 mg/kg dry soil
BF 500-7 *		--	NOEC ≥ 800 mg/kg dry soil
BAS 516 07 F		--	NOEC = 250 mg/kg dry soil (corresponding to 16.8 mg pyraclostrobin/kg dry soil and 83.8 mg total a.s./kg dry soil)
BAS 516 07 F	*Hypoaspis aculeifer*	--	NOEC ≥ 500 mg/kg dry soil (corresponding to ≥ 33.5 mg pyraclostrobin/kg dry soil and ≥167 mg total a.s./kg dry soil)

Test substance	Test species	EU agreed endpoints	Endpoints used in risk assessment
Field studies			
BAS 516 07 F	earthworm field population	--	No unacceptable effects up to 4.5 kg product/ha (corresponding to 302 g pyraclostrobin/ha and 1504 g total a.s./ha)

1) Toxicity endpoint is re-adjusted using a soil factor of 2 to address the organic content of the soil (peat 10%), and the log P_{ow} of the substance is > 2.

* Study summary is presented in M-CA 8.4.

Overall summary

Data on BAS 516 07 F are evaluated and appropriate risk assessments are provided for the active substance pyraclostrobin, the relevant metabolites and the formulation BAS 516 07 F for the already registered use pattern in potatoes (critical GAP: maximum 4 x 17 g pyraclostrobin per ha).

The endpoints and calculated toxicity/exposure ratios are shown in Table 10.4-2.

Table 10.4-2: Toxicity/exposure ratios for earthworms and other soil non-target macro-organisms

Test substance	Use pattern [g/ha]	Species	Test type	Endpoint [mg/kg dry soil]	PEC [mg/kg dry soil]	TER	Trigger
Pyraclostrobin	4 x 17	*Eisenia fetida*	56-d reproduction test	NOEC $_{CORR}$ = 11.6	0.015	773	5
BF 500-6	--	*Eisenia fetida*	56-d reproduction test	NOEC $_{CORR}$ ≥ 160	0.008	≥ 20000	
BF 500-7	--	*Eisenia fetida*	56-d reproduction test	NOEC $_{CORR}$ ≥ 160	0.005	≥ 32000	
BAS 516 07 F	pyraclostrobin: 4 x 17	*Eisenia fetida*	56-d reproduction test	NOEC = 2.7	0.015	180	
	total a.s.: 4 x 84			NOEC = 13.4	0.260	52	
BAS 516 07 F	pyraclostrobin: 4 x 17	*Folsomia candida*	28-d reproduction test	NOEC = 16.8	0.015	1117	
	total a.s.: 4 x 84			NOEC = 83.8	0.260	322	
BF 500-6	--	*Folsomia candida*	28-d reproduction test	NOEC ≥ 1000	0.008	≥ 125000	
BF 500-7	--	*Folsomia candida*	28-d reproduction test	NOEC ≥ 800	0.005	≥ 160000	
BAS 516 07 F	pyraclostrobin: 4 x 17	*Hypoaspis aculeifer*	14-d reproduction test	NOEC ≥ 33.5	0.015	≥ 2233	
	total a.s.: 4 x 84			NOEC ≥ 167	0.260	≥ 642	

Acute studies on earthworms were performed with pyraclostrobin, the metabolites BF 500-6 and BF 500-7 (see SANCO/1420/2001-final, Monograph 12945/ECCO/BBA/01) and with the formulation BAS 516 07 F. According to Regulation 1107/2009 the tier 1 data requirement for acute toxicity on earthworms has been replaced by a requirement for chronic toxicity (i.e. reproduction) on earthworms. However, from previous submissions according to the former Directive EC 91/414 data on the acute toxicity of BAS 516 07 F to earthworms is available, which is for completeness presented as additional information, although acute endpoints are not used anymore in the current risk assessment (see below for respective chronic studies).

Chronic studies on earthworms were carried out with pyraclostrobin, the metabolites BF 500-6 and BF 500-7 (see M-CA 8.4) as well as with the formulation BAS 516 07 F.

Furthermore, chronic studies on collembolans were carried out with BAS 516 07 F and with the metabolites BF 500-6 and BF 500-7 (for metabolites see M-CA 8.4). Moreover, a chronic study on soil mites and in addition a field study on earthworms has been carried out with BAS 516 07 F.

In the risk assessment, all TER values exceeded the trigger value of 5 for chronic exposure.

Overall conclusion:

It is concluded that the use of BAS 516 07 F in potatoes will not pose unacceptable risks to populations of earthworms or other soil macro-organisms.

CP 10.4.1 Earthworms

Toxicity

Acute and chronic earthworm toxicity studies have been carried out with pyraclostrobin, its metabolites BF 500-6 and BF 500-7, as well as with the formulation BAS 516 07 F. Furthermore, an earthworm field study has been carried out with BAS 516 07 F. Further details on the studies with the formulation are given below (M-CP 10.4.1, M-CP 10.4.1.1 and M-CP 10.4.1.2) and on the studies with the active substance and metabolites in M-CA 8.4. The acute endpoints of the study with BAS 516 07 F are presented as additional information. The earthworm endpoints are summarized in Table 10.4.1-1.

For substances with log P_{ow} values > 2 and a high content of organic material in the artificial soil (i.e. 10% peat), the resulting endpoints have to be corrected by a soil factor of 2 (f_{oc}) in the risk assessment in order to address lower contents of organic material in soil.

The log P_{ow} for the active substance is > 2 (i.e. 3.99). Therefore, the correction was done for the endpoints obtained from the studies where the artificial soil contained more than 5% peat.

Table 10.4.1-1: Summary of earthworm endpoints for BAS 516 07 F, pyraclostrobin and metabolites

Test substance	Endpoint	Value [mg/kg dry soil]	Reference (BASF DocID)
Acute toxicity			
Pyraclostrobin	$LC_{50\ CORR}$	283	1999/10708
BF 500-6	$LC_{50\ CORR}$	> 500	1999/11308
BF 500-7	$LC_{50\ CORR}$	> 500	1999/11309
Pyraclostrobin in BAS 516 07 F [1)]	$LC_{50\ CORR}$	> 34 [2)]	2001/1001837
total a.s. in BAS 516 07 F [1)]		> 168 [2)]	
Chronic toxicity			
Pyraclostrobin	$NOEC_{CORR}$	11.6	2014/1000461
BF 500-6	$NOEC_{CORR}$	≥ 160	2013/1003174
BF 500-7	$NOEC_{CORR}$	≥ 160	2013/1224029
Pyraclostrobin in BAS 516 07 F	NOEC	2.7	2006/1015860
total a.s. in BAS 516 07 F		13.4	

[1)] Study summary is presented below as additional information.
[2)] Endpoint recalculated based on the nominal content of 6.7% pyraclostrobin and 26.7% boscalid in BAS 516 07 F.

Exposure

Table 10.4.1-2: Critical use pattern of BAS 516 07 F

Crop	Application time (BBCH code)	Number of applications	Interval [d]	Application rate per treatment		
				Boscalid [g a.s./ha]	Pyraclostrobin [g a.s./ha]	BAS 516 07 F [g/ha]
potatoes	51 - 89	4	10	67	17	250

The exposure to soil organisms was estimated by calculating the maximum predicted environmental concentrations in soil (PEC_{soil}). For multiple applications, the worst-case maximum PEC_{soil} will be the one immediately after the final application. The worst-case use pattern of BAS 516 07 F foresees four applications to potatoes with a maximum dose rate of 250 g/ha, corresponding to 17 g pyraclostrobin/ha and 67 g boscalid/ha.

For details see M-CP 9.1. The resulting maximum PEC_{soil} values are presented in Table 10.4.1-3.

Table 10.4.1-3: PEC_{soil} values for pyraclostrobin, boscalid and relevant metabolites

Test substance	$PEC_{soil, max}$ [mg/kg dry soil]	$PEC_{soil, plateau}$ [mg/kg dry soil]	$PEC_{soil, accu}$ [mg/kg dry soil]
Pyraclostrobin	0.015	--	--
BF 500-6	0.004	0.004	0.008
BF 500-7	0.003	0.002	0.005
Boscalid	0.069	--	0.245

Risk assessment for earthworms

The potential long-term risk of BAS 516 07 F to earthworms was assessed by calculating long-term TER (TER_{LT}) values by comparing the NOEC values and the maximum instantaneous PEC_{soil} using the following equation:

$$TER_{LT} = \frac{NOEC\,[mg/kg\ dry\ soil]}{PEC_{soil}\,[mg/kg\ dry\ soil]}$$

The resulting TER_{LT} values are presented below:

Table 10.4.1-4: Long-term TER values for earthworms

Test substance	NOEC [mg/kg dry soil]	PEC_{soil} [mg/kg dry soil]	TER_{LT}	TER trigger
Pyraclostrobin	11.6	0.015	773	5
BF 500-6	≥ 160	0.008	≥ 20000	
BF 500-7	≥ 160	0.005	≥ 32000	
Pyraclostrobin in BAS 516 07 F	2.7	0.015	180	
total a.s. in BAS 516 07 F	13.4	0.260	52	

The long-term TER values for BAS 516 07 F, pyraclostrobin and relevant soil metabolites are above the trigger value of 5. Therefore, chronic risk for earthworms arising from long-term use of BAS 516 07 F is negligible. However, for additional information, an earthworm field study was carried out with BAS 516 07 F.

The test site in this study was an arable field. At an application rate of up to 4.5 kg BAS 516 07 F/ha (equivalent to 302 g pyraclostrobin/ha and 1504 g total a.s./ha), no unacceptable effects to earthworm populations were observed (for details see M-CP 10.4.1.2). The intended maximum use of BAS 516 07 F, i.e. 4 x 250g/ha (equivalent to 4 x 17 g pyraclostrobin/ha and 4 x 84 g total a.s./ha) in potatoes is therefore covered by the tested field rate. Thus, it can be concluded that the use of BAS 516 07 F will be of low risk to natural earthworm communities.

Based on the standard risk assessment and on data from the field study, it is concluded that earthworm communities will be at low risk following the use of BAS 516 07 F when applied to potatoes according to the recommended use pattern.

Earthworms – acute toxicity

This study was a former requirement under Council Directive 91/414/EEC and SANCO/10329/2002 rev 2. However, under Regulation (EC) 1107/2009 it is not required anymore. Therefore, the study is considered as additional information.

Report:	CP 10.4.1/1 Wachter S., 2001b Acute toxicity of BAS 516 00 F on earthworms, Eisenia foetida using an artificial soil test 2001/1001837
Guidelines:	OECD 207, ISO 11268-1
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

Adult earthworms of the species *Eisenia fetida*, were exposed to BAS 516 00 F. The test item was mixed into artificial soil containing 10% peat at rates of 198, 296, 444, 667 and 1000 mg BAS 516 00 F/kg dry soil. For the control treatment, the soil was left untreated.

The worms were placed on the surface of the soil. Four replicates were prepared for each treatment group and the control, each containing 10 worms. Assessment of mortality and behavioral effects were made 7 and 14 days after treatment. Assessment of worm weight was made after 14 days.

After 14 days of exposure, no mortality was observed in the control group. Up to 1000 mg BAS 516 00 F/kg dry soil, a maximum of 20% mortality was observed. The biomass development was statistically significantly inhibited at the tested concentrations of 667 and 1000 mg BAS 516 00 F/kg dry soil. No particular behavioral abnormalities could be observed.

In a 14-d toxicity study with BAS 516 00 F to earthworms (*Eisenia fetida*) the LC_{50} was determined to be > 1000 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).
Test species:	Earthworm (*Eisenia fetida*), adult worms (with clitellum and weight > 300 mg) less than one year old; source GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany.

B. STUDY DESIGN

Test design:	14 d exposure in treated artificial soil; different concentrations of the test item are mixed homogeneously into the soil which is filled in glass vessels before the earthworms are introduced on top of the soil; 5 concentrations; 4 replicates/concentration with 10 worms each. Assessment of worm mortality and behavioral effects after 7 and 14 d, measurement of weight change as sub-lethal parameter after 14 d.
Endpoints:	LC_{50} (50% mortality of earthworms after exposure over 14 days), behavioral effects, weight change.
Test concentrations:	Control, 198, 296, 444, 667 and 1000 mg BAS 516 00 F/kg dry soil.
Test conditions:	Artificial soil according to OECD 207 (10% peat); pH 5.6 - 5.7; water content at test initiation 32.6% - 35.9% (of dry soil); 31.2% - 35.0% at test termination; temperature: 18°C – 22°C.
Statistics:	Descriptive statistics, ANOVA followed by Dunnett-test (α = 0.05) for weight change.

II. RESULTS AND DISCUSSION

After 14 days of exposure, no mortality was observed in the control group. Up to 1000 mg BAS 516 00 F/kg dry soil, a maximum of 20% mortality was observed. The biomass development was statistically significant inhibited at the tested concentrations of 667 and 1000 mg BAS 516 00 F/kg dry soil (Dunnett-test, $\alpha = 0.05$). The results are summarized in Table 10.4.1-5.

Table 10.4.1-5: Effects of BAS 516 00 F on earthworm (*Eisenia fetida*) mortality and biomass (14 d)

BAS 516 00 F [mg/kg dry soil]	Control	198	296	444	667	1000
Mortality [%]	0	0	0	2.5	2.5	20
Weight change [%]	-10.3	-12.7	-12.9	-16.3	-20.1 *	-21.7 *
Endpoints [mg/kg dry soil]						
LC_{50}	> 1000					

* Statistically significant differences compared to the control (Dunnett-test, $\alpha = 0.05$).

III. CONCLUSION

In a 14-d toxicity study with BAS 516 00 F to earthworms (*Eisenia fetida*) the LC_{50} was determined to be > 1000 mg/kg dry soil.

CP 10.4.1.1 Earthworms – sub-lethal effects

Report:	CP 10.4.1.1/1 Friedrich S., 2006a Sublethal toxicity of BAS 516 07 F to the earthworm Eisenia fetida in artificial soil with 5% peat 2006/1015860
Guidelines:	OECD 222
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of BAS 516 07 F on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* were investigated in a 56-days extended laboratory study. Six application rates (2.5, 5, 10, 20, 40 and 60 mg BAS 516 07 F/kg dry soil) were incorporated into the soil (5% peat) with four replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of adult worm mortality and biomass development was carried out after 28 days, assessment of behavior, pathological symptoms and reproduction rate (number of juveniles) was carried out after 56 days.

No mortality was observed in any of the treatment groups. Body weights of the earthworms exposed to BAS 516 07 F were not statistically significantly different compared to the control. No statistically significant effects on reproduction were observed up to and including the concentration of 40 mg test item/kg dry soil. In the highest test item concentration of 60 mg test item/kg dry soil the number of juveniles was statistically significantly reduced compared to the control group. Neither behavioral abnormalities nor effects on feeding activity were observed in any of the treatment groups.

In a 56-day earthworm reproduction study with BAS 516 07 F on earthworms, the NOEC was determined to be 40 mg BAS 516 07 F/kg dry soil and the EC_{50} was > 60 mg BAS 516 07 F/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 516 07 F, batch 1789, content of a.s.: pyraclostrobin (BAS 500 F): 6.8% (nominal 6.7%); boscalid (BAS 510 F; Reg. no.:300 355): 26.7% (nominal 26.7%).
Test species:	Earthworm (*Eisenia fetida andrei*), adult worms (with clitellum and weight of 300 mg – 454 mg), approximately 3 months old; source: in-house culture.

B. STUDY DESIGN

Test design:	56-day test in treated artificial soil according to OECD 222 (5% peat only); different concentrations of the test item were incorporated into the soil; 7 treatment groups (6 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. Assessment of worm mortality and biomass development after 28 days. Behavior, pathological symptoms and reproduction rate were determined after another 28 days (assessed 56 days after application).
Endpoints:	Mortality, weight change, reproduction rate, feeding activity and behavioral effects
Reference item:	Benlate (Benomyl WP, 500 g/kg). The effects of the reference item were investigated in a separate study.
Test rates:	Control, 2.5, 5, 10, 20, 40 and 60 mg BAS 516 07 F/kg dry soil (nominal).
Test conditions:	Artificial soil according to OECD 222 (with reduced content of peat: 5%); pH 6.3 at test initiation, 6.2 - 6.4 at test termination; water content 24.7% - 24.9% at test initiation and 24.7% - 25.4% at test termination; temperature: 17C - 21C; photoperiod: 16 h light : 8 h dark, light intensity: 570 lux, food: horse manure.
Statistics:	Descriptive statistics, Dunnett-test for weight change and reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

No mortality was observed in any of the treatment groups. Body weights of the earthworms exposed to BAS 516 07 F were not statistically significantly different compared to the control (Dunnett-test, $\alpha = 0.05$). No statistically significant effects on reproduction were observed up to and including the concentration of 40 mg test item/kg dry soil. In the test item concentration of 60 mg test item/kg dry soil the reproduction was statistically significantly reduced compared to the control group (Dunnett-test, $\alpha = 0.05$). Neither behavioral abnormalities nor effects on feeding activity were observed in any of the treatment groups. The results are summarized in Table 10.4.1.1-1.

Table 10.4.1.1-1: Effects of BAS 516 07 F on earthworms (*Eisenia fetida andrei)* in a 56-day reproduction study

BAS 516 07 F [mg/kg dry soil]	**Control**	**2.5**	**5**	**10**	**20**	**40**	**60**
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Weight change (day 28) [%]	65.7	63.6	60.5	61.9	64.0	61.9	58.4
No. of juveniles (day 56)	113	118.3	107.3	112.3	112.8	98.5	78.8 *
Reproduction in [%] of control (day 56)	100	104.6	94.9	99.3	99.8	87.2	69.7
Endpoints [mg/kg dry soil]							
EC_{50}	> 60						
NOEC (day 56)	40						

* Statistically significant different compared to the control (Dunnett-test; $\alpha = 0.05$).

III. CONCLUSION

In a 56 day earthworm reproduction study with BAS 516 07 F on earthworms, the NOEC was determined to be 40 mg BAS 516 07 F/kg dry soil and the EC_{50} was > 60 mg BAS 516 07 F/kg dry soil.

CP 10.4.1.2 Earthworms – field studies

Report:	CP 10.4.1.2/1 Hamberger A., 2011a Field study to evaluate the effects of BAS 516 07 F on earthworms in Southern Germany 2011/1043488
Guidelines:	ISO 11268-3 (1999)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

An arable land site was chosen as a natural habitat of earthworms. The treatments with 1.5, 3.0 and 4.5 kg BAS 516 07 F/ha were assigned randomly to the plots within each replicate. Over the experimental period from March 2009 until April 2010 four earthworm samplings were evaluated. Earthworm extraction was achieved by hand sorting combined with formalin extraction.
Prior to the treatment, the test site yielded sufficiently high numbers of earthworms of 199.2 individuals per m^2 representing various common earthworm species such as *Lumbricus terrestris* and *Aporrectodea longa* (anecic species) and *Aporrectodea caliginosa* and *A. rosea* (endogeic species).
No statistically significant differences between BAS 516 07 F treatments and the untreated control could be detected in terms of population density or biomass 1, 6 and 12 months after application.

The results of this field study on natural earthworm biocoenosis showed that after application of BAS 516 07 F at 1.5, 3.0 and 4.5 kg/ha no significant long-term effects on natural earthworm populations in terms of abundance and total biomass occurred. No obvious effects on the composition of earthworm population could be observed.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 07 F; batch no. 11041; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 26.7% (nominal 26.9%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.7% (nominal 6.5%).

Test species: Naturally occurring field population of earthworms comprising all mobile stages (juvenile and adult earthworms) including endogeic species such as *Aporrectodea caliginosa* and *A. rosea* as well as anecic species such as *Lumbricus terrestris* and *Aporrectodea longa*.

B. STUDY DESIGN

Test site: Arable field, in Bodelshausen, Southern Germany. The last crop planted was mustard sown in September 2008. The site had received chemical applications in previous years prior to the study but none were applied during the study apart from the test item and toxic standard.

Test design: Randomized block design with 5 treatment groups (control, 3 test item rates and toxic standard) each with four replicates. A number of 20 plots, each 12 m x 13 m, were arranged. The test item was applied using a boom sprayer (Schachtner).

Endpoints: Total abundance and biomass of earthworms.

Reference item: Nociolex (50.0% carbendazim; nominal).

Test rates: Untreated control; Treatment groups: BAS 516 07 F applied at 1.5 kg/ha; 3.0 kg/ha; 4.5 kg/ha; the reference item was applied at 20.0 kg/ha.

Application date: 16.04.2009.

Sampling dates: Pre-sampling: 23-16 days before application (24.03. to 31.03.2009); 1st sampling: 39-43 days after application (25.05. to 29.05.2009); 2nd sampling: 179-182 days after application (12.10. to 15.10.2009); 3rd sampling: 355-358 days after application (06.04. to 09.04.2010).

Extraction method: Hand sorting combined with formalin extraction in the excavated hole; 4 samples per plot were taken at each sampling date.

Test conditions:	Natural field conditions; soil texture: clay 5.3 – 14.9%, silt 48.5 – 69.5%, sand 17.8 – 46.3%, pH 7.6 - 7.7, 48.9 – 64.6% of the maximum water holding capacity; C_{org}: 2.63 – 3.47%; mean air temperature during the trial period: 8.8C.
Statistics:	Descriptive statistics; differences between test item treatments and control: ANOVA followed by Dunnett's t-test, Kruskal-Wallis, Wilcoxon-test or Bonferroni U-test ($\alpha = 0.05$), differences between toxic standard and control: Student or Satterthwaite t-test or U-test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Prior to the treatment, the test site yielded sufficiently high numbers of earthworms of 199.2 individuals per m^2 representing various common earthworm species such as *Lumbricus terrestris* and *Aporrectodea longa* (anecic species) and *Aporrectodea caliginosa* and *A. rosea* (endogeic species).
Surface monitoring on days 1-3 after application showed that there was no acute primary effect on earthworms by the test item. No alive, moribund and dead earthworms were found on the soil surface in any of the plots treated with BAS 516 07 F and in the control plots.
No statistically significant differences between BAS 516 07 F treatments and the untreated control could be detected in terms of population density or biomass 1, 6 and 12 months after application. Dominant adult earthworm species found in the field site were the endogeic species *Aporrectodea caliginosa* and the anecic species *Lumbricus terrestris*. No statistically significant reductions in the abundance and biomass of these earthworm species could be observed in the test item treatments compared to the control. Twelve months after application, the biomass of *Aporrectodea longa* at 3.0 kg BAS 516 07 F/ha was statistically significantly reduced. However, no significant reduction in biomass could be found for *A. longa* at the highest application rate at all sampling dates. Therefore, the significant reduction in biomass for 3.0 kg BAS 516 07 F/ha for *A. longa* cannot be considered to be due to the treatment with BAS 516 07 F. Moreover, there was no significant reduction in abundance found for *A. longa* in all test item treatments at all sampling dates. The results are summarized in Table 10.4.1.2-1.

Table 10.4.1.2-1: Summary of total earthworm abundance and biomass in a field study with BAS 516 07 F

Treatment	Pre sampling 24. - 31.03.2009	First sampling 25. - 29.05.2009	Second sampling 12. - 15.10.2009	Third sampling 06. - 09.04.2010
Total earthworm abundance [Ind./m²]				
Control	202.0	233.5	379.0	352.8
1.5 kg/ha BAS 516 07 F	201.0	269.8	411.3	373.8
% of control	**99.5**	**115.5**	**108.5**	**106.0**
3.0 kg/ha BAS 516 07 F	196.3	232.3	326.8	316.8
% of control	**97.2**	**99.5**	**86.2**	**89.8**
4.5 kg/ha BAS 516 07 F	198.3	237.3	332.0	344.5
% of control	**98.1**	**101.6**	**87.6**	**97.7**
reference item	--	15.1% of control *	49.5% of control *	63.0% of control
Total earthworm biomass [g/m²]				
Control	148.6	150.4	139.7	177.3
1.5 kg/ha BAS 516 07 F	155.2	163.9	135.4	145.2
% of control	**104.4**	**108.9**	**96.9**	**81.9**
3.0 kg/ha BAS 516 07 F	143.6	132.6	109.9	169.7
% of control	**96.6**	**88.1**	**78.7**	**95.7**
4.5 kg/ha BAS 516 07 F	149.7	136.3	130.0	138.5
% of control	**100.8**	**90.6**	**93.0**	**78.1**
reference item	--	9.3% of control *	49.0% of control	72.4% of control *

* Statistically significantly different compared to the control (Student or Satterthwaite t-test or U-test, $\alpha = 0.05$).

Validity of the reference item was proved by clear statistically significant differences in earthworm numbers at the first and second sampling (Student or Satterthwaite t-test or U-test, $\alpha = 0.05$).

III. CONCLUSION

The results of this field study on natural earthworm biocoenosis showed that after application of BAS 516 07 F at 1.5, 3.0 and 4.5 kg/ha no significant long-term effects on natural earthworm populations in terms of abundance and total biomass occurred. No obvious effects on the composition of earthworm population could be observed.

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

According to SANCO/10329/2002 rev2 final the test on 'sub-lethal effects on collembola or soil mites' is required if *(a)* the $DT_{90,\ field}$ is between 100 and 365 days and *(b)* the standard HQ for arthropods (*T. pyri* and *A. rhopalosiphi*) is above 2.

Toxicity and Exposure

The worst-case $DT_{90,\ field}$ values of pyraclostrobin and the metabolites BF 500-6 and BF 500-7 exceed 100 days. Although the standard HQ value for *T. pyri* and *A. rhopalosiphi* indicated no in-field and no off-field risk (please refer to M-CP 10.3), tests with other soil macro-organisms than earthworms were performed to gain additional information for the risk assessment. Reproduction studies with BAS 516 07 F were carried out with *Folsomia candida* and additionally with *Hypoaspis aculeifer*. The chronic endpoints are summarized in Table 10.4.2-1.

Table 10.4.2-1: Summary of chronic endpoints on *Folsomia candida* and *Hypoaspis aculeifer* for BAS 516 07 F

Test substance	Test species	Endpoint	Value [mg/kg dry soil]	Reference (BASF DocID)
BF 500-6 [1)]	*Folsomia candida*	NOEC	≥ 1000	2013/1068054
BF 500-7 [1)]	*Folsomia candida*	NOEC	≥ 800	2013/1224030
Pyraclostrobin in BAS 516 07 F [1)]	*Folsomia candida*	NOEC	16.8 [2)]	2006/1015861
total a.s. in BAS 516 07 F [1)]			83.8 [2)]	
Pyraclostrobin in BAS 516 07 F [1)]	*Hypoaspis aculeifer*	NOEC	≥ 33.5 [2)]	2014/1010834
total a.s. in BAS 516 07 F [1)]			≥ 167 [2)]	

[1)] Test was conducted with only 5% peat in the test substrate.
[2)] Endpoint recalculated based on the nominal content of 6.7% pyraclostrobin and 26.7% boscalid in BAS 516 07 F.

Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)

For the risk assessment, the chronic toxicity data (NOEC for reproduction) are compared to the predicted environmental concentration considering long-term exposure following multi-year use of BAS 516 07 F. The results are presented in Table 10.4.2-2.

Table 10.4.2-2: Long-term TER values for *Folsomia candida* and *Hypoaspis aculeifer*

Test substance	Test species	NOEC [mg/kg dry soil]	PEC_{soil} [mg/kg dry soil]	TER_{LT}	TER trigger
BF 500-6	*Folsomia candida*	≥ 1000	0.008	≥ 125000	5
BF 500-7	*Folsomia candida*	≥ 800	0.005	≥ 160000	
Pyraclostrobin in BAS 516 07 F	*Folsomia candida*	16.8	0.015	1120	
total a.s. in BAS 516 07 F		83.8	0.260	322	
Pyraclostrobin in BAS 516 07 F	*Hypoaspis aculeifer*	≥ 33.5	0.015	≥ 2233	
total a.s. in BAS 516 07 F		≥ 167	0.260	≥ 642	

All long-term TER values exceed the Commission Regulation (EU) 546/2011 trigger value of 5, indicating that no unacceptable effects are expected for other non-target soil meso- and macrofauna (other than earthworms) when BAS 516 07 F is applied to potatoes according to the proposed use pattern.

CP 10.4.2.1 Species level testing

Report: CP 10.4.2.1/1
Friedrich S., 2006c
Effects of BAS 516 07 F on the reproduction of the collembolans Folsomia candida in artificial soil with 5% peat
2006/1015861
Guidelines: ISO 11267 (1999)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of BAS 516 07 F on mortality and reproduction of the collembolans *Folsomia candida* were investigated in a laboratory study over 28 days. Five application rates (62.5, 125, 250, 500 and 1000 mg BAS 516 07 F/kg dry soil) were incorporated into the soil (5% peat only) with 5 replicates per treatment (each containing 10 juvenile collembolans). An untreated control with 5 replicates was included. Assessment of adult springtail mortality and reproduction rate (number of juveniles) was carried out after 28 days.

Statistically significant mortality was observed at the two highest concentrations tested. In the treatment groups of 500 and 100 mg/kg dry soil mortality rates of 0% to 50% were observed, respectively, compared to 0% in the control. A mean of 775.2 juveniles was counted in the control. In the treatment groups the mean number of juveniles ranged from 637.6 to 786.6 at 62.5 to 500 mg/kg soil, respectively, and was not statistically significantly different compared to the control. The reproduction was significantly inhibited at the highest test concentration of 1000 mg/kg dry soil.

In a 28-d reproduction study on Collembola (*Folsomia candida)* with BAS 516 07 F the NOEC for mortality was 250 mg/kg dry soil and 500 mg/kg dry soil for reproduction. The LC_{50} was determined to be 955 mg BAS 516 07 F/kg dry soil and the EC_{50} (reproduction) was determined to be > 1000 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 516 07 F, batch 1789, content of a.s.: pyraclostrobin (BAS 500 F; Reg. no.:304 428): 6.8% (nominal 6.7%); boscalid (BAS 510 F; Reg. no.:300 355): 26.7% (nominal 26.7%).
Test species:	Collembola (*Folsomia candida*), juveniles (10-12 days old); source: Biologische Bundesanstalt (BBA), Berlin-Dahlem.

B. STUDY DESIGN

Test design:	28-day test in treated artificial soil according to ISO 11267 (5% peat only); artificial soil filled in glass vessels was treated with different concentrations of the test item before collembolans were introduced; 6 treatment groups (5 test item concentrations, control); with 5 replicates each and each containing 10 juvenile collembolans. Assessment of adult collembolan mortality and reproduction rate (number of juveniles) after 28 days.
Endpoints:	Mortality, reproduction rate.
Reference item:	Betosip (phenmedipham, 114 g/L). The effects of the reference item were investigated in a separate study.
Test rates:	Control, 62.5, 125, 250, 500 and 1000 mg BAS 516 07 F/kg dry soil (nominal).
Test conditions:	Artificial soil according to ISO 11267 (with reduced content of peat: 5%); 20% kaolinite clay, 0.3% $CaCO_3$ and 74.7% quartz sand; pH 6.3 - 6.4 at test initiation and 6.3 - 6.4 at test termination; water content 24.4% - 25.9% at test initiation and 24.2% - 24.7% at test termination; temperature: 18C - 21C; photoperiod: 16 h light : 8 h dark, light intensity: 570 lux; food: 2 mg granulated dry yeast at the start of the test and after 14 days.
Statistics:	Descriptive statistics, Fisher`s Exact Test for mortality data, Dunnett-test for reproduction data (α = 0.05), Probit analysis for determination of the LC_{50} value.

II. RESULTS AND DISCUSSION

Statistically significant mortality was observed at the two highest concentrations tested (Fisher`s Exact Binominal Test, α = 0.05). In the treatment groups of 500 and 100 mg/kg dry soil mortality rates of 0% to 50% were observed, respectively, compared to 0% in the control. A mean of 775.2 juveniles was counted in the control. In the treatment groups the mean number of juveniles ranged from 637.6 to 786.6 at 62.5 mg to 500 mg/kg dry soil, respectively, and was not statistically significantly different compared to the control. The reproduction was significantly inhibited at the highest test concentration of 1000 mg/kg dry soil (Dunnett-test, α = 0.05). The results are summarized in Table 10.4.2.1-1.

Table 10.4.2.1-1: Effect of BAS 516 07 F on collembolans (*Folsomia candida)* in a 28-day reproduction study

BAS 516 07 F [mg/kg dry soil]	**Control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality (day 28) [%]	0	0	0	0	22 *	50 *
No. of juveniles (day 28)	775.2	772.4	786.6	738.8	637.6	450.4 *
Reproduction (day 28) [%]	--	100	101	95	82	58
Endpoints [mg/kg dry soil]						
$NOEC_{mortality}$ (day 28)	250					
$NOEC_{reproduction}$ (day 28)	500					
LC_{50} (95% CL) [1)]	955 (774 - 1179)					
$EC_{50,\ reproduction}$	> 1000					

* Statistically significantly different compared to the control (Fisher`s Exact Test for mortality, Dunnett-test for reproduction, α = 0.05).

1) Median effect concentration calculated using probit analysis (with 95% Confidence Limits).

III. CONCLUSION

In a 28 d reproduction study on Collembola (*Folsomia candida*) with BAS 516 07 F the NOEC for mortality was 250 mg/kg dry soil and 500 mg/kg dry soil for reproduction. The LC_{50} was determined to be 955 mg BAS 516 07 F/kg dry soil and the EC_{50} (reproduction) was determined to be > 1000 mg/kg dry soil.

Report:	CP 10.4.2.1/2 Schulz L., 2014b Effects of BAS 516 07 F on the reproduction of the predatory mite Hypoaspis aculeifer 2014/1010834
Guidelines:	OECD 226 (2008)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of BAS 516 07 F on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. The test item was mixed into artificial soil at rates of 31.25, 62.5, 125, 250 and 500 mg BAS 516 07 F/kg dry soil. Test item treatments were replicated four times each. As a control treatment, the soil was left untreated in eight replicates. Each treatment contained 10 adult female soil mites. Assessments of mortality and reproduction were carried out after 14 days of exposure.

Mortality rates of 2.5 - 7.5% were recorded in the test item treatment groups. In the control group the mortality rate was 5.0%. The observed mortality rates for adult mortality in the test item treatment groups compared to control were not statistically significantly different. Differences between the behavior and the morphology of the mites in the control and the test item treatment groups could not be observed.
Reproduction rates in the 31.25, 62.5, 125, 250 and 500 mg BAS 516 07 F/kg dry soil were 223.5, 220.8, 185.3, 211.8, 212.0 juveniles, respectively. The mean reproduction in the control reached 206.1 juveniles. BAS 516 07 F showed no statistically significantly differences on reproduction at all tested concentrations.

In a 14-day reproduction study with BAS 516 07 F on predatory soil mites (*Hypoaspis aculeifer*), the NOEC for mortality and reproduction was determined to be ≥ 500 mg/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 07 F, batch 12-000110, content of a.s.: pyraclostrobin (BAS 500 F; Reg. no.:304 428): 6.7% (nominal 6.7%); boscalid (BAS 510 F; Reg. no.:300 355): 27.1% (nominal 26.7%).

B. STUDY DESIGN

Test species: *Hypoaspis aculeifer* (CANESTRINI), adult mites with an age difference of 3 days; source: in-house culture.

Test design: 14-day chronic laboratory test (according to OECD 226) on effects of BAS 516 07 F on mortality and reproduction of soil mites. 5 different concentrations of the test item were homogenously mixed into artificial soil (5% peat) which was then filled in glass vessels before the soil mites were introduced on top of the soil; 6 treatment groups (5 test item concentrations, control); 8 replicates for the control and 4 replicates for test item treatments, each with 10 soil mites; assessment of adult mortality and reproduction effects (number of juveniles) after 14 days.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate (analysed purity: 99.8%, tolerance ± 1.0%). The effects of the reference item were investigated in a separate study.

Test rates: Control, 31.25, 62.5, 125, 250 and 500 mg BAS 516 07 F/kg dry soil.

Test conditions: Artificial soil according to OECD 226 (5% peat); pH 5.9 – pH 6.3 at test initiation, pH 5.8 - 5.9 at test termination; water content at test initiation 54.04% - 55.94% of maximum water holding capacity (WHC) and 52.95% - 55.71% of maximum WHC at test termination; temperature: 19.7°C - 20.5°C; photoperiod: 16 h light : 8 h dark; light intensity: 517 lux. Feeding of mites with *Tyrophagus putrescentiae* (SCHRANK) at the beginning and *ad libitum* in the course of the test.

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni Correction for mortality ($\alpha = 0.05$, one-sided greater), Dunnett t-test for reproduction ($\alpha = 0.05$, one-sided smaller).

II. RESULTS AND DISCUSSION

Mortality rates of 2.5 - 7.5% were recorded in the test item treatment groups. In the control group the mortality rate was 5.0%. The observed mortality rates for adult mortality in the test item treatment groups compared to control were not statistically significantly different (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). Differences between the behavior and the morphology of the mites in the control and the test item treatment groups could not be observed.
Reproduction rates in the 31.25, 62.5, 125, 250 and 500 mg BAS 516 07 F/kg dry soil were 223.5, 220.8, 185.3, 211.8, 212.0 juveniles, respectively. The mean reproduction in the control reached 206.1 juveniles. BAS 516 07 F showed no statistically significantly differences on reproduction at all tested concentrations (Dunnett-t-test, $\alpha = 0.05$, one-sided smaller).

The results are summarized in table Table 10.4.2.1-2:

Table 10.4.2.1-2: Effects of BAS 516 07 F on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

BAS 516 07 F [mg/kg dry soil]	**Control**	**31.25**	**62.5**	**125**	**250**	**500**
Mortality (day 14) [%]	5.0	2.5	7.5	7.5	5.0	5.0
No. of juveniles (day 14)	206.1	223.5	220.8	185.3	211.8	212.0
Reproduction [% of control] (day 14)	--	108	107	90	103	103
Endpoint [mg BAS 516 07 F/kg dry soil]						
$NOEC_{mortality}$	≥ 500					
$NOEC_{reproduction}$	≥ 500					
LC_{50}	> 500					
EC_{50}	> 500					

III. CONCLUSION

In a 14-day reproduction study with BAS 516 07 F on predatory soil mites (*Hypoaspis aculeifer*), the NOEC for mortality and reproduction was determined to be ≥ 500 mg/kg dry soil.

CP 10.4.2.2 Higher tier testing

Further studies are not triggered.

CP 10.5 Effects on soil nitrogen transformation

The new representative formulation BAS 516 07 F was not evaluated within a previous Annex I inclusion process. It is a water dispersible granule (WG) containing 67 g/kg pyraclostrobin and 267 g/kg boscalid.

The EU agreed endpoints for the soil metabolites of the active substance pyraclostrobin as described in the EU Review Report (SANCO/1420/2001-final, September 2004) plus endpoints from new studies with the new representative formulations BAS 500 06 F and BAS 516 07 F are used for the risk assessment on soil micro-organisms (see Table 10.5-1).

Table 10.5-1: Ecotoxicological endpoints for soil micro-organisms

Test substance	Test design [1)]	EU agreed endpoints	Endpoints used in risk assessment
Pyraclostrobin [2)]	C	NOEC = 3.33 mg a.s./kg dry soil, (equivalent to 2.5 kg a.s./ha)	--
	N	NOEC = 3.33 mg a.s./kg dry soil, (equivalent to 2.5 kg a.s./ha)	--
Pyraclostrobin [3)]	N	--	NOEC = 3.33 mg a.s./kg dry soil (equivalent to 2.5 kg a.s./ha)
BF 500-6	C	NOEC = 1.0 mg/kg dry soil, equivalent to 0.75 kg/ha	
	N	NOEC = 1.0 mg/kg dry soil, equivalent to 0.75 kg/ha	NOEC = 1.0 mg/kg dry soil, equivalent to 0.75 kg/ha
BF 500-7	C	NOEC = 0.5 mg/kg dry soil, equivalent to 0.375 kg/ha	
	N	NOEC = 0.5 mg/kg dry soil, equivalent to 0.375 kg/ha	NOEC = 0.5 mg/kg dry soil, equivalent to 0.375 kg/ha
BAS 516 07 F [4)]	N	--	NOEC = 24 mg/kg dry soil, equivalent to 18 kg/ha

1) C = Carbon transformation, N = Nitrogen transformation.
2) Study was conducted with the pyraclostrobin solo-formulation BAS 500 00 F (250 g pyraclostrobin/L).
3) Study was conducted with the pyraclostrobin solo-formulation BAS 500 06 F (200 g pyraclostrobin/L).
4) The study was performed with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).

Toxicity

The effects of the active substance, the relevant metabolites and the formulated product to soil micro-organisms are summarized in Table 10.5-2. Further details on the studies on nitrogen transformation with the formulated product are given in the summary provided below. According to Regulation 1107/2009 the study on carbon transformation was omitted from the formal data requirements. However, from previous submissions according to the former Directive EC 91/414 data on carbon transformation is available for BAS 516 07 F and is therefore presented at the end of this chapter as additional information without consideration in the risk assessment.

Table 10.5-2: Toxicity of BAS 516 07 F, BF 500-6 and BF 500-7 to soil micro-organisms

Test substance	Endpoint	NOEC	Reference (BASF DocID)
BF 500-6	Effects on nitrogen and carbon transformation	1.0 mg/kg dry soil	1999/11311 1999/11120
BF 500-7	Effects on nitrogen and carbon transformation	0.5 mg/kg dry soil	1999/11311 1999/11120
Pyraclostrobin [1)]	Effects on nitrogen transformation	3.33 mg a.s./kg dry soil (equivalent to 2.5 kg a.s./ha)	2012/1129443
BAS 516 07 F [2)]	Effects on nitrogen transformation	24.0 mg/kg dry soil (equivalent to 18.0 kg/ha)	2001/1005959
	Effects on carbon transformation [3)]	24.0 mg/kg dry soil (equivalent to 18.0 kg/ha)	2001/1005960

1) Study was carried out with BAS 500 06 F as a surrogate for pyraclostrobin. For the study summary please refer to the formulation dossier of BAS 500 06 F, M-CP 10.5.
2) The study was performed with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).
3) Not considered in risk assessment since not a formal data requirement according to Regulation 1107/2009

Exposure

Table 10.5-3: Critical use pattern of BAS 516 07 F

Crop	Application time (BBCH code)	Number of applications	Interval [d]	Application rate per treatment		
				Boscalid [g a.s./ha]	Pyraclostrobin [g a.s./ha]	BAS 516 07 F [g/ha]
potatoes	51 - 89	4	10	67	17	250

The predicted environmental concentrations of pyraclostrobin and relevant metabolites in soil (PEC_{soil}) were calculated as described in M-CP 9.1. The resulting maximum PEC_{soil} values are presented in Table 10.5-4.

Table 10.5-4: PEC_{soil} values for pyraclostrobin, relevant metabolites and boscalid

Test substance	$PEC_{soil, max}$ [mg/kg dry soil]	$PEC_{soil, plateau}$ [mg/kg dry soil]	$PEC_{soil, accu}$ [mg/kg dry soil]
Pyraclostrobin	0.015	--	--
BF 500-6	0.004	0.004	0.008
BF 500-7	0.003	0.002	0.005
Boscalid	0.069	--	0.245

Risk assessment for Soil Nitrogen Transformation

BAS 516 07 F (tested as minor change formulation BAS 516 00 F) had no significant effect on soil micro-organisms at 24 mg BAS 516 07 F/kg dry soil, corresponding to 1.6 mg pyraclostrobin/kg dry soil and 8.0 mg total a.s./kg dry soil. These concentrations are approximately 107-times higher than the maximum $PEC_{soil, max}$ of 0.015 mg pyraclostrobin/kg dry soil - and approximately 31-times higher than the sum of the highest PEC-values for both a.s., i.e. 0.260 mg total a.s./kg dry soil. This supports the conclusion that under field conditions, the use of BAS 516 07 F in potatoes at the recommended rates poses no unacceptable risk to non-target soil micro-organisms.

Furthermore, the NOEC values for the metabolites of 1.0 mg BF 500-6/kg dry soil and 0.5 mg BF 500-7/kg dry soil are 125- and 100-times higher than the maximum $PEC_{soil, accu}$ values of 0.008 mg BF 500-6/kg dry soil and 0.005 mg BF 500-7/kg dry soil, respectively.

It is concluded that the proposed use of BAS 516 07 F will not pose an unacceptable risk to non-target soil micro-organisms, when applied in potatoes according to the recommended use pattern.

Report:	CP 10.5/1 Wachter S., 2001a Assessment of the side effects of BAS 516 00 F on the activity of the soil microflora; nitrogen turnover 2001/1005959
Guidelines:	BBA VI 1-1 (1990)
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

The effect of BAS 516 00 F on nitrogen transformation was tested in a lucerne-enriched loamy sand soil and a loamy silt soil.
BAS 516 00 F was applied to samples of the soils, in a laboratory, at nominal application rates of 2.4 mg and 24 mg BAS 516 00 F/kg dry soil. The treated soils and untreated controls were incubated at 20 ± 2°C in the dark for 28 days. Triplicate samples of each treatment were removed for analysis of NH_4-nitrogen and NO_3-nitrogen, using calibrated ion-selective electrodes connected to an Orion expandable ion analyzer, 0, 14, 28 and 56 days after application.

No adverse effects of BAS 516 00 F on nitrogen transformation could be observed in both soils at both tested rates (single and ten-fold application rate) after 28 days (loamy silt soil) and 56 days (loamy sand soil).

Based on the results of this study, BAS 516 00 F caused no long-term effects (OECD 216) on the soil nitrogen turnover in both field soils tested at concentrations equivalent to field application rates of 1.8 kg BAS 516 00 F/ha (corresponding to 0.12 kg pyraclostrobin/ha and 0.48 kg boscalid/ha) and ten-fold that rate (i.e. 18 kg BAS 516 00 F/ha).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).

Test species: Biologically active agricultural soils: 1) loamy sand: pH 6.9, 1.06% C_{org}, 40.8% WHC. 2) loamy silt: pH 6.5, 2.78% C_{org}, 48.2% WHC.

B. STUDY DESIGN

Test design: Determination of the N-transformation in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil and a reference item treated soil. Three replicates per treatment and concentration. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen from the nitrification process was determined by using calibrated ion-selective electrodes connected to an Orion expandable ion analyzer EA 940. Sampling scheme: 0, 14, 28 and 56 days after treatment, aliquots were withdrawn and subjected to the measurement.

Test concentrations: Control, 2.4 mg BAS 516 00 F per kg dry soil (corresponding to an application rate of 1.8 kg BAS 516 00 F/ha) and 24 mg BAS 516 00 F per kg soil (corresponding to an application rate of 18 kg BAS 516 00 F/ha) and reference item. The reference item was applied at a rate of 6.7 mg Dinoterb/kg dry soil (corresponding to an application rate of 5.0 kg Dinoterb/ha). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

Reference item: Herbogil liquide (Dinoterb, 250 g/L).

Test conditions: Soil moisture: 40% (loamy sand soil) to 60% (loamy silt soil) of its water holding capacity. Soil samples were incubated at 20°C ± 2°C while stored in glass bottles.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of BAS 516 00 F on nitrogen transformation could be observed in both soils at both tested rates (single and ten-fold application rate) after 28 days (loamy silt soil) and 56 days (loamy sand soil). The results are summarized in Table 10.5-5.

Table 10.5-5: Effects of BAS 516 00 F on soil micro-organisms (nitrogen transformation) on days 14 and 28 of incubation (additionally on day 56 for the loamy sand soil)

Soil (days)	Control	2.4 mg BAS 516 00 F/kg dry soil (equivalent to 1.8 kg/ha)		24 mg BAS 516 00 F/kg dry soil (equivalent to 1.8 kg/ha)	
	NO_3-N [mg/100 g dry soil]	NO_3-N [mg/100 g dry soil]	% Deviation from control [1)]	NO_3-N [mg/100 g dry soil]	% Deviation from control [1)]
Loamy sand (14 d)	1.88	2.87	+52.69	3.31	+75.97
Loamy sand (28 d)	3.39	4.10	+21.03	4.65	+37.26
Loamy sand (56 d)	4.99	4.69	-5.93	5.21	+4.42
Loamy silt (14 d)	7.83	7.70	-1.73	8.31	+6.07
Loamy silt (28 d)	8.54	8.44	-1.16	8.22	-3.73

[1)] Based on NO_3-nitrogen production; - = inhibition; + = stimulation.

A clear effect of the reference item Dinoterb was observed in both soils (54 and 29% increase).

III. CONCLUSION

Based on the results of this study BAS 516 00 F caused no long-term effects (OECD 216) on the soil nitrogen turnover in both field soils tested at concentrations equivalent to field application rates of 1.8 kg BAS 516 00 F/ha (corresponding to 0.12 kg pyraclostrobin/ha and 0.48 kg boscalid/ha) and ten-fold that rate (i.e. 18 kg BAS 516 00 F/ha).

Report:	CP 10.5/2 Wachter S., 2001c Assessment of the side effects of BAS 516 00 F on the activity of the soil microflora; short-term respiration 2001/1005960
Guidelines:	BBA VI 1-1 (1990)
GLP:	yes (certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

The effect of BAS 516 00 F on carbon transformation was investigated in the field on a loamy sand soil and a loamy silt soil.

BAS 516 00 F was applied to samples of the soil, in a laboratory, at nominal application rates of 2.4 mg/kg and 24 mg/kg of dry soil. BAS 516 00 F treated soils and untreated controls were incubated at 20 ± 2°C in the dark for 28 days. Three replicate samples of each treatment were removed for analysis of carbon transformation (oxygen consumption), using an OxiTop® system, 0, 14 and 28 days after application.

No adverse effects of BAS 516 00 F on C-transformation could be observed in both soils at both tested rates (single and ten-fold application rate) after 28 days.

Based on the results of this study BAS 516 00 F caused no long-term effects (OECD 217) on the soil respiration in both field soils tested at concentrations equivalent to field application rates of 1.8 kg BAS 516 00 F/ha (corresponding to 0.48 kg boscalid/ha and 0.12 kg pyraclostrobin/ha) and ten-fold that rate (i.e. 18 kg BAS 516 00 F/ha).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 516 00 F; batch no. 2000-2; content of a.s.: boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%); pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%).
Test species:	Biologically active agricultural soils: 1) loamy sand: pH 6.9, 1.06% C_{org}, 40.8% WHC; 2) loamy silt: pH 6.5, 2.78% C_{org}, 48.2% WHC.

B. STUDY DESIGN

Test design:	Determination of C-transformation in soil after addition of glucose. Comparison of test item treated soil with a non-treated and a reference item treated soil. Three replicates per treatment and concentration. An OxiTop® system was used to measure the CO_2 production over a period of maximum 24 hours at different sampling intervals. Sampling scheme: 0, 14 and 28 days after treatment, aliquots were withdrawn and subjected to the measurement.
Test concentrations:	Control, 2.4 mg BAS 516 00 F per kg dry soil (corresponding to an application rate of 1.8 kg BAS 516 00 F/ha), and 24 mg BAS 516 00 F per kg soil (corresponding to an application rate of 18 kg BAS 516 00 F/ha) and reference substance. The reference item was applied at a rate of 6.7 mg dinoterb/kg dry soil (corresponding to an application rate of 5.0 kg dinoterb/ha). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.
Reference item:	Herbogil liquide (dinoterb, 250 g/L).
Test conditions:	Soil moisture: 40% (loamy sand soil) to 60% (loamy silt soil) of its water holding capacity. Soil samples were incubated at 20°C ± 2°C while stored in glass bottles.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

No adverse effects of BAS 516 00 F on C-transformation could be observed in both soils at both tested rates (single and ten-fold application rate) after 28 days (see Table 10.5-6).

Table 10.5-6: Effects of BAS 516 00 F on soil micro-organisms (carbon transformation) on days 14 and 28 of incubation

Soil (days)	Control	2.4 mg BAS 516 00 F/kg dry soil (equivalent to 1.8 kg/ha)		24 mg BAS 516 00 F/kg dry soil (equivalent to 1.8 kg/ha)	
	O_2 consumption [mg/h/100 g dry soil]	O_2 consumption [mg/h/100 g dry soil]	% Deviation from control [1)]	O_2 consumption [mg/h/100 g dry soil]	% Deviation from control [1)]
Loamy sand (14 d)	0.43	0.41	-5.17	0.39	-10.41
Loamy sand (28 d)	0.44	0.43	-1.99	0.43	-1.60
Loamy silt (14 d)	2.33	2.12	-9.02	2.15	-8.05
Loamy silt (28 d)	1.84	1.71	-6.79	1.57	-14.53

[1)] Based on O_2 consumption; - = inhibition; + = stimulation

The reference item dinoterb produced in both soils the expected level of effect (26 and 16% inhibition).

III. CONCLUSION

Based on the results of this study, BAS 516 00 F caused no long-term effects (OECD 217) on the soil respiration in both field soils tested at concentrations equivalent to field application rates of 1.8 kg BAS 516 00 F/ha (corresponding to 0.48 kg boscalid/ha and 0.12 kg pyraclostrobin/ha) and ten-fold that rate (i.e., 18 kg BAS 516 00 F/ha).

CP 10.6 Effects on terrestrial non-target higher plants

The new representative formulation BAS 516 07 F was not evaluated within a previous Annex I inclusion process. It is a water dispersible granule (WG) containing 67 g/kg pyraclostrobin and 267 g/kg boscalid.

Endpoints from two studies with the new representative formulation BAS 516 07 F are used for the risk assessment on non-target higher plants (see Table 10.6-1).

Table 10.6-1: Ecotoxicological endpoints for non-target higher plants

Test substance	Test system	Test species	EU agreed endpoints	Endpoints used in risk assessment
BAS 516 07 F	Vegetative vigor	Carrot, lettuce, oilseed rape, cabbage, soybean, tomato, onion, ryegrass, wheat and maize	--	ER_{50} > 8.0 kg/ha
BAS 516 07 F	Seedling emergence	Carrot, lettuce, oilseed rape, cabbage, soybean, tomato, onion, ryegrass, wheat and maize	--	ER_{50} > 8.0 kg/ha

Toxicity

Studies on potential effects of BAS 516 07 F on vegetative vigour and seedling emergence have been carried out with ten plant species. Furthermore, a study on vegetative vigour of six plant species was conducted with the minor change formulation BAS 516 00 F (for the detailed composition of both products please refer to Document JCP 1.4.1). In conclusion there were no unacceptable effects on vegetative vigour and seedling emergence at rates up to and including 8.0 kg BAS 516 07 F/ha for all ten species and no unacceptable effects on vegetative vigour up to and including 5.4 kg BAS 516 00 F/ha for all six species tested (the latter study given only for completeness and not being used in the risk assessment). Further details of the studies are given in M-CP 10.6.2.

Table 10.6-2: Summary of effects on terrestrial non-target plants following exposure to BAS 516 07 F

Test substance	Test species	Test system	ER_{50} [kg/ha]	Reference (BASF DocID)
BAS 516 07 F [1)]	Carrot, sunflower, pea, cabbage, onion, oat	Vegetative vigor	> 5.4	2001/1005953
BAS 516 07 F	Carrot, lettuce, oilseed rape, cabbage, soybean, tomato, onion, ryegrass, wheat and maize	Vegetative vigor	> 8.0	2014/1010836
BAS 516 07 F	Carrot, lettuce, oilseed rape, cabbage, soybean, tomato, onion, ryegrass, wheat and maize	Seedling emergence	> 8.0	2014/1010835

1) Test was carried out with BAS 516 00 F, which is a minor change formulation of BAS 516 07 F (the detailed composition of both products is given in Document JCP 1.4.1).

Exposure

Table 10.6-3: Critical use pattern

Crop	Application time (BBCH code)	Number of applications	Interval [d]	Application rate per treatment		
				Boscalid [g a.s./ha]	Pyraclostrobin [g a.s./ha]	BAS 516 07 F [g/ha]
potatoes	51 - 89	4	10	67	17	250

Effects on non-target plants are of concern in the off-field environment, where they may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the BBA [*BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden.*] from the spray-drift predictions of Ganzelmeier & Rautmann (2000) [*Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application*]. Only a single application was considered, because factors like plant growth will reduce residues per unit area between multiple applications.
For a single application to vegetables (i.e. potatoes), 2.77% of the application rate was assumed to reach areas at 1 m from the edge of the crop (worst-case scenario). The highest single application rate of BAS 516 07 F is 0.250 kg product/ha, giving a maximum off-field predicted environmental rate ($PER_{off\text{-}field}$) of 0.007 kg product/ha.

Risk assessment for terrestrial non-target higher plants

BAS 516 07 F is a fungicide and therefore this product is not expected to have any significant herbicidal activity. Profiling studies of the effects on post- and pre-emergence of non-target higher plants were conducted and showed no effects on any of ten species tested at rates up to and including 8.0 kg BAS 516 07 F/ha.

According to the Terrestrial Guidance Document [*Anonymous (2002b). Guidance Document on terrestrial ecotoxicology under council directive 91/414/EEC. SANCO/10329/2002. 17 October 2002*], the risk to non-target plants should be considered acceptable if less than 50% effect on all ten species is seen at the maximum single application rate. Less than 50% effect on vegetative vigor and seedling emergence were observed on all tested species at the highest tested rate of 8.0 kg BAS 516 07 F/ha. The calculated maximum $PER_{off\text{-}field}$ of 0.007 kg product/ha is far below these test rates.

In conclusion, BAS 516 07 F poses no unacceptable risk to terrestrial non-target plants in off-crop areas following the intended use in potatoes.

CP 10.6.1 Summary of screening data

Tests on non-target plants have been conducted. The data point is covered by M-CP 10.6.2.

CP 10.6.2 Testing on non-target plants

Report:	CP 10.6.2/1 Oberwalder C., Schmidt O., 2001a BAS 516 00 F: Effects on non-target plants in the greenhouse - A limit test 2001/1005953
Guidelines:	EPA 850.4150, EPA 850.4000, OECD 208 B (Draft 1999), OECD 208 A (Draft 1999), EPA 712-C-96-163
GLP:	no

Executive Summary

In a vegetative vigor test four species of dicotyledonous plants (cabbage, carrot, sunflower, pea) and two species of monocotyledonous plants (oats, onion) were exposed to BAS 516 00 F. Nominal application rates tested were 1.8 and 5.4 kg BAS 516 00 F per ha. Following the application, the plants were cultivated for 14 days under greenhouse conditions. Assessment of phytotoxicity was carried out 7 and 14 days after application. Fresh weight was determined at study termination 14 DAA.

After treatment with BAS 516 00 F slight injuries could be seen in carrot plants (13% at 5.4 kg/ha) at study termination. Besides that, no clearly treatment related or only negligible visible phytotoxic effects were observed in the plant species tested. For all plant species the plant weight was at the same level as the weight of the control plants. No statistically significant differences were observed. Hence the observed plant damages were without impact on plant growth.

Based on the results of this study it can be concluded that BAS 516 00 F has only negligible phytotoxic potential to terrestrial non-target plants if applied at a rate of 5.4 kg BAS 516 00 F/ha. The ER_{50} value for all plant species is > 5.4 kg BAS 516 00 F/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 00 F; batch no. 2000-2; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.6% (nominal 6.7%); boscalid (BAS 510 F, Reg. No. 300 355): 27.9% (nominal 26.7%).

Plant species: Oats *Avena sativa*; onion *Allium cepa*; cabbage *Brassica oleracea*; pea *Pisum sativum*; carrot *Daucus carota*; sunflower *Helianthus annuus*.

B. STUDY DESIGN

Test design: Limit test; three treatment groups: two treatment rates plus a water treated control; 4 replicates/treatment group; 1 pot/replicate, 3-5 plants per pot (species dependent); greenhouse cultivation; BAS 516 00 F was applied post-emergence at growth stage BBCH 11-14 using a laboratory spray cabin at a water rate of 400 L/ha. Following the application, the plants were cultivated for 14 days in the greenhouse. Assessments for phytotoxicity (e.g. scorch, stunting, deformations) were done approximately 7 days after application (DAA) and 14 DAA. Immediately after the 14 DAA assessment the fresh weight of the plant biomass above ground was determined.

Test rates: 1.8 kg BAS 516 00 F/ha (equivalent to 121 g Pyraclostrobin/ha and 481 g Boscalid/ha) and 5.4 kg BAS 516 00 F/ha (equivalent to 362 g Pyraclostrobin/ha and 1442 g Boscalid/ha).

Test conditions: Greenhouse conditions: temperatures between 14°C and maximum 31°C; humidity: about 80%; photoperiod: 16 h light, 8 h dark; additional light when outdoor illumination was less than 4500 lux.

Statistics: Descriptive statistics, HSD test($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After treatment with BAS 516 00 F slight injuries could be seen in carrot plants (13% at 5.4 kg/ha) at study termination. Besides that, no clearly treatment related or only negligible visible phytotoxic effects were observed in the plant species tested. For all plant species the plant weight was at the same level as the weight of the control plants. No statistically significant differences were observed (HSD test; $\alpha = 0.05$). Hence the observed plant damages were without impact on plant growth (see Table 10.6.2-1 below).

Table 10.6.2-1: Effect of BAS 516 00 F on plant biomass and plant condition (visible damage) 14 DAA

Treatment	Cabbage	Carrot	Sunflower	Pea	Oats	Onion
Mean plant weight [% of control]						
Control	100.0	100.0	100.0	100.0	100.0	100.0
1.8 kg/ha	98.1	92.0	86.8	100.6	99.8	99.4
5.4 kg/ha	99.1	76.9	100.9	90.6	91.1	138.8
Mean visible damage [% damage compared to control]						
Control	0	0	0	0	0	0
1.8 kg/ha	0	3	8	0	0	0
5.4 kg/ha	1	13	8	1	0	0

III. CONCLUSION

Based on the results of this study it can be concluded that BAS 516 00 F has only negligible phytotoxic potential to terrestrial non-target plants if applied at a rate of 5.4 kg BAS 516 00 F/ha. The ER_{50} value for all plant species is > 5.4 kg BAS 516 00 F/ha.

Report:	CP 10.6.2/2 Stroemel C., Brockmann A., 2014a Effect of BAS 516 07 F on vegetative vigour of ten species of terrestrial plants under greenhouse conditions 2014/1010836
Guidelines:	OECD 227 July 2006, EPA 850.4150, EPA 712-C-011
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

Executive Summary

In a vegetative vigor test, six species of dicotyledonous plants (carrot, lettuce, oilseed rape, cabbage, soyabean, tomato) and four species of monocotyledonous plants (onion, rye grass, wheat, maize) were exposed to BAS 516 07 F to evaluate the phytotoxic potential of the test item. BAS 516 07 F was applied post-emergence at BBCH 12 – 14 in a dose-response design at test rates of 0.5, 1.0, 2.0, 4.0 and 8.0 kg BAS 516 07 F /ha. The plants were cultivated for 21 days after application under greenhouse conditions. Assessment of phytotoxicity was done 7, 14 and 21 days after application; plant length and dry weight was determined at study termination.

All tested species showed no phytotoxic symptoms after application of BAS 516 07 F up to and including 8.0 kg/ha. No plant mortality was observed for all tested plant species following the application of BAS 516 07 F up to and including the highest tested rate. No dose response for plant survival could be calculated for all tested species. Plant length was not substantially influenced by BAS 516 07 F up to and including 8.0 kg/ha for all tested plant species. However, a significant reduced plant length of 6% was found for carrot after application of 8.0 kg BAS 516 07 F/ha and between 3 and 5% in all treatments of wheat (except 2.0 kg/ha). Based on the actual data these reduced plant length can be considered as ecologically non-relevant. Therefore, based on expert judgement the NOER for all tested plant species determined to be equal to or higher than the highest testes rate of 8.0 kg BAS 516 07 F/ha. No reduction of plant dry weight was observed for all tested plant species after application of up to and including 8.0 kg BAS 516 07 F/ha.

Based on the results of this study, conducted under worst-case greenhouse conditions, it can be concluded that post-emergence application of BAS 516 07 F with rates up to and including 8.0 kg/ha did not cause adverse effects in terms of phytotoxicity, plant length and plant weight for carrot, lettuce, oilseed rape, cabbage, soybean, tomato, onion, ryegrass, wheat and maize. For all tested species, ER_{50} values for plant survival, plant length and plant weight was > 8.0 kg/ha.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 07 F; batch no. 12-000110; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.7% (nominal 6.7%); boscalid (BAS 510 F, Reg. No. 300 355): 27.1% (nominal 26.7%)

B. STUDY DESIGN

Test species: Carrot (*Daucus carota*), lettuce (*Lactuca sativa*), oilseed rape (*Brassica napus*), cabbage (*Brassica oleracea*), soybean (*Glycine max*), tomato (*Lycopersicon esculentum*), onion (*Allium cepa*), rye grass (*Lolium multiflorum*), wheat (*Triticum aestivum*) and maize (*Zea mays*).

Test design: Rate-response design; 6 treatments (5 test item rates, control) per plant species; 5 replicates per treatment, comprising 1-2 pot with 3 or 8 plants per replicate depending on species at application; BAS 516 07 F was applied post-emergence at BBCH 12 - 14 using a laboratory spray cabin (applied water volume 238 or 236 L/ha); greenhouse cultivation for 21 days after application; assessments of plant damage and plant survival were done 7, 14 and 21 days after application (DAA); Plant length and shoot dry weight was determined at 21 DAA.

Endpoints: NOER, ER_{25}, ER_{50}.

Test rates: Control (tap water), 0.5, 1.0, 2.0, 4.0 and 8.0 kg BAS 516 07 F/ha.

Test conditions: Greenhouse conditions: daily average temperature: 19.6°C to 24.4°C (extremes 11°C and 37°C only for short time); daily mean relative humidity: 37.1% to 74.5% (extremes 14% and 90% relative humidity, mean relative humidity lower than 45% for lettuce, oilseed rape, cabbage, tomato, wheat and maize for some days); photoperiod: day length ≥ 16 hours; additional light supply automatically when outdoor illumination was less than 10 klux.

Statistics: Descriptive statistics. Welch t-test for inhomogeneous variances with Bonferroni adjustment, Dunnett's t-test for homogenous variances.

II. RESULTS AND DISCUSSION

All tested species showed no phytotoxic symptoms after application of BAS 516 07 F up to and including 8.0 kg/ha. No plant mortality was observed for all tested plant species following the application of BAS 516 07 F up to and including the highest tested rate. No dose response for plant survival could be calculated for all tested species. Plant length was not influenced by BAS 516 07 F up to and including 8.0 kg/ha for all tested plant species. A statistically significant reduced plant length of 6% was found for carrot after application of 8.0 kg BAS 516 07 F/ha (Dunnett´s t-test, α=0.05). In wheat all treatments (except 2.0 kg/ha) are indicated as statistically significant different from untreated control with reductions between 3 and 5% (Dunnett´s t-test, α=0.05). Based on the actual data these reduced plant length can be considered as ecologically non-relevant. No negative influence of BAS 516 07 F on plant dry weight was observed for all tested plant species after application of 8.0 kg BAS 516 07 F/ha.

The results are summarized in Table 10.6.2-2 and Table 10.6.2-3.

Table 10.6.2-2: Effect of BAS 516 07 F on phytotoxicity, plant length and plant dry weight 21 DAA

Treatment [kg/ha]	Carrot	Lettuce	Oilseed rape	Cabbage	Soybean	Tomato	Onion	Ryegrass	Wheat	Maize
Phytotoxic effects [%]										
control	0	0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0	0	0
1.0	0	0	0	0	0	0	0	0	0	0
2.0	0	0	0	0	0	0	0	0	0	0
4.0	0	0	0	0	0	0	0	0	0	0
8.0	0	0	0	0	0	0	0	0	0	0
Plant length [% to control]										
0.5	99.6	98.8	99.5	101.1	99.6	95.6	107.3	101.3	95.9 *	104.2
1.0	97.4	98.5	98.5	99.6	105.5	96.3	104.2	98.3	94.9 *	101.8
2.0	97.3	98.8	99.3	103.8	102.4	94.9	107.2	102.9	97.4	106.3
4.0	97.2	101.1	101.6	103.5	103.2	97.2	105.1	102.0	96.7 *	105.1
8.0	93.5 *	100.3	107.9	102.1	100.5	96.8	102.0	101.8	96.8 *	101.2
Plant dry weight [% to control]										
0.5	100.5	100.5	104.9	96.0	99.0	95.7	111.0	100.2	103.7	101.9
1.0	101.5	105.2	106.1	96.7	104.0	96.2	106.2	95.3	99.9	104.9
2.0	94.6	102.1	105.0	102.2	102.9	91.4	113.0	102.1	101.0	120.2
4.0	96.3	98.0	103.4	101.7	99.0	96.4	109.7	103.4	104.7	112.1
8.0	94.8	97.1	108.8	98.9	99.3	94.5	104.5	101.7	101.2	100.2

* Statistically significantly different to the untreated control (Dunnett´s t-test, α=0.05)

Table 10.6.2-3: NOER, ER_{25}, and ER_{50} of BAS 516 07 F for non-target plants 21 DAA

Endpoint [kg/ha]	Carrot	Lettuce	Oilseed rape	Cabbage	Soybean	Tomato	Onion	Ryegrass	Wheat	Maize
Phytotoxicity										
NOER	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0
Plant length										
NOER	≥ 8.0 [1)]	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0 [1)]	≥ 8.0
ER_{25}	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0
ER_{50}	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0
Plant dry weight										
NOER	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0
ER_{25}	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0
ER_{50}	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0

[1)] Expert judgement

III. CONCLUSION

Based on the results of this study, conducted under worst-case greenhouse conditions, it can be concluded that post-emergence application of BAS 516 07 F with rates up to and including 8.0 kg/ha did not cause adverse effects in terms of phytotoxicity, plant length and plant weight for carrot, lettuce, oilseed rape, cabbage, soybean, tomato, onion, ryegrass, wheat and maize. For all tested species, ER_{50} values for plant survival, plant length and plant weight was > 8.0 kg/ha.

Report:	CP 10.6.2/3 Stroemel C., Brockmann A., 2014b Effect of BAS 516 07 F on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions 2014/1010835
Guidelines:	OECD 208 (2006), EPA 850.4100, EPA 712-C-012
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

Executive Summary

In a seedling emergence test, six species of dicotyledonous plants (carrot, lettuce, oilseed rape, cabbage, soyabean, tomato) and four species of monocotyledonous plants (onion, rye grass, wheat, maize) were exposed to BAS 516 07 F. The test item was applied pre-emergence in a dose-response-design with concentrations 0.5, 1.0, 2.0, 4.0 and 8.0 kg/ha in 234 L water/ha. Following the application, the plants were cultivated for 21 days (28 days for onion and carrot) under greenhouse conditions. Assessments on plant emergence, survival and length were carried out at 7, 14, 21 DAA (14, 21 and 28 DAA for onion and carrot) and on dry weight at 21 DAA (28 DAA for onion and carrot).

None of the tested plant species showed phytotoxic symptoms after application up to and including 8.0 kg BAS 516 07 F/ha pre emergence. No reduction of plant length was observed for all tested plant species following the application of BAS 516 07 F. The statistically significant difference found for oilseed rape at rates of 1.0 kg and 2.0 kg BAS 516 07 F/ha, but not at higher rates can be considered as not treatment related. No negative influence of BAS 516 07 F on plant dry weight was observed for all tested plant species up to and including 8.0 kg BAS 516 07 F/ha.

Based on the results of this study, conducted under worst-case greenhouse conditions, it can be concluded that pre-emergence application of BAS 516 07 F caused no unacceptable adverse effects up to and including the highest rate of 8.0 kg/ha to the seedling emergence and survival of all tested plant species.
The NOER for phytotoxicity of all tested plant species was determined to be ≥ 8.0 kg BAS 516 07 F/ha. The ER_{25} and ER_{50} values for plant length, plant dry weight and seedling emergence were determined to be > 8.0 kg BAS 610 06 F/ha for all tested plant species.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 516 07 F; batch no. 12-000110; content of a.s.: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 6.7% (nominal 6.7%); boscalid (BAS 510 F, Reg. No. 300 355): 27.1% (nominal 26.7%).

B. STUDY DESIGN

Test species: Carrot (*Daucus carota*); lettuce (*Lactuca sativa*); oilseed rape (*Brassica napus ssp. napus*); cabbage *(Brassica oleracea. var. capitata f. alba);* soybean (*Glycine max);* tomato (*Lycopersicon esculentum);* onion *(Allium cepa*); ryegrass *(Lolium multiflorum);* wheat *(Triticum aestivum)*; maize (*Zea mays*)

Test design: Dose-response design; 6 treatment groups (5 test item rates, water-treated control) with 4 replicates; 1-2 pots/replicate with 5 or 10 seeds/pot; greenhouse cultivation; application pre-emergence with 234 L water/ha using a laboratory spray chamber. Following the application, all plants were grown for at least 21 days (28 for onion and carrot). Plant emergence, survival and length were assessed at 7, 14, 21 DAA (14, 21 and 28 DAA for onion and carrot), dry weight at 21 DAA (28 DAA for onion and carrot).

Endpoints: NOER, ER_{25}, ER_{50}.

Test rates: Control (tap water), 0.5, 1.0, 2.0, 4.0 and 8.0 kg BAS 516 07 F/ha.

Test conditions: Greenhouse conditions: daily average temperature ranged from 19.9°C to 24.9°C (extremes ranged from 13°C to 35°C, 35°C for maize and cabbage for one hour at one day, for oilseed rape and wheat for 1 hour at two days); daily mean relative humidity ranged from 36.9% to 59.2% (extremes 14% and 84% relative humidity, mean relative humidity lower than 45% for carrot, oilseed rape, soybean, tomato, onion and wheat at several days); photoperiod: day length ≥ 16 hours; additional light supply automatically when outdoor illumination was less than 10 klux.

Statistics: Descriptive statistics. Dunnett's t-test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

No control mortality > 10% and no other adverse effects on control plants were observed.

None of the tested plant species showed phytotoxic symptoms after application up to and including 8.0 kg BAS 516 07 F/ha pre emergence. No reduction of plant length was observed for all tested plant species following the application of BAS 516 07 F. The statistically significant difference found for oilseed rape at rates of 1.0 kg and 2.0 kg BAS 516 07 F/ha, but not at higher rates can be considered as not treatment related (Dunnett's t-test, $\alpha = 0.05$). No negative influence of BAS 516 07 F on plant dry weight was observed for all tested plant species up to and including 8.0 kg BAS 516 07 F/ha. The results are summarized in Table 10.6.2-4 and Table 10.6.2-5.

Table 10.6.2-4: Effect of BAS 516 07 F on phytotoxicity, seedling emergence, plant length and plant biomass 21 DAA (28 DAA for onion and carrot)

Treatment [kg/ha]	Carrot	Lettuce	Oilseed rape	Cabbage	Soybean	Tomato	Onion	Ryegrass	Wheat	Maize
Phytotoxic effects [%]										
Control	0	0	0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0	0	0	0
1.0	0	0	0	0	0	0	0	0	0	0
2.0	0	0	0	0	0	0	0	0	0	0
4.0	0	0	0	0	0	0	0	0	0	0
8.0	0	0	0	0	0	0	0	0	0	0
Plant length [% of control]										
0.5	100.4	96.5	95.1	104.0	99.5	105.6	100.8	104.0	101.9	97.9
1.0	100.4	103.3	93.6 *	107.3	99.4	102.3	100.0	101.5	101.6	98.4
2.0	102.8	98.1	93.5 *	103.8	97.4	100.8	101.8	103.3	102.3	98.8
4.0	100.9	99.5	95.4	99.3	97.2	100.2	101.1	101.0	102.1	98.1
8.0	103.8	96.8	96.5	107.5	98.2	103.2	97.4	97.6	100.4	98.8
Plant dry weight [% of control]										
0.5	101.7	109.6	101.7	122.1	100.4	107.1	97.7	101.0	103.3	101.6
1.0	104.5	105.1	97.8	130.3	106.0	110.5	96.1	89.9	96.1	95.0
2.0	110.6	116.2	99.1	121.5	103.0	110.0	97.3	109.1	99.3	105.9
4.0	107.5	97.9	92.7	116.2	96.2	104.0	91.7	95.1	101.0	101.4
8.0	107.9	97.9	102.5	133.3	102.1	98.5	95.1	91.8	97.8	98.4
Seedling emergence [%]										
Control	90	83	93	85	78	90	93	93	100	100
0.5	88	78	90	95	83	85	95	93	100	100
1.0	93	80	95	93	80	100	98	88	98	100
2.0	95	83	98	95	83	93	90	98	98	100
4.0	90	80	95	95	75	88	95	93	98	98
8.0	95	80	95	93	80	85	88	93	100	98

* Statistically significantly different to the untreated control (Dunnett´s t-test, α=0.05)

Table 10.6.2-5: NOER, ER_{25} and ER_{50} of BAS 516 07 F for non-target plants 21 DAA (28 DAA for onion and carrot)

Endpoints [kg/ha]	Carrot	Lettuce	Oilseed rape	Cabbage	Soybean	Tomato	Onion	Ryegrass	Wheat	Maize
Plant survival										
NOER	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0
Plant length										
NOER	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0
ER_{25}	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0
ER_{50}	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0
Plant dry weight										
NOER	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0
ER_{25}	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0
ER_{50}	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0
Seedling emergence										
NOER	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0	≥ 8.0
ER_{25}	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0
ER_{50}	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0	> 8.0

III. CONCLUSION

Based on the results of this study, conducted under worst-case greenhouse conditions, it can be concluded that pre-emergence application of BAS 516 07 F caused no unacceptable adverse effects up to and including the highest rate of 8.0 kg/ha to the seedling emergence and survival of all tested plant species.
The NOER for phytotoxicity of all tested plant species was determined to be ≥ 8.0 kg BAS 516 07 F/ha. The ER_{25} and ER_{50} values for plant length, plant dry weight and seedling emergence were determined to be > 8.0 kg BAS 610 06 F/ha for all tested plant species.

CP 10.6.3 Extended laboratory studies on non-target plants

Further tests on non-target plants are not triggered.

CP 10.6.4 Semi-field and field tests on non-target plants

Further tests on non-target plants are not triggered.

CP 10.7 Effects on other terrestrial organisms (flora and fauna)

Further studies are not triggered.

CP 10.8 Monitoring data

According to the knowledge of the applicant, there are currently no monitoring studies available, which are assessing ecotoxicological effects of BAS 516 07 F.

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BAS 516 07 F

DOCUMENT M-CP, Section 11

LITERATURE DATA

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CP 11 LITERATURE DATA

Based on their trade names, a number of end-use products containing pyraclostrobin were included in the literature search for the active substance. For further information, please refer to M-CA 9.

BAS 516 07 F

DOCUMENT M-CP, Section 12

CLASSIFICATION AND LABELLING OF THE PLANT PROTECTION PRODUCT

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CP 12 CLASSIFICATION AND LABELLING OF THE PLANT PROTECTION PRODUCT

Physico-chemical properties

Table 12-1: Physico-chemical data relevant for classification of BAS 516 07 F

Parameter	Findings (triggered risk phrase)	Reference (BASF DocID)
Explosive properties	The product has no explosive properties.	2005/1011601 2011/1065573
Oxidizing properties	The product has no oxidizing properties.	2005/1011601 2011/1065573
Flammability	Not easily flammable Auto-ignition temperature: 246°C	2005/1011601 2011/1065573
Content of hydrocarbons	< 10 %	Doc JCP 1.4.1
Viscosity	Not applicable	-
Surface tension	65.5 mN/m at 0.01%, 46.0 mN/m at 1.0% (both at 20°C)	2004/1024836

Remarks:
No classification is triggered for BAS 516 07 F by its physical/chemical properties.

Toxicology

Table 12-2: Toxicological data relevant for classification of BAS 516 07 F

Study type/species	Results	Classification		Reference
		EU Dir. 67/548/EEC 2001/59/EC	Reg. EC 1272/2008 (CLP)	(BASF DocID)
Acute oral toxicity, rat	LD_{50} > 2000 mg/kg bw	-	-	2008/1004838
Acute dermal toxicity, rat	LD_{50} > 2000 mg/kg bw	-	-	2001/1003723
Acute inhalation toxicity, rat	LC_{50} > 5.6 mg/L (both sexes)	-	-	2001/1001824
Skin irritation, rabbit	Non-irritating to rabbit skin	-	-	2007/1056989
Eye irritation, rabbit	Non-irritating to rabbit eye	-	-	2007/1056988
Skin sensitization LLNA assay, mouse	Non-sensitizing	-	-	2014/1001403

Remarks:
The acute dermal and inhalation toxicity studies have been conducted with BAS 516 00 F, a formulation that is very similar to BAS 516 07 F. Therefore studies performed with BAS 516 00 F are considered to be also valid for BAS 516 07 F. Information on the detailed composition of both formulations can be found in Doc JCP 1.4.1.
BAS 516 07 F is of low toxicity by the oral, dermal and inhalation route of exposure. It is non-irritant to the skin and the eye and does not cause skin sensitization.
Neither the classification of the active substances nor the classification of individual co-formulants has any further influence on the classification of the product BAS 516 07 F. Detailed information about the co-formulants is given in Documents JCP and JGH.

Ecotoxicology/Environment

Table 12-3: Ecotoxicological data relevant for classification of BAS 516 07 F

Study type/species	Results	Classification		Reference
		EU Dir. 67/548/EEC 2001/59/EC	Reg. EC 1272/2008 (CLP)	(BASF DocID)
Rainbow Trout (96 h)	LC_{50} = 0.088 mg/L	R50	H400	2000/1018726
D. magna (48 h)	EC_{50} = 0.120 mg/L	R50	H400	2007/1008605
Green alga (72 h)	E_RC_{50} = 10.8 mg/L	R52	-	2009/1117877
Biodegradation	Not readily biodegradable	R53	H410	1999/10655* 1999/10290**

* study conducted with pyraclostrobin
** study conducted with boscalid

Remarks:
The acute toxicity study in rainbow trout has been conducted with BAS 516 00 F, a formulation that is very similar to BAS 516 07 F. Therefore studies performed with BAS 516 00 F are considered to be also valid for BAS 516 07 F. Information on the detailed composition of BAS 516 00 F and BAS 516 07 F can be found in Doc JCP 1.4.1.
The statement on biodegradation has been derived from the properties of the active substances (both, pyraclostrobin and boscalid are not readily biodegradable). Neither the classification of the active substances nor the classification of individual co-formulants has any further influence on the classification of the product BAS 516 07 F. Detailed information about the co-formulants is given in Documents JCP and JGH.

Label Elements

Table 12-4: Proposed Risk and Safety Phrases according to Directive 67/548/EEC or 1999/45/EC

Pictogram(s)		
Hazard symbol(s):	N	
Indication(s) of danger:	Dangerous for the environment.	
Risk phrases:	R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
Safety phrases	S2	Keep out of the reach of children.
	S13	Keep away from food, drink and animal feeding stuffs.
	S20/21	When using do not eat, drink or smoke.
	S29/35	Do not empty into drains, this material and its container must be disposed of in a safe way.
	S57	Use appropriate container to avoid environmental contamination.

Table 12-5: Proposed Hazard and Precautionary Statements according to Globally Harmonized System/Regulation (EC) No 1272/2008 [CLP]

Pictogram(s)		
Signal word	Warning	
Hazard statements	H400	Very toxic to aquatic life.
	H410	Very toxic to aquatic life with long lasting effects.
	EUH401	To avoid risks to human health and the environment, comply with the instructions for use.
Precautionary Statements		
Prevention	-	-
Response	P391	Collect spillage.
Storage	-	-
Disposal	P501	Dispose of contents/container to hazardous or special waste collection point.

Table 12-6: Labelling proposed according to Directive 67/548/EEC or 1999/45/EC

Hazard symbol(s):	N
Indications of danger:	Dangerous for the environment
Risk phrases:	R50/53
Safety phrases:	S2, 13, 20/21, 29/35, 57

Table 12-7: Labelling proposed according to GHS (Globally Harmonized System) Regulation (EC) No 1272/2008

Signal word	Warning
Hazard Statement	H400, H410 EUH401
Precautionary Statements (Prevention):	-
Precautionary Statements (Response):	P391
Precautionary Statements (Storage):	-
Precautionary Statements (Disposal):	P501

BAS 516 07 F

DOCUMENT OCP

COMPLETENESS CHECK FORM

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OCP Evaluation Form Plant Protection Product
for use in checking that all test and study reports required in accordance with SANCO/11803 have been provided

Active Substance: Pyraclostrobin, boscalid Applicant: BASF SE Date: 18/Jul/2014

Preparation: BAS 516 07 F

As the representative formulation has changed from the original dossier to the supplementary dossier, all studies and references for BAS 516 07 F submitted within the supplementary dossier are new and are therefore not individually identified as new.

SANCO/ 11803 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
1	Identity of the plant protection product				
1.1	Applicant	yes			☐
1.2	Producer of the plant protection product and the active substances	yes			☐
1.3	Trade name or proposed trade name and producer's development code number of the plant protection product if appropriate	yes			☐
1.4	Detailed quantitative and qualitative information on the composition of the plant protection product				
1.4.1	Composition of the plant protection product	yes			☐
1.4.2	Information on the active substances	yes			☐
1.4.3	Information on safeners, synergists and co-formulants	yes	Doc J		☐
1.5	Type and code of the plant protection product	yes			☐
1.6	Function	yes			☐
2	Physical and chemical properties of the plant protection product				
2.1	Appearance	yes			☐
2.2	Explosive and oxidising properties	yes			☐
2.3	Flammability and self-heating	yes			☐
2.4	Acidity/alkalinity and pH value	yes			☐
2.5	Viscosity and surface tension	yes			☐
2.6	Relative density and bulk density	yes			☐
2.7	Storage stability and shelf-life: effects of temperature on technical characteristics of the plant protection product	yes			☐
2.8	Technical characteristics of the plant protection product				
2.8.1	Wettability	yes			☐
2.8.2	Persistent foaming	yes			☐
2.8.3	Suspensibility, spontaneity and dispersion stability	yes			☐
2.8.4	Degree of dissolution and dilution stability	not relevant	M-CP 2.8.4		☐
2.8.5	Particle size distribution, dust content, attrition and mechanical stability				
2.8.5.1	Particle size distribution	yes			☐
2.8.5.2	Dust content	yes			☐
2.8.5.3	Attrition	yes			☐
2.8.5.4	Hardness and integrity	not relevant	M-CP 2.8.5.4		☐

SANCO/ 11803 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
2.8.6	Emulsifiability, re-emulsifiability, emulsion stability	not relevant	M-CP 2.8.6		☐
2.8.7	Flowability, pourability and dustability	yes			☐
2.9	Physical and chemical compatibility with other products including plant protection products with which its use is to be authorised	yes			☐
2.10	Adherence and distribution to seeds	not relevant	M-CP 2.10		☐
2.11	Other studies	not relevant	M-CP 2.11		☐
3	Data on application				
3.1	Fields of use envisaged	yes			☐
3.2	Effects on harmful organisms	yes			☐
3.3	Details of intended use	yes			☐
3.4	Application rate and concentration of the active substance	yes			☐
3.5	Method of application	yes			☐
3.6	Number and timing of applications and duration of protection	yes			☐
3.7	Necessary waiting periods and other precautions to avoid phytotoxic effects on succeeding crops	yes			☐
3.8	Proposed instructions for use	yes			☐
4	Further information on the plant protection product				
4.1	Safety intervals and other precautions to protect humans, animals and the environment	yes			☐
4.2	Recommended methods and precautions	yes			☐
4.3	Emergency measures in the case of an accident	yes			☐
4.4	Packaging, compatibility of the plant protection product with proposed packaging materials	yes			☐
4.5	Procedures for destruction or decontamination of the plant protection product and its packaging				
4.5.1	Neutralisation procedure	yes			☐
4.5.2	Controlled incineration	yes			☐
5	Analytical methods				
5.1	Methods used for the generation of pre-authorisation data				
5.1.1	Analysis of the plant protection product	yes			☐
5.1.2	Methods for the determination of residues	yes	M-CA 4.1.2		☐
5.2	Methods for post-authorisation control and monitoring purposes	yes	M-CA 4.2		☐
6	Efficacy data				
6.1	Preliminary test	not relevant	not required		☐
6.2	Testing effectiveness	not relevant	not required		☐
6.3	Information on the occurrence or possible occurrence of the development of resistance	not relevant	not required		☐
6.4	Adverse effects on treated crops				
6.4.1	Phytotoxicity to target plants (including different cultivars), or to target plant products	not relevant	not required		☐
6.4.2	Effects on the yield of treated plants or plant products	not relevant	not required		☐
6.4.3	Effects on the quality of plants or plant product	not relevant	not required		☐
6.4.4	Effects on transformation processes	not relevant	not required		☐
6.4.5	Impact on treated plants or plant products to be used for propagation	not relevant	not required		☐

SANCO/ 11803 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
6.5	Observations on other undesirable or unintended side-effects				
6.5.1	Impact on succeeding crops	not relevant	not required		☐
6.5.2	Impact on other plants, including adjacent crops	not relevant	not required		☐
6.5.3	Effects on beneficial and other non-target organisms	not relevant	not required		☐
7	Toxicological studies				
7.1	Acute toxicity				
7.1.1	Oral toxicity	yes			☐
7.1.2	Dermal toxicity	yes			☐
7.1.3	Inhalation toxicity	yes			☐
7.1.4	Skin irritation	yes			☐
7.1.5	Eye irritation	yes			☐
7.1.6	Skin sensitisation	yes			☐
7.1.7	Supplementary studies on the plant protection product	not relevant	M-CP 7.1.7		☐
7.1.8	Supplementary studies for combinations of plant protection products	not relevant	M-CP 7.1.8		☐
7.2	Data on exposure				
7.2.1	Operator exposure				
7.2.1.1	Estimation of operator exposure	yes			☐
7.2.1.2	Measurement of operator exposure	not relevant	M-CP 7.2.1.2		☐
7.2.2	Bystander and resident exposure				
7.2.2.1	Estimation of bystander and resident exposure	yes			☐
7.2.2.2	Measurement of bystander and resident exposure	not relevant	M-CP 7.2.2.2		☐
7.2.3	Worker exposure				
7.2.3.1	Estimation of worker exposure	yes			☐
7.2.3.2	Measurement of worker exposure	not relevant	M-CP 7.2.3.2		☐
7.3	Dermal absorption	yes			☐
7.4	Available toxicological data relating to co-formulant	yes	Doc J		☐
8	Metabolism and residues data	yes	M-CA 6		
9	Fate and behaviour in the environment				
9.1	Fate and behaviour in soil				
9.1.1	Rate of degradation in soil	yes	M-CA 7.1.2		☐
9.1.1.1	Laboratory studies	yes	M-CA 7.1.2.1		☐
9.1.1.2	Field studies	yes	M-CA 7.1.2.2		☐
9.1.2	Mobility in the soil				
9.1.2.1	Laboratory studies	yes	M-CA 7.1.4.1		☐
9.1.2.2	Lysimeter studies	not relevant	M-CA 9.1.2.2		☐
9.1.2.3	Field leaching studies	not relevant	M-CA 9.1.2.3		☐
9.1.3	Estimations of concentrations in soil	yes			☐
9.2	Fate and behaviour in water and sediment				
9.2.1	Aerobic mineralisation in surface water	not relevant	M-CA 7.2.2.2		☐
9.2.2	Water/sediment study	not relevant	M-CA 7.2.2.3		☐
9.2.3	Irradiated water/sediment study	not relevant	M-CA 7.2.2.4		☐
9.2.4	Estimation of concentrations in groundwater				
9.2.4.1	Calculation of concentrations in groundwater	yes			☐
9.2.4.2	Additional field tests	not relevant	M-CP 9.2.4.2		☐

SANCO/ 11803 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
9.2.5	Estimation of concentrations in surface water and sediment	yes			☐
9.3	Fate and behaviour in air				
9.3.1	Route and rate of degradation in air and transport via air	yes	M-CA 7.3		☐
9.4	Estimation of concentrations for other routes of exposure	not relevant	M-CP 9.4		☐
10	Ecotoxicological studies				
10.1	Effects on birds and other terrestrial vertebrates				
10.1.1	Effects on birds				
10.1.1.1	Acute oral toxicity	yes			☐
10.1.1.2	Higher tier data for birds	yes			☐
10.1.2	Effects on terrestrial vertebrates other than birds				
10.1.2.1	Acute oral toxicity to mammals	yes			☐
10.1.2.2	Higher tier data on mammals	yes			☐
10.1.3	Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)	yes			
10.2	Effects on aquatic organisms				
10.2.1	Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes	yes			☐
10.2.2	Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms	not relevant	M-CP 10.2.2		☐
10.2.3	Further testing on aquatic organisms	not relevant	M-CP 10.2.3		☐
10.3	Effects on arthropods				
10.3.1	Effects on bees				
10.3.1.1	Acute toxicity to bees				
10.3.1.1.1	Acute oral toxicity to bees	yes			☐
10.3.1.1.2	Acute contact toxicity to bees	yes			☐
10.3.1.2	Chronic toxicity to bees	not relevant	M-CP 10.3.1.2		☐
10.3.1.3	Effects on honey bee development and other honey bee life stages	yes			☐
10.3.1.4	Sublethal effects	not relevant	M-CP 10.3.1.4		☐
10.3.1.5	Cage and tunnel tests	not relevant	M-CP 10.3.1.5		☐
10.3.1.6	Field tests with honeybees	not relevant	M-CP 10.3.1.6		☐
10.3.2	Effects on non-target arthropods other than bees				
10.3.2.1	Standard laboratory testing for non-target arthropods	yes			☐
10.3.2.2	Extended laboratory testing, aged residue studies with non-target arthropods	yes			☐
10.3.2.3	Semi-field studies with non-target arthropods	not relevant	M-CP 10.3.2.3		☐
10.3.2.4	Field studies with non-target arthropods	yes			☐
10.3.2.5	Other routes of exposure for non-target arthropods	not relevant	M-CP 10.3.2.5		☐
10.4	Effects on non-target soil meso- and macrofauna				
10.4.1	Earthworms				
10.4.1.1	Earthworms – sub-lethal effects	yes			☐

SANCO/ 11803 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
10.4.1.2	Earthworms – field studies	yes			☐
10.4.2	Effects on non-target soil meso- and macrofauna (other than earthworms)				
10.4.2.1	Species level testing	yes			☐
10.4.2.2	Higher tier testing	not relevant	M-CP 10.4.2.2		☐
10.5	Effects on soil nitrogen transformation	yes			☐
10.6	Effects on terrestrial non-target higher plants				
10.6.1	Summary of screening data	yes	M-CP 10.6.2		☐
10.6.2	Testing on non-target plants	yes			☐
10.6.3	Extended laboratory studies on non-target plants	not relevant	M-CP 10.6.3		☐
10.6.4	Semi-field and field tests on non-target plants	not relevant	M-CP 10.6.4		☐
10.7	Effects on other terrestrial organisms (flora and fauna)	yes			☐
10.8	Monitoring data	not relevant	M-CP 10.8		☐
11	Literature data	yes	M-CA 9		☐
12	Classification and labelling	yes			☐

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT A

Statement of the context in which the dossier is submitted

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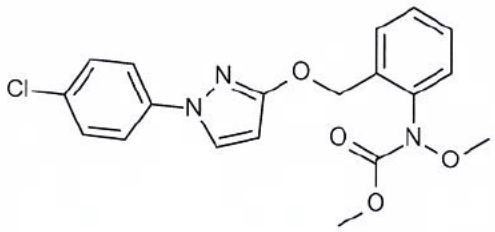
A supplementary dossier (Documents A to O) is herewith submitted as an application for the renewal of approval in accordance with Regulation (EC) No. 1107/2009 and Regulation (EU) No. 844/2012 for the active substance:

Pyraclostrobin (BAS 500 F)

Common name and chemical identity are shortly summarized below:

Common name:	Pyraclostrobin
Chemical names	
IUPAC:	Methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)-(N-methoxy)carbamate
CA:	Carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester
CAS:	175013-18-0
CIPAC:	657
Empirical formula:	$C_{19}H_{18}ClN_3O_4$
Structural formula:	
Molecular mass:	387.8 g/mol

Pyraclostrobin is a broad spectrum fungicide, which is active against fungal development stages both on the plant surface and within the tissues. It has a protective as well as an eradicative/curative action. Pyraclostrobin belongs to the group of active substances which is collectively known as strobilurins. The biochemical mode of action of the strobilurins is the inhibition of mitochondrial respiration.
In addition to the fungicidal effects plant physiology is also affected by the application of pyraclostrobin. Among these effects higher yield and better product quality in absence of diseases, improvement of the assimilation rate and delayed senescence have been reported. Furthermore, some studies showed better stress tolerance to abiotic stresses (e.g. drought or frost).
Pyraclostrobin is used world-wide in a large variety of crops including cereals, oilseeds, legumes, fruits and vegetables.

Pyraclostrobin was included in Annex I of Directive 91/414/EEC on 1 June 2004 (entry into force) under Inclusion Directive 2004/30/EC for the use as fungicide. This use was amended on 22 April 2009 (entry into force) by Inclusion Directive 2009/25/EC to the use as fungicide or plant growth regulator. Both Annex I inclusion decisions were based on the same endpoints and provisions.

The Review Report (Pyraclostrobin - SANCO/1420/2001-Final) is dated 08 September 2004 and provides endpoints agreed during first inclusion evaluation (Appendix II to the Review Report).

Member States were required to pay particular attention to the following areas:
- should pay particular attention to the protection of aquatic organisms, especially fish,
- should pay particular attention to the protection of terrestrial arthropods and earthworms

Due to the improvement of physical and chemical properties, the representative formulation will be changed to a 200 g/L EC solo formulation (coded BAS 500 06 F) representing the use in important and widely grown "row crops" (cereals and maize). In addition a second (new) representative formulation (coded BAS 516 07 F and containing boscalid as second active substance) has been chosen to represent the many uses in "specialty crops" were typically lower application rates are used.

The intended maximum application rate is 1.25 L product/ha for the EC solo formulation BAS 500 06 F corresponding to 250 g a.s./ha and 0.25 kg product/ha for the WG formulation BAS 516 07 F corresponding to 17 g pyraclostrobin/ha.

BASF SE makes this submission in good faith and is looking forward to working with Germany as Rapporteur Member State, as outlined in Commission Regulation (EU) No. 686/2012.

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[redacted] [redacted]

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT B

Documentation relating to the joint submission of dossiers

Compiled by:

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Pyraclostrobin is an existing active substance marketed by BASF SE. This application is submitted for the renewal of approval of pyraclostrobin in accordance with Regulation (EU) No. 1107/2009. BASF SE is the only applicant as stated in a letter of the Rapporteur Member State Germany (dated March 7, 2014) and owner of a complete data package regarding the active substance.

BASF
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Pyraclostrobin

DOCUMENT C

Existing or proposed labels

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In agreement with the Rapporteur Member State (Germany) in this document general information is provided that is recommended to be contained on national product labels for BAS 500 06 F and BAS 516 07 F with focus on the representative uses. Furthermore, please consider that on the specific country labels additional detailed guidance for the use of the product is given considering the national requirements. These instructions will be covered by the dRRs to be submitted for the re-registration of the plant protection products BAS 500 06 F and BAS 516 07 F following the renewal of approval for the active substance pyraclostrobin.

General draft product label (”master label”) for BAS 500 06 F

Emulsifiable concentrate (EC) containing 200 g/L pyraclostrobin.

Classification and labelling

Causes serious eye irritation.
Causes skin irritation.
Harmful if inhaled.
Harmful if swallowed.
May cause an allergic skin reaction.
May be fatal if swallowed and enters airways.
Very toxic to aquatic life.
Very toxic to aquatic life with long lasting effects.
To avoid risks to human health and the environment, comply with the instructions for use.

Only for use as agricultural fungicide.

Instructions for the use in cereals

BAS 500 06 F is a fungicide with protectant and curative properties for disease control in winter and spring wheat, winter and spring barley, oats, rye and triticale.

Time of application:
Apply BAS 500 06 F at the start of foliar disease attack. Applications can be made starting from mid tillering (BBCH 25) until end of flowering (latest application at BBCH 69). The recommended spray interval is 21 days depending on disease pressure and the general spray program strategy.

Maximum number of applications: 2

Maximum individual application rate: 1.25 L per ha

Water volume: 100 – 400 L of water per ha

Instructions for the use in maize

BAS 500 06 F is a fungicide with protectant properties for disease control in maize (forage and grain).

Time of application:
Best results are achieved by an application of BAS 500 06 F before the start of foliar disease attack or at very early signs of infection. Application can be made after beginning of stem elongation (BBCH 30) and up to and including mid-flowering (BBCH 65).

Maximum number of applications : 1

Maximum individual application rate: 1 L per ha

Water volume: 100 – 400 L of water per ha

Resistance management
The general spray program strategy has to consider the use of other products providing a different mode of action.

Succeeding crops
No minimum waiting periods needs to be considered for succeeding crops.

Re-entry
Re-entry is possible after the spray deposits on the crops have dried given the worker is wearing adequate work clothing.

Personal precautions
Do not breathe vapour or spray. Use personal protective clothing. Avoid contact with skin, eyes and clothing. During use do not eat, drink or smoke. Hands should be washed thoroughly with soap and water before breaks.

Environmental precautions
Do not discharge into subsoil or soil. Do not discharge into drains, surface waters or ground-water.

Storage
Segregate from food and animal feed. Protect the product from heat, frost and direct sunlight. Storage temperature should not exceed 40 °C. Ensure ventilation of stores.

Mixing and spraying
Do not prepare more spray solution than required. Fill the tank half with clean water and start agitation. Shake container, add the required amount of product and the necessary additional amount of water. Continue agitation until spraying is complete. When tank mixes are used, add each product separately and consider any specific instructions given e.g. for the order of mixing. During spraying, buffer zones to surface water have to be respected.

Compatibility
BAS 500 06 F is at least compatible with the following products:
Basagran DP, Biathlon, Duplosan DP, Duplosan KV, Sumicidin Alpha, Fastac, Cycocel 720, Terpal C, Capalo, Adexar, Champion, Osiris, Diamant

Disposal
Any surplus spray solution containing BAS 500 06 F should be diluted with water at a ratio of 1:10 and sprayed onto the previously treated area according to the use instructions.
Empty packaging should be triple rinsed. The rinsing water must be added to the spray liquid.
Empty and rinsed containers should be delivered to local container collection stations or must be disposed according to local regulations.
The spray equipment should be cleaned thoroughly immediately after use by draining the system completely and by rinsing spray tank, boom and nozzles two to three times with clean water.

General draft product label (”master label”) for BAS 516 07 F

Water dispersible granule (WG) containing 6.7% w/w pyraclostrobin and 26.7% w/w boscalid.

Classification and labelling

Very toxic to aquatic life.
Very toxic to aquatic life with long lasting effects.
To avoid risks to human health and the environment, comply with the instructions for use.

Only for use as agricultural fungicide.

Instructions for the use in potatoes

BAS 516 07 F is a fungicide used in potatoes mainly for the control of Alternaria spp..

Time of application:
Apply BAS 516 07 F at the start of disease attack with Alternaria spp.. The last application should be done latest 3 days before harvest. The recommended spray interval is about 10 – 21 days depending on disease pressure and the general spray program strategy.

Maximum number of applications: 4

Maximum individual application rate: 0.25 kg per ha

Water volume: 150 – 400 L of water per ha

Resistance management

The general spray program strategy has to consider the use of other products providing a different mode of action.

Succeeding crops

No minimum waiting periods needs to be considered for succeeding crops.

Re-entry
Re-entry is possible after the spray deposits on the crops have dried given the worker is wearing adequate work clothing.

Personal precautions
Avoid dust formation. Use personal protective clothing. Avoid contact with skin, eyes and clothing. During use do not eat, drink or smoke. Hands should be washed thoroughly with soap and water before breaks.

Environmental precautions
Do not discharge into subsoil or soil. Do not discharge into drains, surface waters or ground-water.

Storage
Segregate from food and animal feed. Protect the product from heat, moisture and direct sunlight. Storage temperature should not exceed 40 °C. Ensure ventilation of stores.

Mixing and spraying
Do not prepare more spray solution than required. Fill the tank half with clean water and start agitation. Add the required amount of product and the necessary additional amount of water. Continue agitation until spraying is complete. When tank mixes are used, add each product separately and consider any specific instructions given e.g. for the order of mixing. During spraying, buffer zones to surface water have to be respected.

Compatibility
BAS 516 07 F is at least compatible with the following products:
Acrobat Plus, Polyram, Delan, Kumulus, Decis, Fastac, Stratos Ultra, Perfekthion, Rovral, Alverde, Forum, Aramo

Disposal
Any surplus spray solution containing BAS 516 07 F should be diluted with water at a ratio of 1:10 and sprayed onto the previously treated area according to the use instructions.
Empty packaging should be triple rinsed. The rinsing water must be added to the spray liquid.
Empty and rinsed containers should be delivered to local container collection stations or must be disposed according to local regulations.
The spray equipment should be cleaned thoroughly immediately after use by draining the system completely and by rinsing spray tank, boom and nozzles two to three times with clean water.

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT D1

Intended uses supported in the EU for which data have been provided

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The intended use patterns (GAPs) of the representative crops for which data and risk assessments will be provided in the supplemental dossier are already registered in several EU Member States for BAS 500 06 F and BAS 516 07 F. The intended maximum application rate is 1.25 L product/ha for the EC solo formulation BAS 500 06 F corresponding to 250 g a.s./ha and 0.25 kg product/ha for the WG formulation BAS 516 07 F containing pyraclostrobin and boscalid as active substances, corresponding to 17 g pyraclostrobin/ha.

In the following tables an overview of the GAPs is provided for all representative uses either already registered, or submitted and still under evaluation by the authorities, the latter being marked by "*". Tables showing only the already registered representative uses can be found in M-CP 3 of the supplemental product dossiers.

Summary of representative uses registered or under evaluation in the EU for BAS 500 06 F

GAP rev 1.0., date: 2014-Jun-03

PPP (code)	**BAS 500 06 F**	**Formulation type:**	**EC**
active substance 1	**Pyraclostrobin**	**Conc. of as 1:**	**200 g/L**
Applicant:	**BASF SE**	**professional use**	☒
Zone(s):	**EU**	**non professional use**	☐
Verified by MS:	**y/n**		

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
Cereals												
1	DK	Barley (spring, winter)	F	*Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 69	a) 1 b) 1	a) 1.25 b) 1.25	a) 0.25 b) 0.25	100 - 400	42	
2	DK	Oats Rye	F	*Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	42	
3	DK	Triticale Wheat	F	*Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
4	EE	Barley (spring, winter) Oats Wheat (spring, winter)	F	*Blumeria graminis* *Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis* *Septoria tritici*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
5	FI	Barley (spring, winter) Oats Rye Triticale Wheat (spring, winter, durum)	F	*Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.2 b) 2.4	a) 0.24 b) 0.48	150 - 300	35	A range of 0.3 – 1.2 L/ha is registered.
6	LT, LV, SE	Barley (spring, winter) Oats Rye Triticale Wheat (spring, winter, durum)	F	*Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	A range of 0.8 – 1.25 L/ha is registered.
7	AT*	Barley (spring, winter) Rye Triticale	F	*Puccinia recondita* *Puccinia hordei* *Pyrenophora teres* *Rhynchosporium secalis* sunburn injury	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
8	AT*	Wheat (spring, winter, durum, spelt)	F	*Drechslera tritici-repentis* *Puccinia recondita* *Puccinia striiformis*	Spraying	25 – 61 (*P.recondita*: 25-69)	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
9	BE*, LU*	Barley (spring, winter) Oats Rye Triticale Wheat (spring, winter, durum, spelt)	F	*Drechslera tritici-repentis* *Puccinia coronata* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis* sunburn injury	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
10	CZ*, NL*	Barley (spring, winter) Rye Triticale Wheat (spring, winter, durum, spelt)	F	*Drechslera tritici-repentis* *Puccinia coronata* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis* sunburn injury	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
11	DE*	Barley Rye Wheat	F	*Drechslera tritici-repentis* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 – 61 *(P.recondita:* 25-69*)*	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
12	DE*	Barley	F	decrease of nonparasitic leaf spots	Spraying	32 - 61	a) 1 b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
13	DE*	Triticale	F	*Puccinia recondita*	Spraying	25 – 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
14	HU*, PL*, RO*, SK*, SI*	Barley (spring, winter) Rye Triticale Wheat (spring, winter, durum, spelt)	F	*Drechslera tritici-repentis* *Puccinia coronata* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis* sunburn injury	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	A range of 0.8 – 1.25 L/ha is registered.
15	IE, UK	Barley (spring, winter) Oats (winter, spring)	F	*Puccinia coronata* *Puccinia hordei* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 59	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	200 - 400	35	Including physiological effects

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
16	IE, UK	Wheat (spring, winter, durum, spelt)	F	*Fusarium* (ear) *Puccinia recondita* *Puccinia striiformis* *Septoria nodorum* (UK), *Septoria tritici* (UK)	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.5	200 - 400	35	Including physiological effects
17	IE	Rye Triticale	F	*Puccinia recondita* *Puccinia striiformis* *Puccinia triticina* *Rhynchosporium secalis*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.5	200 - 400	35	Including physiological effects
18	BG*, IT, GR	Barley (spring, winter) Triticale Wheat (spring, winter, durum)	F	*Blumeria graminis* *Fusarium spp.* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis* *Septoria spp.*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	Including physiological effects A range of 1 – 1.25 L/ha is registered.
19	ES, FR, PT	Barley Oats Rye Triticale Wheat	F	*Fusarium spp.* *Puccinia coronata* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis* *Septoria spp.*	Spraying	25 - 69	a) 2 (21d) b) 2)	a) 1.10 b) 2.20	a) 0.22 b) 0.44	100 - 400	35	Maximum 1 application against *Pyrenophora teres* in barley

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
Maize												
20	DK*, SE	Maize (forage and grain)	F	*Exserohilum turcicum* *Kabatiella zeae*	Spraying	30 - 65	a) 1 b) 1	a) 1.00 b) 1.00	a) 0.2 b) 0.2	100 - 400	n.a.	PHI defined by growth stage at application.
21	UK*	Maize (forage and grain)	F	*Puccinia sorghum* *Kabatiella zeae* *Setosphaeria turcica*	Spraying	30 - 65	a) 1 b) 1	a) 1.00 b) 1.00	a) 0.2 b) 0.2	200 - 400	n.a.	Including physiological effects PHI defined by growth stage at application.
21	BG	Maize (forage and grain)	F	*Exserohilum turcicum* *Puccinia sorghi*	Spraying	30 - 65	a) 1 b) 1	a) 1.00 b) 1.00	a) 0.2 b) 0.2	100 - 400	n.a.	PHI defined by growth stage at application. A range of 0.7 – 1 L/ha is registered.
23	ES*, GR, IT, PT*	Maize (forage and grain)	F	*Exserohilum turcicum* *Puccinia sorghi*	Spraying	30 - 65	a) 1 b) 1	a) 1.00 b) 1.00	a) 0.2 b) 0.2	100 - 400	n.a.	Including physiological effects PHI defined by growth stage at application.

*evaluation ongoing

Summary of representative uses registered or under evaluation in the EU for BAS 516 07 F

GAP rev. 1.0, date: 2014-Jun-09

PPP (code)	**BAS 516 07 F**	**Formulation type:**	**WG**
active substance 1	**Pyraclostrobin**	**Conc. of as 1:**	**67 g/kg**
active substance 2	**Boscalid**	**Conc. of as :**	**267 g/kg**
Applicant:	**BASF**	**professional use**	☒
Zone(s):	**EU**	**non professional use**	☐
Verified by MS:	**y/n**		

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:**
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
1	DK	Potatoes	F	*Alternaria* spp.	Spraying	47-75	a) 4 (5d) b) 4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	150	3	
2	EE	Potatoes	F	*Alternaria* spp.	Spraying	47-75	a) 4 (7d) b) 4	a) 0.25 a) 1.00	a) 17 + 67 b) 67 + 267	150	3	
3	LT	Potatoes	F	*Alternaria* spp.	Spraying	47-75	a) 4 (7d) b) 4	a) 0.20 b) 0.80	a) 13 + 53 b) 54 + 214	250-1000	14	
4	LV	Potatoes	F	*Alternaria* spp.	Spraying	first symptoms	a) 4 (7d) b) 4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	250-400	3	
5	SE	Potatoes	F	*Alternaria* spp.	Spraying	47-75	a) 4 (10d) b) 4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	150-400	3	
6	DE, AT	Potatoes	F	*Alternaria* spp.	Spraying	51-89	a) 4 (10d) b)4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	200-400	3	used as critical GAP in risk assessments
7	BE	Potatoes	F	*Alternaria* spp.	Spraying	first symptoms	a) 4 (10d) b) 4	a) 0.20 b) 0.80	a) 13 + 53 b) 54 + 214	300-400	3	

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
8	NL	Potatoes	F	*Alternaria* spp.	Spraying	40 - 95	a) 4 (14d) b) 4	a) 0.20 b) 0.80	a) 13 + 53 b) 54 + 214	200-400	0	
9	HR	Potatoes	F	*Alternaria* spp.	Spraying	41-89	a) 4 b)4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	300	14	

BASF
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Pyraclostrobin

DOCUMENT D2

List of currently authorized uses and extent of use

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In agreement with the RMS Germany not all use patterns (GAPs) of all pyraclostrobin containing products currently registered in the EU are shown in this document, because a very long list of several hundred GAPs for all products, countries and crops will be not helpful for the evaluation of pyraclostrobin, which is in general based on selected representative uses, whose GAPs are shown in detail in Document D1.

However, in the following tables all registered crops or crop groups are listed for all currently registered products containing pyraclostrobin. [redacted] For each product-crop combination all EU member states with currently valid registrations are listed. Furthermore information is given on the general application method and whether a field, greenhouse or in-door use is registered.

Current practice is not known to considerably and/or consistently deviate from the registered uses as described on the approved labels. Consequently it is not necessary to compare the registered and actual use patterns in a table.

REGISTERED USES

PPP trade name: **Cabrio** **PPP code:** **BAS 500 00 F**

Formulation type: **EC (emulsifiable concentrate)**

Conc. of as: **250 g/L pyraclostrobin**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	DE	Grapes	F	Spraying	[redacted]

PPP trade name: **Cabrio Olivio WG, Eland, Insignia** **PPP code:** **BAS 500 02 F**

Formulation type: **WG (water dispersible granules)**

Conc. of as: **20 % pyraclostrobin**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	IT	Olives	F	Spraying	[redacted]
2	BE, FR, IT, LU, UK	Turf	F	Spraying	

PPP trade name: **Comet Pro, Comet 20 EC, Comet 200, Envelis 20 EC, Modem 200, Retengo, Retengo New, Solaram 200**

PPP code: **BAS 500 06 F**

Formulation type: **EC (emulsifiable concentrate)**

Conc. of as: **200 g/L pyraclostrobin**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	DK, EE, ES, FI, FR, GR, IE, IT, LT, LV, PT, SE, UK	Cereals	F	Spraying	[redacted]
2	BG, GR, IT, SE	Maize (forage and grain)	F	Spraying	
3	FI, SE	Sugar beets	F	Spraying	

PPP trade name: **Cabrio, Cabrio EC, Comet 250 EC, Tucana, Varen**

PPP code: **BAS 500 13 F**

Formulation type: **EC (emulsifiable concentrate)**

Conc. of as: **250 g/L pyraclostrobin**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	IT	Pome fruits	F	Spraying	[redacted]
2	ES, IT, PT	Grapes	F	Spraying	
3	IT	Cereals	F	Spraying	

PPP trade name: **Comet, Flyer, Ley, Modem, Platoon 250, Solaram, Tucana, Vivid**

PPP code: **BAS 500 14 F**

Formulation type: **EC (emulsifiable concentrate)**

Conc. of as: **250 g/L pyraclostrobin**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	BE, DE, DK, EE, FR, IE, LV, LT, NL, SE, UK	Cereals	F	Spraying	[redacted]
2	SE	Maize (forage and grain)	F	Spraying	
3	SE	Sugar beets	F	Spraying	
4	DK	Grass for seed production	F	Spraying	

PPP trade name: **Opera**

PPP code: **BAS 512 00 F**

Formulation type: **SE (suspo-emulsion)**

Conc. of as 1: **133 g/L pyraclostrobin**
Conc. of as 2: **50 g/L epoxiconazole**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	DE, NL	Cereals	F	Spraying	[redacted]
2	DE	Sugar beets	F	Spraying	

PPP trade name: **Bauxit, Ibex, Opera**

PPP code: **BAS 512 04 F**

Formulation type: **SE (suspo-emulsion)**

Conc. of as 1: **133 g/L pyraclostrobin**
Conc. of as 2: **50 g/L epoxiconazole**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	FR	Pulses, dry	F	Spraying	[redacted]
2	FR	Cereals	F	Spraying	

PPP trade name: **Abacus SP, Comet Duo, Diamant Max, Envoy, Favia, Opera Max, Opera Max 147,5 SE, Opera N, Opera New, Opera Top**

PPP code: **BAS 512 07 F**

Formulation type: **SE (suspo-emulsion)**

Conc. of as 1: **85 g/L pyraclostrobin**
Conc. of as 2: **62.5 g/L epoxiconazole**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	BG, CZ, EE, FR, GR, HU, IT, LV, NL, PL, RO, SK, UK	Cereals	F	Spraying	[redacted]
2	EE, LV	Maize (forage and grain)	F	Spraying	
3	CZ	Grasses for biomass production	F	Spraying	

PPP trade name: **Gemstone, Lumen**

PPP code: **BAS 512 15 F**

Formulation type: **SE (suspo-emulsion)**

Conc. of as 1: **80 g/L pyraclostrobin**
Conc. of as 2: **62.5 g/L epoxiconazole**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	IE, UK	Cereals	F	Spraying	

PPP trade name: **Bauxit, Ibex, Opera, Optan 183 SE, Retengo Plus, Retengo Plus 183 SE**

PPP code: **BAS 512 16 F**

Formulation type: **SE (suspo-emulsion)**

Conc. of as 1: **133 g/L pyraclostrobin**
Conc. of as 2: **50 g/L epoxiconazole**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	FR	Pulses, dry	F	Spraying	
2	AT, DE, FR, HR, IT, NL, PL, UK	Cereals	F	Spraying	
3	AT, CZ, HR, HU, IT, NL, PL, PT, RO, SK	Maize (forage and grain)	F	Spraying	
4	AT, FR	Fodder beets	F	Spraying	
5	AT, BE, DE, HR, IT, NL, PL, UK	Sugar beets	F	Spraying	
6	BE	Grass for seed production	F	Spraying	

PPP trade name: **Optimo**

PPP code: **BAS 513 01 F**

Formulation type: **SE (suspo-emulsion)**

Conc. of as 1: **133 g/L pyraclostrobin**
Conc. of as 2: **50 g/L epoxiconazole**
Conc. of as 3: **67 g/L kresoxim-methyl**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	DE	Cereals	F	Spraying	[redacted]

PPP trade name: **Signum**

PPP code: **BAS 516 00 F**

Formulation type: **WG (water dispersible granules)**

Conc. of as 1: **6.7 % pyraclostrobin**
Conc. of as 2: **26.7 % boscalid**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	ES	Strawberries	F	Spraying	[redacted]
2	ES	Fruiting vegetables	F	Spraying	
3	ES	Leaf vegetables	F	Spraying	

PPP trade name: **Bellis, Bellis 25.2/12.8 WG, Bellis 38 WG, Velisto 25.2/12.8 WG**

PPP code: **BAS 516 04 F**

Formulation type: **WG (water dispersible granules)**

Conc. of as 1: **12.8 % pyraclostrobin**
Conc. of as 2: **25.2 % boscalid**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	AT, BE, CZ, DE, ES, FR, GR, HR, HU, IE, IT, NL, PL, PT, RO, SI, UK	Pome fruits	F	Spraying	
2	AT, BE, CZ, DE	Hops	F	Spraying	

PPP trade name: **Aragon, Bellis Drupacee, Signum, Signum S, Signum WG, Signum 26.7/6.7 WG, Signum 33 WG, Terminet, Trigramm 26.7/6.7 WG**

PPP code: **BAS 516 07 F**

Formulation type: **WG (water dispersible granules)**

Conc. of as 1: **6.7 % pyraclostrobin**
Conc. of as 2: **26.7 % boscalid**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	CY, GR, IT	Tree nuts	F	Spraying	
2	FI, HR	Pome fruits	F	Spraying	
3	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, GR, HR, HU, IT, LV, MT, NL, PL, PT, RO, SE, SK	Stone fruits	F	Spraying	
4	BE, DE, DK, FR, HR, HU, IE, IT, LV, NL, PL, SI, UK	Berries	F/G	Spraying	
5	AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, GR, HR, HU, IE, IT, LV, LT, MT, NL, PL, PT, SE, SI, UK	Strawberries	F/G	Spraying	
6	BE, DE, DK, EE, HR, LV, LT, NL, SE	Potatoes	F	Spraying	
7	AT, BE, CY, DE, DK, EE, ES, FI, FR, GR, HR, HU, IE, IT, LV, LT, NL, PL, PT, SE, SI, UK	Root and tuber vegetables	F	Spraying	
8	AT, BE, CY, DE, EE, FI, FR, GR, HR, IT, NL, PL, PT, SK	Bulb vegetables	F	Spraying	
9	BE, CY, ES, FR, GR, HR, IT, MT, NL, PL, PT, RO	Fruiting vegetables	F/G	Spraying	
10	AT, BE, DE, DK, EE, FI, FR, HR, HU, IE, IT, LV, LT, MT, NL, PL, PT, SE, SI, UK	Brassica vegetables	F	Spraying	
11	AT, BE, CY, DE, DK, EE, ES, FI, FR, GR, HR, HU, IE, IT, LT, MT, NL, PL, PT, SE, SI, UK	Leaf vegetables	F/G/I	Spraying	
12	AT, BE, CY, DE, GR, IT, MT, NL, PL	Fresh herbs	F/G	Spraying	
13	CY, EE, FI, GR, IE, LT, LV, SE, SI, UK	Legume vegetables	F	Spraying	
14	AT, BE, CZ, DE, DK, EE, ES, FI, FR, GR, HU, HR, IT, LT, MT, NL, PL, PT	Stem vegetables	F	Spraying	
15	DK, FI, HR, IE, LV, LT, SE, UK	Pulses, dry	F	Spraying	
16	PL	Edible flowers	G	Spraying	
17	BE, MT, NL, PL	Spices	F	Spraying	
18	BE	Chicory roots	F	Spraying	
19	AT	Turf	F	Spraying	

PPP trade name: **Aneto, Cabrio Plus, Cabrio Top, Tucana Top**

PPP code: **BAS 518 01 F**

Formulation type: **WG (water dispersible granules)**

Conc. of as 1: **5 % pyraclostrobin**
Conc. of as 2: **55 % metiram**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	AT, BG, CZ, DE, ES, FR, HR, HU, IT, PT, RO, SI, SK	Grapes	F	Spraying	
2	IT, RO	Tomatoes	F	Spraying	

PPP trade name: **Comet Fly, Jenton**

PPP code: **BAS 528 00 F**

Formulation type: **EC (emulsifiable concentrate)**

Conc. of as 1: **100 g/L pyraclostrobin**
Conc. of as 2: **375 g/L fenpropimorph**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	FR, IE, UK	Cereals	F	Spraying	[redacted]

PPP trade name: **Diamant**

PPP code: **BAS 529 02 F**

Formulation type: **SE (suspo-emulsion)**

Conc. of as 1: **114.3 g/L pyraclostrobin**
Conc. of as 2: **214.3 g/L fenpropimorph**
Conc. of as 3: **42.9 g/L epoxiconazole**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	FR	Cereals	F	Spraying	[redacted]

PPP trade name: **Diamant**

PPP code: **BAS 529 03 F**

Formulation type: **SE (suspo-emulsion)**

Conc. of as 1: **114.3 g/L pyraclostrobin**
Conc. of as 2: **214.3 g/L fenpropimorph**
Conc. of as 3: **42.9 g/L epoxiconazole**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	AT, DE	Cereals	F	Spraying	[redacted]

PPP trade name: **Demeo, Diamant**

PPP code: **BAS 529 04 F**

Formulation type: **SE (suspo-emulsion)**

Conc. of as 1: **114.3 g/L pyraclostrobin**
Conc. of as 2: **214.3 g/L fenpropimorph**
Conc. of as 3: **42.9 g/L epoxiconazole**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	AT, BE, DE, IE, UK	Cereals	F	Spraying	[redacted]

PPP trade name: **Cabrio Duo, Cabrio Duo EC, Cabrio Duo 4/7.2 EC, Cabrio Duo 112 EC, Cassiopeia, Coach Plus, Coach Plus 4/7-2 EC, Optimo Tech**

PPP code: **BAS 536 01 F**

Formulation type: **EC (emulsifiable concentrate)**

Conc. of as 1: **40 g/L pyraclostrobin**
Conc. of as 2: **72 g/L dimethomorph**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	BE, CY, ES, FR, GR, IT, PL, PT	Potatoes	F	Spraying	
2	BE, CY, DK, ES, FR, GR, IT, PT, UK	Bulb vegetables	F	Spraying	
3	BE, CY, DK, ES, FR, GR, IT, PT	Fruiting vegetables	F/G	Spraying	
4	CY, ES, GR, IT, PT	Leaf vegetables	F/G	Spraying	
5	FR, IT	Stem vegetables	F	Spraying	

PPP trade name: **Cabrio Team, Cabrio Team 6.7/12 WG, Forum Team, Simvia 6.7/12 WG**

PPP code: **BAS 536 02 F**

Formulation type: **WG (water dispersible granules)**

Conc. of as 1: **6.7 % pyraclostrobin**
Conc. of as 2: **12 % dimethomorph**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	ES, GR, IT, PT	Grapes	F	Spraying	

PPP trade name: **Securo**

PPP code: **BAS 537 02 F**

Formulation type: **SC (suspension concentrate)**

Conc. of as 1: **100 g/L pyraclostrobin**
Conc. of as 2: **300 g/L folpet**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	NL	Ornamentals	F/G	Flower bulb dipping	

PPP trade name: **Cabrio Star, Cabrio Ultra, Equerre**

PPP code: **BAS 537 03 F**

Formulation type: **SE (suspo-emulsion)**

Conc. of as 1: **40 g/L pyraclostrobin**
Conc. of as 2: **400 g/L folpet**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	FR, IT	Grapes	F	Spraying	[redacted]

PPP trade name: **Cabrio Star, Cabrio Ultra, Equerre**

PPP code: **BAS 537 04 F**

Formulation type: **SE (suspo-emulsion)**

Conc. of as 1: **40 g/L pyraclostrobin**
Conc. of as 2: **400 g/L folpet**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	FR, PT	Grapes	F	Spraying	[redacted]

PPP trade name: **Maccani 4/12 WG, Tercel**

PPP code: **BAS 584 00 F**

Formulation type: **WG (water dispersible granules)**

Conc. of as 1: **4 % pyraclostrobin**
Conc. of as 2: **12 % dithianon**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	CY, CZ, DE, GR, HR, IT, SK, UK	Pome fruits	F	Spraying	[redacted]

PPP trade name: **Maccani, Tercel 16 WG**

PPP code: **BAS 584 01 F**

Formulation type: **WG (water dispersible granules)**

Conc. of as 1: **4 % pyraclostrobin**
Conc. of as 2: **12 % dithianon**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	BE, DE, HU, PL, RO, SI	Pome fruits	F	Spraying	[redacted]

PPP trade name: **Nebula XL, Rubis, Viverda**

PPP code: **BAS 667 00 F**

Formulation type: **OD (oil dispersion)**

Conc. of as 1: **60 g/L pyraclostrobin**
Conc. of as 2: **50 g/L epoxiconazole**
Conc. of as 3: **140 g/L boscalid**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	BE, DE, DK, EE, FR, LV, LT, UK	Cereals	F	Spraying	

PPP trade name: **Adexar Plus, Ceriax, Voxan**

PPP code: **BAS 702 03 F**

Formulation type: **EC (emulsifiable concentrate)**

Conc. of as 1: **61 g/L pyraclostrobin**
Conc. of as 2: **41.6 g/L epoxiconazole**
Conc. of as 3: **41.6 g/L fluxapyroxad**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	BE, CZ, FR, HU, IE, PL, UK	Cereals	F	Spraying	

PPP trade name: **Vortex**

PPP code: **BAS 702 04 F**

Formulation type: **EC (emulsifiable concentrate)**

Conc. of as 1: **61 g/L pyraclostrobin**
Conc. of as 2: **41.6 g/L epoxiconazole**
Conc. of as 3: **41.6 g/L fluxapyroxad**

1	2	3	4	5	6
Use-No.	**Member state(s)**	**Crop or crop group**	**F G or I**	**Application method**	**Remarks:**
1	UK	Cereals	F	Spraying	

Pyraclostrobin

DOCUMENT D3

Intended uses supported in the EU for which data will be provided

Compiled by:

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In agreement with the RMS Germany not all use patterns (GAPs) of all pyraclostrobin containing products intended to be registered in the EU after renewal of approval are shown in this document, because a very long list of several hundred GAPs for all products, countries and crops will be not helpful for the evaluation of pyraclostrobin, which is in general based on selected representative uses, whose GAPs are shown in detail in Document D1.

However, in the following table all crops or crop groups and their corresponding use patterns are listed, which are assumed to potentially represent a critical GAP (worse case) for the risk assessments that needs to be provided for intended product re-registrations following the renewal of approval for the active substance. The information is provided in a format close to the one used in the D1-Document. It is explicitly emphasized that currently not all GAPs for product re-registration are finally decided. Consequently the list of GAPs shown below can only represent the current status and changes are still possible.

Summary of critical GAPs per crop or crop group

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:**
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
Cereals												
1	HU, LT, LV, PL, RO, SE, SI, SK	Barley (spring, winter), rye, triticale, wheat (spring, winter, durum, spelt)	F	*Drechslera tritici-repentis* *Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis* sunburn injury	SP	BBCH 30 - 69	a) 2 (21) b) 2	a) 0.8 - 1.25 b) 1.6 - 2.50	a) 0.16 - 0.25 b) 0.32 - 0.50	100 / 400	35	BAS 500 06 F 200 g/L pyraclostrobin including physiological effects
Maize												
2	AT, BE, BG, CZ, DE, ES, GR, HR, HU, IT, NL, PL, PT, RO, SI, SK, UK	Maize (forage and grain)	F	*Exserohilum turcicum* *Puccinia sorghi*	SP	BBCH 30 - 65	a) 1 b) 1	a) 0.7 - 1.0 b) 0.7 - 1.0	a) 0.14 - 0.2 b) 0.14 - 0.2	100 / 400	F	BAS 500 06 F 200 g/L pyraclostrobin PHI defined by growth stage at last application. including physiological effects
Grapes												
3	ES, GR, IT, PT	Wine grapes	F	*Plasmopara viticola* *Uncinula necator*	SP	BBCH 15 - 83	a) 3 (12) b) 3	a) 1.5 b) 4.5	a) 0.1(*) + 0.18(**) b) 0.3(*) + 0.54(**)	200 / 1200	35	BAS 536 02 F *67 g/kg pyraclostrobin ** 120 g/kg dimethomorph Do not use the treated leaves for consumption.

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
4	BG, ES, HR, IT, PT	Wine grapes	F	*Guignardia bidwellii* *Phomopsis viticola* *Pseudopezicula tracheiphila*	SP	BBCH 05 - 83	a) 3 (10) b) 3	a) 1.5 b) 4.5	a) 0.075(*) + 0.825(**) b) 0.225(*) + 2.475(**)	200 / 1200	35	BAS 518 01 F *50 g/kg pyraclostrobin **550 g/kg metiram Do not use in vineyards with a closed horizontal leaf area between rows. Earliest BBCH
5	FR	Wine grapes	F	*Plasmopara viticola* *Uncinula necator*	SP	BBCH 53 - 83	a) 1 b) 1	a) 2.0 b) 2.0	a) 0.1(*) + 1.1(**) b) 0.1(*) + 1.1(**)	100 / 300	35	BAS 518 01 F *50 g/kg pyraclostrobin **550 g/kg metiram 10-14 day spray interval with other products Do not use in vineyards with a closed horizontal leaf area between rows. Highest spray broth concentration
6	BG	Wine grapes	F	*Plasmopara viticola*	SP	BBCH 53 - 83	a) 3 (7) b) 3	a) 1.5 - 2.0 b) 4.5 - 6.0	a) 0.075 - 0.1(*) + 0.825 - 1.1(**) b) 0.225 - 0.3(*) + 2.475 - 3.3(**)	200 / 1200	35	BAS 518 01 F *50 g/kg pyraclostrobin **550 g/kg metiram Do not use in vineyards with a closed horizontal leaf area between rows. 7 – 10 days spray interval for 1.5 kg/ha and 10-14 day for 2 kg/ha Shortest appl. interval

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
7	ES, GR, IT, PT	Table grapes	F	*Plasmopara viticola* *Uncinula necator*	SP	BBCH 15 - 79	a) 3 (12) b) 3	a) 1.5 b) 4.5	a) 0.1(*) + 0.18(**) b) 0.3(*) + 0.54(**)	200 / 1200	56	BAS 536 02 F *67 g/kg pyraclostrobin ** 120 g/kg dimethomorph Do not use the treated leaves for consumption. Water volume linked to BBCH: BBCH 15-69: 200-1200 L/ha BBCH 71-79: 500-1200 L/ha
Orchards												
8	ES, GR, IT, PT, FR, HR	Olive (table, oil)	F	*Colletotrichum acutatum* *C. gloeosporioides* *Spilocaea oleagina*	SP	BBCH 15 - 85	a) 3 (21) b) 3	a) 0.5 b) 1.5	a) 0.1 b) 0.3	1000 / 1500	28	BAS 500 02 F 200 g/kg pyraclostrobin In table and oil olives 1-2 appl. are made until BBCH 71 (April - June). In Fall season, 1 appl. is made a) in oil olives during fruit ripening (Sep-Oct) or b) in table olives after harvest.
9	GR	Pome fruits (apple)	F	*Podosphaera leucotricha* *Venturia inaequalis*	SP	BBCH 09 - 75	a) 4 (10) b) 4	a) 0.8 b) 3.2	a) 0.1(*) + 0.2(**) b) 0.4(*) + 0.8(**)	1000 / 1500	7	BAS 516 04 F *128 g/kg pyraclostrobin **252 g/kg boscalid Earliest BBCH

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
10	SI	Pome fruits (apple, pear)	F	Apple: *Podosphaera leucotricha* *Venturia inaequalis* Pear: *Stemphylium vesicarium* *Venturia pirina* Storage diseases: *Alternaria* spp. *Botrytis cinerea* *Gloeosporium* spp. *Monilia* spp. *Penicillium* spp.	SP	BBCH (31)55 - 89	a) 3 (7) b) 3	a) 0.8 b) 2.4	a) 0.1(*) + 0.2(**) b) 0.3(*) + 0.6(**)	300 / 1000	7	BAS 516 04 F *128 g/kg pyraclostrobin **252 g/kg boscalid Last BBCH
11	NL	Pome fruits (apple, pear)	F	Apple: *Podosphaera leucotricha* *Venturia inaequalis* Pear: *Venturia pirina* Storage diseases: *Gloeosporium* spp. *Penicillium* spp.	SP	BBCH 56 – 87	a) 4 (7) b) 4	a) 0.8 b) 3.2	a) 0.1(*) + 0.2(**) b) 0.4(*) + 0.8(**)	200 / 1500	14	BAS 516 04 F *128 g/kg pyraclostrobin **252 g/kg boscalid Shortest appl. interval
12	CZ	Pome fruits (apple, pear)	F	Apple: *Podosphaera leucotricha* *Venturia* spp. Pear: *Venturia* spp. Storage diseases	SP	BBCH 54 – 85	a) 4 (8) b) 4	a) 0.8 b) 3.2	a) 0.1(*) + 0.2(**) b) 0.4(*) + 0.8(**)	200 / 1000	7	BAS 516 04 F *128 g/kg pyraclostrobin **252 g/kg boscalid Highest appl. number

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
13	FI	Stone fruits (cherry, plum)	F	*Blumeriella jaapii* *Colletotrichum gloeosporioides* *Monilinia fructigena* *Monilinia laxa*	SP	BBCH 60 - 67, 77 - 87	a) 3 (5) b) 3	a) 1.0 b) 3.0	a) 0.067(*) + 0.267(**) b) 0.201(*)+ 0.801(**)	600 / 1000	3	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Smallest appl. interval
14	FR	Stone fruits (apricot, nectarine, peach)	F	*Sphaerotheca pannosa*	SP	BBCH 10 - 85	a) 3 (7) b) 3	a) 0.6 b) 1.8	a) 0.04(*) + 0.16(**) b) 0.121(*)+ 0.481(**)	1000 / 1000	7	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Earliest BBCH
15	NL	Stone fruits (tree nursery)	F	*Colletotrichum cereale* *Fusarium* *Sclerotinia homoeocarpa*	SP	BBCH 19 - 89	a) 3 (7) b) 3	a) 0.75 b) 2.25	a) 0.05(*) + 0.2(**) b) 0.151(*)+ 0.601(**)	500 / 1500	NA	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid crop < 150 cm Last BBCH
16	DE, EE	Stone fruits (cherry, plum)	F	*Blumeriella jaapii* *Colletotrichum gloeosporioides* *Monilinia fructigena* *Monilinia laxa*	SP	BBCH 60 - 67, 77 - 81	a) 3 (14) b) 3	a) 0.75 b) 2.25	a) 0.05(*) + 0.2(**) b) 0.151(*)+ 0.601(**)	200 / 400	3	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Highest spray broth concentration
17	IT	Tree nuts (almond, hazelnut, pistachio)	F	*Botryosphaeria dothidea* *Coryneum spp.* *Monilinia spp.* *Nut grey necrosis* *Taphrina deformans*	SP	up to BBCH 87 (PHI)	a) 2 (10) b) 2	a) 1.0 b) 2.0	a) 0.067(*) + 0.267(**) b) 0.134(*)+ 0.534(**)	1000 / 1500	28	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Last BBCH

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
18	CY, GR	Tree nuts (hazelnut, walnut)	F	*Alternaria sp.* *Cladosporium sp.* *Colletotrichum sp.* *Fusarium lateritium* *Gnomonia leptostyla*	SP	BBCH 61 - 79	a) 2 (10) b) 2	a) 1.0 b) 2.0	a) 0.067(*) + 0.267(**) b) 0.134(*) + 0.534(**)	600 / 1500	28	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Highest spray broth concentration
Ornamentals - flower bulb dipping												
19	NL	Lily	F	*Penicillium spp.*	Dipping	BBCH 00 - 09 (Mar-Apr)	a) 1 b) 1	a) 10.5 b) 10.5	a) 1.05(*) + 3.15(**) b) 1.05(*) + 3.15(**)	700	-	BAS 537 02 F *100g/L pyraclostrobin **300 g/L folpet
20	NL	Ornamentals (others)	F, G	*Fusarium oxysporum*	Dipping	BBCH 00 - 09 (Feb-Nov)	a) 1 b) 1	a) 10.7 b) 10.7	a) 1.07(*) + 3.21(**) b) 1.07(*) + 3.21(**)	715	-	BAS 537 02 F *100g/L pyraclostrobin **300 g/L folpet Excluded are "grofbollige narcis" and protected soil bound lilies.
Berries + strawberries												
21	CZ	Cane fruits (blackberry)	F	*Rhabdospora ruborum*	SP	Visible symptoms	a) 3 (7) b) 3	a) 1.0 b) 3.0	a) 0.067(*) + 0.267(**) b) 0.201(*) + 0.801(**)	100 / 1000	14	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Highest appl. number
22	PL	Cane fruits (raspberry)	F	*Botrytis cinerea* *Didymella aplanata*	SP	BBCH 51 - 90	a) 2 (7) b) 2	a) 1.8 b) 3.6	a) 0.121(*) + 0.481(**) b) 0.241(*) + 0.961(**)	600 / 700	3	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Highest appl. rate

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
23	SI	Other fruits and berries (black currant)	F	*Botrytis cinerea* *Drepanopeziza ribis* *Sphaerotheca mors-uvae*	SP	2 appl. before harvest (after first symptoms occurred, preventively from beginning of flowering) and 1 appl. after harvest (before falling of leaves)	a) 3 (10) b) 3	a) 1.5 b) 4.5	a) 101(*) + 0.401(**) b) 0.302(*) + 1.202(**)	200 / 1000	?	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Highest appl. rate per season
24	PL	Other fruits and berries (black currant)	F	*Cronartium ribicola* *Drepanopeziza ribis*	SP	BBCH 51 - 90	a) 2 (7) b) 2	a) 1.8 b) 3.6	a) 0.121(*) + 0.481(**) b) 0.241(*) + 0.961(**)	600 / 800	3	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Highest appl. rate per use
25	CY, GR	Strawberry	G	*Botrytis cinerea* *Spaerotheca macularis*	SP	BBCH 61 - 89	a) 3 (5) b) 2	a) 1.5 b) 3.0	a) 0.101(*) + 0.401(**) b) 0.201(*) + 0.801(**)	1000 / 1000	3	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Highest appl. number
26	DK, EE, SE	Strawberry	F	*Botrytis cinerea* *Colletotrichum acutatum* *Sphaerotheca macularis*	SP	BBCH 60 - 81	a) 2 (5) b) 2	a) 1.8 b) 3.6	a) 0.121(*) + 0.481(**) b) 0.241(*) + 0.961(**)	400 / 2000	3	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Highest appl. rate + spray broth concentration
27	AT, DE	Strawberry	F, G	*Diplocarpon earliana* *Mycosphaerella fragariae*	SP	BBCH 13 - ?	a) 1 b) 1	a) 1.8 b) 1.8	a) 0.121(*) + 0.481(**) b) 0.121(*) + 0.481(**)	max. 2000	3	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Earliest BBCH

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
28	NL	Strawberry	F	*Botryotinia fuckeliana*	SP	BBCH 60 - 89	a) 2 (7) b) 2	a) 1.8 b) 3.6	a) 0.121(*) + 0.481(**) b) 0.241(*) + 0.961(**)	500 / 600	1	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Shortest PHI
Vegetables												
29	BE, ES, FR, GR, IT, PL, PT	Fruiting vegetables (aubergine, tomato)	F, G	*Alternaria* spp. *Colletotrichum* spp. *Leveillula taurica* *Phytophthora infestans*	SP	BBCH 11 - 89	a) 3 (7) b) 3	a) 2.0 - 2.5 b) 6.0 - 7.5	a) 0.8 - 0.1(*) + 0.144 - 0.18(**) b) 0.24 - 0.3(*) + 0.432 - 0.54(**)	200 / 1000	3	BAS 536 01 F *40 g/L pyraclostrobin **72 g/L dimethomorph Earliest BBCH
30	PL	Fruiting vegetables (tomato)	G	*Botrytis cinerea* *Phytophtera infestans*	SP	BBCH 55 - 61	a) 2 (7) b) 2	a) 2.0 (0.2/100 L) b) 4.0	a) 0.134(*) + 0.534(**) b) 0.268(*) + 1.068(**)	1000 / 2000 (100 - 200L / 100m²)	3	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Highest appl. rate
31	NL	Fruiting vegetables (aubergine, tomato)	G	*Botryotinia fuckeliana* *Leveillula taurica*	SP	BBCH 51 - 89	a) 3 (7) b) 3	a) 1.5 b) 4.5	a) 0.101(*) + 0.401(**) b) 0.302(*) + 1.202(**)	500 / 1500	1	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid non soil bound Shortest PHI

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
32	ES, GR, IT	Cucurbits – edible peel	F, G	*Erysiphe cichoracearum* *Pseudoperonospora cubensis* *Sphaerotheca fuligenea*	SP	BBCH 11 - 89	a) 3 (7) b) 3	a) 2.0 - 2.5 b) 6.0 - 7.5	a) 0.8 - 0.1(*) + 0.144 - 0.18(**) b) 0.24 - 0.3(*) + 0.432 - 0.54(**)	250 / 1000	1	BAS 536 01 F *40 g/L pyraclostrobin **72 g/L dimethomorph
33	ES, GR, IT	Cucurbits - inedible peel	F	*Alternaria spp.(only IT)* *Pseudoperonospora cubensis*	SP	BBCH 11 - 89	a) 3 (7) b) 3	a) 2.0 - 2.5 b) 6.0 - 7.5	a) 0.8 - 0.1(*) + 0.144 - 0.18(**) b) 0.24 - 0.3(*) + 0.432 - 0.54(**)	250 / 1000	3	BAS 536 01 F *40 g/L pyraclostrobin **72 g/L dimethomorph
34	ES, GR, IT, PT	Brassica vegetables (flowering brassica)	F	*Alternaria brassicae* *Peronospora parasitica*	SP	BBCH 11 - 49	a) 3 (7) b) 3	a) 2.0 - 2.5 b) 6.0 - 7.5	a) 0.8 - 0.1(*) + 0.144 - 0.18(**) b) 0.24 - 0.3(*) + 0.432 - 0.54(**)	200 / 1000	7	BAS 536 01 F *40 g/L pyraclostrobin **72 g/L dimethomorph
35	ES, GR, IT, PT	Brassica vegetables (head cabbage)	F	*Alternaria brassicae* *Peronospora parasitica*	SP	BBCH 11 - 49	a) 3 (7) b) 3	a) 2.0 - 2.5 b) 6.0 - 7.5	a) 0.8 - 0.1(*) + 0.144 - 0.18(**) b) 0.24 - 0.3(*) + 0.432 - 0.54(**)	200 / 1000	3	BAS 536 01 F *40 g/L pyraclostrobin **72 g/L dimethomorph

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
36	NL	Brassica vegetables (leafy brassica)	F	*Alternaria brassicae* *Alternaria brassicicola*	SP	BBCH 11 - 49	a) 3 (7) b) 3	a) 1.0 b) 3.0	a) 0.067(*) + 0.267(**) b) 0.201(*)+ 0.801(**)	200 / 800	14	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid
37	DK, EE, ES, FI, GR, LT, LV, NO, PL, PT, SE	Bulb vegetables (garlic, onion, shallot)	F	*Alternaria porri (only SEU)* *Peronospora destructor*	SP	BBCH 13 - 48	a) 3 (7) b) 3	a) 2.0 - 2.5 b) 6.0 - 7.5	a) 0.8 - 0.1(*) + 0.144 - 0.18(**) b) 0.24 - 0.3(*) + 0.432 - 0.54(**)	200 / 1000	7	BAS 536 01 F *40 g/L pyraclostrobin **72 g/L dimethomorph
38	GR, IT, ES, PT	Stem vegetables (artichoke)	F	*Ascochyta hortorum* *Bremia lactucae* *Leveillula taurica*	SP	BBCH 50 - 85	a) 3 (7) b) 3	a) 2.0 - 2.5 b) 6.0 - 7.5	a) 0.8 - 0.1(*) + 0.144 - 0.18(**) b) 0.24 - 0.3(*) + 0.432 - 0.54(**)	200 / 1000	3	BAS 536 01 F *40 g/L pyraclostrobin **72 g/L dimethomorph
39	IT	Stem vegetables (asparagus)	F	Grey mould (BOTRCI) Powdery mildew (LEVETA)	SP	after harvest (July-September)	a) 3 (10) b) 3	a) 1.5 b) 4.5	a) 0.101(*) + 0.401(**) b) 0.302(*) + 1.202(**)	500 / 1000	120	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Highest appl. number + shortest interval
40	NL	Stem vegetables (asparagus)	F	*Botryotinia fuckeliana* *Stemphylium spp.*	SP	BBCH 11 - 89	a) 3 (14) b) 3	a) 0.75 b) 2.25	a) 0.05(*) + 0.2(**) b) 0.101(*)+ 0.401(**)	700 / 800	14	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Earliest BBCH, shortest PHI

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
41	AT, CZ, DE	Stem vegetables (asparagus)	F	*Botrytis cinerea*	SP	from BBCH 69	a) 2 (14) b) 2	a) 1.5 b) 3.0	a) 0.101(*) + 0.401(**) b) 0.201(*)+ 0.801(**)	400 / 600	NA	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Highest spray broth concentration
42	PL	Stem vegetables (celery)	F	*Sclerotinia sclerotiorum*	SP	BBCH 15 - 49	a) 2 (10) b) 2	a) 1.5 b) 3.0	a) 0.101(*) + 0.401(**) b) 0.201(*)+ 0.801(**)	600 / 800	14	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Minor use
43	PT	Stem vegetables (fennel)	F	*Sclerotinia sclerotiorum*	SP	BBCH 11 - 49	a) 2 (10) b) 2	a) 1.5 b) 3.0	a) 0.101(*) + 0.401(**) b) 0.201(*)+ 0.801(**)	400 / 1000	14	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid
44	ES, GR, IT, PT	Stem vegetables (leek)	F	*Phytophthora porri*	SP	BBCH 11 - 49	a) 3 (7) b) 3	a) 2.0 - 2.5 b) 6.0 - 7.5	a) 0.8 - 0.1(*) + 0.144 - 0.18(**) b) 0.24 - 0.3(*) + 0.432 - 0.54(**)	200 / 1000	3	BAS 536 01 F *40 g/L pyraclostrobin **72 g/L dimethomorph
45	DK, EE	Stem vegetables (leek)	F	*Alternaria porri* *Cladosporium allii-porri*	SP	BBCH 60 - 69	a) 3 (5) b) 3	a) 1.5 b) 4.5	a) 0.101(*) + 0.401(**) b) 0.302(*) + 1.202(**)	300 / 500	14	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Shortest appl. interval
46	DE	Stem vegetables (rhubarb)	F	*Fungal leaf spot diseases*	SP	BBCH 15 - 49	a) 2 (7) b) 2	a) 1.5 b) 3.0	a) 0.101(*) + 0.401(**) b) 0.201(*)+ 0.801(**)	400 / 1000	14	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Minor use

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
47	DK, EE, ES, FI, FR, GR, LT, LV, NO, PL, PT, SE	Leaf vegetables (lettuce & similar)	F	*Bremia lactucae*	SP	BBCH 10 - 49	a) 3 (7) b) 3	a) 2.0 - 2.5 b) 6.0 - 7.5	a) 0.8 - 0.1(*) + 0.144 - 0.18(**) b) 0.24 - 0.3(*) + 0.432 - 0.54(**)	200 / 1000	7	BAS 536 01 F *40 g/L pyraclostrobin **72 g/L dimethomorph
48	IT	Leaf vegetables (lettuce & similar)	F	*Bremia lactucae*	SP	BBCH 10 - 49	a) 3 (7) b) 3	a) 2.0 b) 6.0	a) 0.8(*) + 0.144(**) b) 0.24(*) + 0.432(**)	200 / 1000	3	BAS 536 01 F *40 g/L pyraclostrobin **72 g/L dimethomorph Shortest PHI
49	DE	Leaf vegetables (rucola)	G	*Botrytis cinerea*	SP	from BBCH 14	a) 2 (7) b) 2	a) 1.5 b) 3.0	a) 0.101(*) + 0.401(**) b) 0.201(*) + 0.801(**)	500 / 1000	3	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Minor use Shortest PHI
50	NO	Other root & tuber vegetables (celeriac)	F	*Septoria*	SP	BBCH 15 - 49	a) 2 (10) b) 2	a) 1.5 b) 3.0	a) 0.101(*) + 0.401(**) b) 0.201(*) + 0.801(**)	400 / 1000	14	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid
51	ES, GR, IT	Herbs (fresh herbs)	F	*Peronospora spp.*	SP	BBCH 11 - 49	a) 3 (7) b) 3	a) 2.0 - 2.5 b) 6.0 - 7.5	a) 0.8 - 0.1(*) + 0.144 - 0.18(**) b) 0.24 - 0.3(*) + 0.432 - 0.54(**)	200 / 1000	7	BAS 536 01 F *40 g/L pyraclostrobin **72 g/L dimethomorph

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L or kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
52	NL	Herbs	F, G	*Botryotinia fuckeliana* *Rhizoctonia solani* *Sclerotiona sclerotiorum*	SP	BBCH 11 - 89	a) 2 (14) b) 2	a) 1.5 b) 3.0	a) 0.101(*) + 0.401(**) b) 0.201(*) + 0.801(**)	200 / 800	14	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid Minor use including greenhouse
53	NL	Spices (caraway)	F	*Mycocentrospora acerina*	SP	BBCH 65 - 69	a) 2 (14) b) 2	a) 1.0 b) 2.0	a) 0.067(*) + 0.267(**) b) 0.134(*)+ 0.534(**)	200 / 400	NA	BAS 516 07 F *67 g/kg pyraclostrobin **267 g/kg boscalid
Hops												
54	AT	Hops	F	*Pseudoperonospora humuli* (secondary infection)	SP	Danger/ warning of infection	a) 3 (8) b) 3	a) BBCH 31 - 37: 0.4 - 1.3 a) BBCH 38 - 89: 1.3 - 2.0 b) 6.0	a) 0.051 - 0.166(*) + 0.101 - 0.328(**) a) 0.166 - 0.256(*) + 0.328 - 0.504(**) b) 0.768(*) + 1.512(**)	High volume: 1000 / 4200 Low volume: 700 / 2800	28	BAS 516 04 F *128 g/kg pyraclostrobin **252 g/kg boscalid

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT E1

Listing of Community and Member States MRLs

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EU MRLs set for the uses of pyraclostrobin

The list of current EU MRLs for pyraclostrobin (mg/kg) as of May 7, 2014 (source: DG Sanco website http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=substance.resultat&s=1**),** is shown below. The residue definition is pyraclostrobin (parent only) for all matrices. * indicates the lower limit of analytical determination and (ft) that a footnote is given at the end of the table.

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
100000	1. FRUIT FRESH OR FROZEN NUTS	
110000	(i) Citrus fruit	
110010	Grapefruit (Shaddocks, pomelos, sweeties, tangelo (except mineola), ugli and other hybrids)	1
110020	Oranges (Bergamot, bitter orange, chinotto and other hybrids)	2
110030	Lemons (Citron, lemon, Buddha's hand (Citrus medica var. sarcodactylis))	1
110040	Limes	1
110050	Mandarins (Clementine, tangerine, mineola and other hybrids tangor (Citrus reticulata x sinensis))	1
110990	Others	1
120000	(ii) Tree nuts	
120010	Almonds	0.02*
120020	Brazil nuts	0.02*
120030	Cashew nuts	0.02*
120040	Chestnuts	0.02*
120050	Coconuts	0.02*
120060	Hazelnuts (Filbert)	0.02*
120070	Macadamia	0.02*
120080	Pecans	0.02*
120090	Pine nuts	0.02*
120100	Pistachios	1
120110	Walnuts	0.02*
120990	Others	0.02*
130000	(iii) Pome fruit	0.5
130010	Apples (Crab apple)	0.5
130020	Pears (Oriental pear)	0.5
130030	Quinces	0.5
130040	Medlar	0.5
130050	Loquat	0.5
130990	Others	0.5

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
140000	(iv) Stone fruit	
140010	Apricots	1
140020	Cherries (Sweet cherries, sour cherries)	3
140030	Peaches (Nectarines and similar hybrids)	0.3
140040	Plums (Damson, greengage, mirabelle, sloe, red date/Chinese date/Chinese jujube (Ziziphus zizyphus))	0.8
140990	Others	0.02*
150000	(v) Berries & small fruit	
151000	(a) Table and wine grapes	
151010	Table grapes	1 (ft)
151020	Wine grapes	2
152000	(b) Strawberries	1.5
153000	(c) Cane fruit	
153010	Blackberries	3
153020	Dewberries (Loganberries, tayberries, boysenberries, cloudberries and other Rubus hybrids)	2
153030	Raspberries (Wineberries, arctic bramble/raspberry, (Rubus arcticus), nectar raspberries (Rubus arcticus x Rubus idaeus))	3
153990	Others	2
154000	(d) Other small fruit & berries	
154010	Blueberries (Bilberries)	4
154020	Cranberries (Cowberries/redbilberries (V. vitis-idaea))	3
154030	Currants (red, black and white)	3
154040	Gooseberries (Including hybrids with other Ribes species)	3
154050	Rose hips	3
154060	Mulberries (Arbutus berry)	3
154070	Azarole (mediteranean medlar) (Kiwiberry (Actinidia arguta))	3
154080	Elderberries (Black chokeberry/appleberry, mountain ash, buckthorn/sea sallowthorn, hawthorn, serviceberries, and other treeberries)	3
154990	Others	3

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
160000	(vi) Miscellaneous fruit	
161000	(a) Edible peel	0.02*
161010	Dates	0.02*
161020	Figs	0.02*
161030	Table olives	0.02*
161040	Kumquats (Marumi kumquats, nagami kumquats, limequats (Citrus aurantifolia x Fortunella spp.))	0.02*
161050	Carambola (Bilimbi)	0.02*
161060	Persimmon	0.02*
161070	Jambolan (java plum) (Java apple/water apple, pomerac, rose apple, Brazilean cherry, Surinam cherry/grumichama (Eugenia uniflora))	0.02*
161990	Others	0.02*
162000	(b) Inedible peel, small	0.02*
162010	Kiwi	0.02*
162020	Lychee (Litchi) (Pulasan, rambutan/hairy litchi, longan, mangosteen, langsat, salak)	0.02*
162030	Passion fruit	0.02*
162040	Prickly pear (cactus fruit)	0.02*
162050	Star apple	0.02*
162060	American persimmon (Virginia kaki) (Black sapote, white sapote, green sapote, canistel/yellow sapote, mammey sapote)	0.02*
162990	Others	0.02*
163000	(c) Inedible peel, large	
163010	Avocados	0.02*
163020	Bananas (Dwarf banana, plantain, apple banana)	0.02*
163030	Mangoes	0.05
163040	Papaya	0.07
163050	Pomegranate	0.02*
163060	Cherimoya (Custard apple, sugar apple/sweetsop, ilama (Annona diversifolia) and other medium sized Annonaceae fruits)	0.02*
163070	Guava (Red pitaya/dragon fruit (Hylocereus undatus))	0.02*
163080	Pineapples	0.02*
163090	Bread fruit (Jackfruit)	0.02*
163100	Durian	0.02*
163110	Soursop (guanabana)	0.02*
163990	Others	0.02*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
200000	2. VEGETABLES FRESH OR FROZEN	
210000	(i) Root and tuber vegetables	
211000	(a) Potatoes	0.02*
212000	(b) Tropical root and tuber vegetables	0.02*
212010	Cassava (Dasheen, eddoe/Japanese taro, tannia)	0.02*
212020	Sweet potatoes	0.02*
212030	Yams (Potato bean/yambean, Mexican yambean)	0.02*
212040	Arrowroot	0.02*
212990	Others	0.02*
213000	(c) Other root and tuber vegetables except sugar beet	
213010	Beetroot	0.1
213020	Carrots	0.5
213030	Celeriac	0.3
213040	Horseradish (Angelica roots, lovage roots, gentiana roots)	0.3
213050	Jerusalem artichokes (Crosne)	0.06
213060	Parsnips	0.3
213070	Parsley root	0.1
213080	Radishes (Black radish, Japanese radish, small radish and similar varieties, tiger nut (Cyperus esculentus))	0.5
213090	Salsify (Scorzonera, Spanish salsify/Spanish oysterplant, edible burdock)	0.1
213100	Swedes	0.02*
213110	Turnips	0.02*
213990	Others	0.02*
220000	(ii) Bulb vegetables	
220010	(a) Garlic	0.3
220020	(b) Onions (Other bulb onions, silverskin onions)	1.5
220030	(c) Shallots	0.3
220040	(d) Spring onions and welsh onions (Other green onions and similar varieties)	1.5
220990	(e) Others	0.02*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
230000	(iii) Fruiting vegetables	
231000	(a) Solanacea	
231010	Tomatoes (Cherry tomatoes, Physalis spp., gojiberry, wolfberry (Lycium barbarum and L. chinense), tree tomato)	0.3
231020	Peppers (Chilli peppers)	0.5
231030	Aubergines (egg plants) (Pepino, antroewa/white eggplant (S. macrocarpon))	0.3
231040	Okra (lady's fingers)	0.02*
231990	Others	0.02*
232000	(b) Cucurbits — edible peel	0.5
232010	Cucumbers	0.5
232020	Gherkins	0.5
232030	Courgettes (Summer squash, marrow (patisson), lauki (Lagenaria siceraria), chayote, sopropo/bitter melon, snake gourd, angled luffa/teroi)	0.5
232990	Others	0.5
233000	(c) Cucurbits-inedible peel	0.5
233010	Melons (Kiwano)	0.5
233020	Pumpkins (Winter squash, marrow (late variety))	0.5
233030	Watermelons	0.5
233990	Others	0.5
234000	(d) Sweet corn (Baby corn)	0.02*
239000	(e) Other fruiting vegetables	0.02*
240000	(iv) Brassica vegetables	
241000	(a) Flowering brassica	0.1
241010	Broccoli (Calabrese, Broccoli raab, Chinese broccoli)	0.1
241020	Cauliflower	0.1
241990	Others	0.1
242000	(b) Head brassica	
242010	Brussels sprouts	0.3
242020	Head cabbage (Pointed head cabbage, red cabbage, savoy cabbage, white cabbage)	0.2
242990	Others	0.02*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
243000	(c) Leafy brassica	1.5
243010	Chinese cabbage (Indian or Chinese) mustard, pak choi, Chinese flat cabbage/ai goo choi), choi sum, Peking cabbage/pe-tsai)	1.5
243020	Kale (Borecole/curly kale, collards, Portuguese Kale, Portuguese cabbage, cow cabbage)	1.5
243990	Others	1.5
244000	(d) Kohlrabi	0.02*
250000	(v) Leaf vegetables & fresh herbs	
251000	(a) Lettuce and other salad plants including Brassicacea	
251010	Lamb's lettuce (Italian corn salad)	10
251020	Lettuce (Head lettuce, lollo rosso (cutting lettuce), iceberg lettuce, romaine (cos) lettuce)	2
251030	Scarole (broad-leaf endive) (Wild chicory, red-leaved chicory, radicchio, curly leaf endive, sugar loaf (C. endivia var. crispum/C. intybus var. foliosum), dandelion greens)	0.4
251040	Cress (Mung bean sprouts, alfalfa sprouts)	10
251050	Land cress	10
251060	Rocket, Rucola (Wild rocket (Diplotaxis spp.))	10
251070	Red mustard	10
251080	Leaves and sprouts of Brassica spp, including turnip greens (Mizuna, leaves of peas and radish and other babyleaf crops, including brassica crops (crops harvested up to 8 true leaf stage), kohlrabi leaves)	10
251990	Others	10
252000	(b) Spinach & similar (leaves)	
252010	Spinach (New Zealand spinach, amaranthus spinach (pak-khom, tampara), tajer leaves, bitterblad/bitawiri)	0.5
252020	Purslane (Winter purslane/miner's lettuce, garden purslane, common purslane, sorrel, glassworth, agretti (Salsola soda))	0.02*
252030	Beet leaves (chard) (Leaves of beetroot)	0.5
252990	Others	0.02*
253000	(c) Vine leaves (grape leaves) (Malabar nightshade, banana leaves, climbing wattle (Acacia pennata))	0.02*
254000	(d) Water cress (Morning glory/Chinese convolvulus/water convolvulus/water spinach/kangkung (Ipomea aquatica), water clover, water mimosa)	0.02*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
255000	(e) Witloof	0.02*
256000	(f) Herbs	2
256010	Chervil	2
256020	Chives	2
256030	Celery leaves (Fennel leaves, coriander leaves, dill leaves, caraway leaves, lovage, angelica, sweet cisely and other Apiacea leaves, culantro/stinking/long coriander/stink weed (Eryngium foetidum))	2
256040	Parsley (leaves of root parsley)	2
256050	Sage (Winter savory, summer savory, Borago officinalis leaves)	2
256060	Rosemary	2
256070	Thyme (Marjoram, oregano)	2
256080	Basil (Balm leaves, mint, peppermint, holy basil, sweet basil, hairy basil, edible flowers (marigold flower and others), pennywort, wild betel leaf, curry leaves)	2
256090	Bay leaves (laurel) (Lemon grass)	2
256100	Tarragon (Hyssop)	2
256990	Others	2
260000	(vi) Legume vegetables (fresh)	0.02*
260010	Beans (with pods) (Green bean/French beans/snap beans, scarlet runner bean, slicing bean, yard long beans, guar beans, soya beans)	0.02*
260020	Beans (without pods) (Broad beans, flageolets, jack bean, lima bean, cowpea)	0.02*
260030	Peas (with pods) (Mangetout/sugar peas/snow peas)	0.02*
260040	Peas (without pods) (Garden pea, green pea, chickpea)	0.02*
260050	Lentils	0.02*
260990	Others	0.02*
270000	(vii) Stem vegetables (fresh)	
270010	Asparagus	0.02*
270020	Cardoons (Borago officinalis stems)	0.02*
270030	Celery	0.02* (ft)
270040	Fennel	0.02*
270050	Globe artichokes (Banana flower)	2
270060	Leek	0.7
270070	Rhubarb	0.02*
270080	Bamboo shoots	0.02*
270090	Palm hearts	0.02*
270990	Others	0.02*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
280000	(viii) Fungi	0.02*
280010	Cultivated fungi (Common mushroom, oyster mushroom, shiitake, fungus mycelium (vegetative parts))	0.02*
280020	Wild fungi (Chanterelle, truffle, morel, cep)	0.02*
280990	Others	0.02*
290000	(ix) Sea weeds	0.02*
300000	3. PULSES, DRY	
300010	Beans (Broad beans, navy beans, flageolets, jack beans, lima beans, field beans, cowpeas)	0.3
300020	Lentils	0.5
300030	Peas (Chickpeas, field peas, chickling vetch)	0.3
300040	Lupins	0.05
300990	Others	0.3
400000	4. OILSEEDS AND OILFRUITS	
401000	(i) Oilseeds	
401010	Linseed	0.2
401020	Peanuts	0.04
401030	Poppy seed	0.2
401040	Sesame seed	0.2
401050	Sunflower seed	0.3
401060	Rape seed (Bird rapeseed, turnip rape)	0.2
401070	Soya bean	0.05
401080	Mustard seed	0.2
401090	Cotton seed	0.3
401100	Pumpkin seeds (Other seeds of Cucurbitaceae)	0.02*
401110	Safflower	0.2
401120	Borage (Purple viper's bugloss/Canary flower (Echium plantagineum), Corn Gromwell (Buglossoides arvensis))	0.2
401130	Gold of pleasure	0.2
401140	Hempseed	0.02*
401150	Castor bean	0.2
401990	Others	0.02*
402000	(ii) Oilfruits	0.02*
402010	Olives for oil production	0.02*
402020	Palm nuts (palmoil kernels)	0.02*
402030	Palmfruit	0.02*
402040	Kapok	0.02*
402990	Others	0.02*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
500000	5. CEREALS	
500010	Barley	1
500020	Buckwheat (Amaranthus, quinoa)	0.02*
500030	Maize	0.02*
500040	Millet (Foxtail millet, teff, finger millet, pearl millet)	0.02*
500050	Oats	1
500060	Rice (Indian/wild rice (Zizania aquatica))	0.02*
500070	Rye	0.2
500080	Sorghum	0.5
500090	Wheat (Spelt, triticale)	0.2
500990	Others (Canary grass seeds (Phalaris canariensis))	0.02*
600000	6. TEA, COFFEE, HERBAL INFUSIONS AND COCOA	
610000	(i) Tea	0.1*
620000	(ii) Coffee beans	0.3 (ft)
630000	(iii) Herbal infusions (dried)	0.1*
631000	(a) Flowers	0.1*
631010	Camomille flowers	0.1*
631020	Hybiscus flowers	0.1*
631030	Rose petals	0.1*
631040	Jasmine flowers (Elderflowers (Sambucus nigra))	0.1*
631050	Lime (linden)	0.1*
631990	Others	0.1*
632000	(b) Leaves	0.1*
632010	Strawberry leaves	0.1*
632020	Rooibos leaves (Ginkgo leaves)	0.1*
632030	Maté	0.1*
632990	Others	0.1*
633000	(c) Roots	0.1*
633010	Valerian root	0.1*
633020	Ginseng root	0.1*
633990	Others	0.1*
639000	(d) Other herbal infusions	0.1*
640000	(iv) Cocoabeans (fermented or dried)	0.1*
650000	(v) Carob (st johns bread)	0.1*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
700000	7. HOPS (dried)	15
800000	8. SPICES	
810000	(i) Seeds	0.1*
810010	Anise	0.1*
810020	Black caraway	0.1*
810030	Celery seed (Lovage seed)	0.1*
810040	Coriander seed	0.1*
810050	Cumin seed	0.1*
810060	Dill seed	0.1*
810070	Fennel seed	0.1*
810080	Fenugreek	0.1*
810090	Nutmeg	0.1*
810990	Others	0.1*
820000	(ii) Fruits and berries	0.1*
820010	Allspice	0.1*
820020	Sichuan pepper (Anise pepper, Japan pepper)	0.1*
820030	Caraway	0.1*
820040	Cardamom	0.1*
820050	Juniper berries	0.1*
820060	Pepper, black, green and white (Long pepper, pink pepper)	0.1*
820070	Vanilla pods	0.1*
820080	Tamarind	0.1*
820990	Others	0.1*
830000	(iii) Bark	0.1*
830010	Cinnamon (Cassia)	0.1*
830990	Others	0.1*
840000	(iv) Roots or rhizome	
840010	Liquorice	0.1*
840020	Ginger	0.1*
840030	Turmeric (Curcuma)	0.1*
840040	Horseradish	(ft)
840990	Others	0.1*
850000	(v) Buds	0.1*
850010	Cloves	0.1*
850020	Capers	0.1*
850990	Others	0.1*
860000	(vi) Flower stigma	0.1*
860010	Saffron	0.1*
860990	Others	0.1*
870000	(vii) Aril	0.1*
870010	Mace	0.1*
870990	Others	0.1*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
900000	9. SUGAR PLANTS	
900010	Sugar beet (root)	0.2
900020	Sugar cane	0.02*
900030	Chicory roots	0.02*
900990	Others	0.02*
1000000	10. PRODUCTS OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS	
1010000	(i) Tissue	0.05*
1011000	(a) Swine	0.05*
1011010	Muscle	0.05*
1011020	Fat	0.05*
1011030	Liver	0.05*
1011040	Kidney	0.05*
1011050	Edible offal	0.05*
1011990	Others	0.05*
1012000	(b) Bovine	0.05*
1012010	Muscle	0.05*
1012020	Fat	0.05*
1012030	Liver	0.05*
1012040	Kidney	0.05*
1012050	Edible offal	0.05*
1012990	Others	0.05*
1013000	(c) Sheep	0.05*
1013010	Muscle	0.05*
1013020	Fat	0.05*
1013030	Liver	0.05*
1013040	Kidney	0.05*
1013050	Edible offal	0.05*
1013990	Others	0.05*
1014000	(d) Goat	0.05*
1014010	Muscle	0.05*
1014020	Fat	0.05*
1014030	Liver	0.05*
1014040	Kidney	0.05*
1014050	Edible offal	0.05*
1014990	Others	0.05*
1015000	(e) Horses, asses, mules or hinnies	0.05*
1015010	Muscle	0.05*
1015020	Fat	0.05*
1015030	Liver	0.05*
1015040	Kidney	0.05*
1015050	Edible offal	0.05*
1015990	Others	0.05*

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
1016000	(f) Poultry chicken, geese, duck, turkey and Guinea fowl, ostrich, pigeon	0.05*
1016010	Muscle	0.05*
1016020	Fat	0.05*
1016030	Liver	0.05*
1016040	Kidney	0.05*
1016050	Edible offal	0.05*
1016990	Others	0.05*
1017000	(g) Other farm animals (Rabbit, kangaroo, deer)	0.05*
1017010	Muscle	0.05*
1017020	Fat	0.05*
1017030	Liver	0.05*
1017040	Kidney	0.05*
1017050	Edible offal	0.05*
1017990	Others	0.05*
1020000	(ii) Milk	0.01*
1020010	Cattle	0.01*
1020020	Sheep	0.01*
1020030	Goat	0.01*
1020040	Horse	0.01*
1020990	Others	0.01*
1030000	(iii) Bird eggs	0.05*
1030010	Chicken	0.05*
1030020	Duck	0.05*
1030030	Goose	0.05*
1030040	Quail	0.05*
1030990	Others	0.05*
1040000	(iv) Honey (Royal jelly, pollen, honey comb with honey (comb honey))	0.05*
1050000	(v) Amphibians and reptiles (Frog legs, crocodiles)	0.05*
1060000	(vi) Snails	0.05*
1070000	(vii) Other terrestrial animal products (Wild game)	0.05*

Footnotes:

Pyraclostrobin (F)
(F) = Fat soluble

0151010 Table grapes
The European Food Safety Authority identified some information on residue trials as unavailable. When reviewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 13 July 2015, or, if that information is not submitted by that date, the lack of it.

0270030 Celery
The European Food Safety Authority identified some information on residue trials as unavailable. When reviewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 13 July 2015, or, if that information is not submitted by that date, the lack of it.

0620000 (ii) Coffee beans
The European Food Safety Authority identified some information on analytical methods as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 13 July 2015, or, if that information is not submitted by that date, the lack of it.

0840040 Horseradish
The applicable maximum residue level for horseradish (Armoracia rusticana) in the spice group (code 0840040) is the one set for horseradish (Armoracia rusticana) in the vegetables category, root and tuber vegetables group (code 0213040) taking into account changes in the levels by processing (drying) according to Art. 20 (1) of Regulation (EC) No 396/2005.

CODEX MRLs set for the uses of pyraclostrobin

The list of current CODEX MRLs for pyraclostrobin can be found on the CODEX ALIMENTARIUS website (http://www.codexalimentarius.net/pestres/data/index.html).

The list (as of May 2014) is given below in this document to allow for direct comparison with the EU-MRLs shown above. CODEX and EU have the same residue definition established.

Commmodity	Pyraclostrobin CODEX MRL [mg/kg]	Note
Alfalfa fodder	30	
Apple	0.5	
Artichoke, Globe	2	
Banana	0.02	(*)
Barley	1	
Beans (dry)	0.2	
Blackberries	3	
Blueberries	4	
Brussels sprouts	0.3	
Cabbages, Head	0.2	
Cantaloupe	0.2	
Carrot	0.5	
Cherries	3	
Citrus fruits	2	excluding kumquats
Citrus oil, edible	10	
Coffee beans	0.3	
Dried grapes (=currants, raisins and sultanas)	5	
Edible offal (mammalian)	0.05	(*)
Egg plant	0.3	
Eggs	0.05	(*)
Flowerhead brassicas (includes Broccoli: Broccoli, Chinese and Cauliflower)	0.1	
Fruiting vegetables, Cucurbits	0.5	
Garlic	0.15	
Grapes	2	
Hops, Dry	15	
Kale	1	
Leek	0.7	
Lentil (dry)	0.5	
Lettuce, Head	2	

Commmodity	Pyraclostrobin CODEX MRL [mg/kg]	Note
Maize	0.02	(*)
Mango	0.05	(*)
Meat (from mammals other than marine mammals)	0.5	(fat)
Milks	0.03	
Nectarine	0.3	
Oats	1	
Oilseed, except peanut	0.4	
Onion, Bulb	1.5	
Papaya	0.15	
Pea hay or pea fodder (dry)	30	
Peach	0.3	
Peanut fodder	50	
Peanut, whole	0.02	(*)
Peas (dry)	0.3	
Peas (pods and succulent=immature seeds)	0.02	(*)
Peppers	0.5	
Pistachio nuts	1	
Plums (including prunes)	0.8	
Potato	0.02	(*)
Poultry meat	0.05	(*) (fat)
Poultry, Edible offal of	0.05	(*)
Radish	0.5	
Radish leaves (including radish tops)	20	
Raspberries, Red, Black	3	
Rye	0.2	
Sorghum	0.5	
Soya bean (dry)	0.05	
Spelt	0.2	
Spring Onion	1.5	
Straw and fodder (dry) of cereal grains	30	
Strawberry	1.5	
Sugar beet	0.2	
Tomato	0.3	
Tree nuts	0.02	(*) except pistachio nuts
Triticale	0.2	
Wheat	0.2	

(*) at or about the limit of determination
(fat) (for meat) The MRL/EMRL applies to the fat of meat.

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT E2

Listing of MRLs established in exporting countries

Compiled by:

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MRLs (worldwide, except EU) set for the uses of pyraclostrobin

The MRLs currently set in the EU can be found in Document E1.

1. Residue definitions

The metabolites are given in Table 1 according to the chemical names found in the internet on the official authority websites. The respective metabolite codes are shown in the footnotes at the end of the table.
In case no clear information on the residue definition could be found on the official authority websites, an expert judgment was done based on BASF-internal knowledge and the official MRL-values given. The residue definition is described in these cases as "Pyraclostrobin (assumed as 'parent only')".

Table 1: Residue definitions for pyraclostrobin

Active Substance: Pyraclostrobin		
Country	**Residue definition plant**	**Residue definition animal**
Argentina	Pyraclostrobin (assumed as 'parent only')	
Australia	Pyraclostrobin (parent only)	Sum of pyraclostrobin and metabolites hydrolysed to 1-(4-chloro-phenyl)-1H-pyrazol-3-ol ** expressed as pyraclostrobin
Brazil	Sum of pyraclostrobin and its metabolite N-[[1-(4-chlorophenyl) pyrazol-3-yl]oxy-o-tolyl]carbamate *	
Canada	All food crops: methyl *N*-[2-[[[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxy]methyl]phenyl]-N-methoxycarbamate, including the metabolite carbamic acid, *N*-[2-[[[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl] oxy] methyl]phenyl]-methyl ester *	All livestock commodities, except poultry related: methyl *N*-[2-[[[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxy]methyl]phenyl*]*-*N*-methoxycarbamate, including the metabolites convertible to 1-(4-chlorophenyl)-1*H*-pyrazol-3-ol and 1-(4-chloro-2-hydroxyphenyl)-1*H*-pyrazol-3-ol ** Eggs, meat and meat byproducts of poultry: methyl *N*-[2-[[[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxy]methyl]phenyl*]*-*N*-methoxycarbamate, including the metabolites convertible to 1-(4-chlorophenyl)-1*H*-pyrazol-3-ol and 1-(3-chloro-4-hydroxyphenyl)-1*H*-pyrazol-3-ol ***
Chile	Pyraclostrobin (assumed as 'parent only')	
China	Pyraclostrobin (assumed as 'parent only')	

Active Substance: Pyraclostrobin		
Country	**Residue definition plant**	**Residue definition animal**
CODEX	Pyraclostrobin (parent only)	Pyraclostrobin (parent only)
Israel	Pyraclostrobin (assumed as 'parent only')	
Japan	Pyraclostrobin (assumed as 'parent only')	Pyraclostrobin (assumed as 'parent only')
New Zealand	Pyraclostrobin (assumed as 'parent only')	Pyraclostrobin (assumed as 'parent only')
Russian Federation	Pyraclostrobin (assumed as 'parent only')	Pyraclostrobin (assumed as 'parent only')
South Africa	Pyraclostrobin (parent) and includes the -desmethoxy metabolite (source: NZ MRL database)	
South Korea	Pyraclostrobin (assumed as 'parent only')	
Taiwan	Pyraclostrobin (assumed as 'parent only')	
USA	Tolerances are established for residues of the fungicide pyraclostrobin, including its metabolites and degradates. Compliance with the tolerance levels specified is to be determined by measuring only the sum of pyraclostrobin (carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy] methyl]phenyl]methoxy-methyl ester) and its desmethoxy metabolite (methyl-N-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy] methyl] phenylcarbamate) *, calculated as the stoichiometric equivalent of pyraclostrobin	Tolerances are established for combined residues of the fungicide pyraclostrobin carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy] methyl]phenyl]methoxy- methyl ester and its metabolites convertible to 1-(4-chlorophenyl)-1H-pyrazol-3-ol and 1-(4-chloro-2-hydroxyphenyl)-1H-pyrazol-3-ol **, expressed as parent compound

* Desmethoxy metabolite 500M07 (synonym: BF 500-3)
** Metabolites 500M04 (synonym: BF 500-5) and 500M85 (synonym: BF 500-8)
*** Metabolites 500M04 (synonym: BF 500-5) and BF 500-9

2. CODEX MRLs set for the uses of pyraclostrobin

The list of current CODEX MRLs for pyraclostrobin can be found on the CODEX ALIMENTARIUS website:

(http://www.codexalimentarius.net/pestres/data/index.html)

The list of current CODEX MRLs (as of May 2014) is given below.

Commmodity	**Pyraclostrobin CODEX MRL [mg/kg]**	**Note**
Alfalfa fodder	30	
Apple	0.5	
Artichoke, Globe	2	
Banana	0.02	(*)
Barley	1	
Beans (dry)	0.2	
Blackberries	3	
Blueberries	4	
Brussels sprouts	0.3	
Cabbages, Head	0.2	
Cantaloupe	0.2	
Carrot	0.5	
Cherries	3	
Citrus fruits	2	excluding kumquats
Citrus oil, edible	10	
Coffee beans	0.3	
Dried grapes (=currants, raisins and sultanas)	5	
Edible offal (mammalian)	0.05	(*)
Egg plant	0.3	
Eggs	0.05	(*)
Flowerhead brassicas (includes Broccoli: Broccoli, Chinese and Cauliflower)	0.1	
Fruiting vegetables, Cucurbits	0.5	
Garlic	0.15	
Grapes	2	
Hops, Dry	15	
Kale	1	
Leek	0.7	
Lentil (dry)	0.5	
Lettuce, Head	2	

Commmodity	Pyraclostrobin CODEX MRL [mg/kg]	Note
Maize	0.02	(*)
Mango	0.05	(*)
Meat (from mammals other than marine mammals)	0.5	(fat)
Milks	0.03	
Nectarine	0.3	
Oats	1	
Oilseed, except peanut	0.4	
Onion, Bulb	1.5	
Papaya	0.15	
Pea hay or pea fodder (dry)	30	
Peach	0.3	
Peanut fodder	50	
Peanut, whole	0.02	(*)
Peas (dry)	0.3	
Peas (pods and succulent=immature seeds)	0.02	(*)
Peppers	0.5	
Pistachio nuts	1	
Plums (including prunes)	0.8	
Potato	0.02	(*)
Poultry meat	0.05	(*) (fat)
Poultry, Edible offal of	0.05	(*)
Radish	0.5	
Radish leaves (including radish tops)	20	
Raspberries, Red, Black	3	
Rye	0.2	
Sorghum	0.5	
Soya bean (dry)	0.05	
Spelt	0.2	
Spring Onion	1.5	
Straw and fodder (dry) of cereal grains	30	
Strawberry	1.5	
Sugar beet	0.2	
Tomato	0.3	
Tree nuts	0.02	(*) except pistachio nuts
Triticale	0.2	
Wheat	0.2	

(*) at or about the limit of determination
(fat) (for meat) The MRL/EMRL applies to the fat of meat.

3. Country MRLs set for the uses of pyraclostrobin

For collecting all current MRL data, different sources were used (country homepages, Homologa database, farmers´ associations and BASF-internal information), a complete reliability check is not possible. In case an official MRL source was identified, this information is given.

Argentina

The list of current MRLs set for pyraclostrobin in Argentina can be found on the following website:

(http://produccion.sanjuan.gov.ar/index.php?option=com_content&view=article&id=200%3A productos-fitosanitarios-registrados-en-argentina-x&catid=92%3Adestacados&Itemid=130).
MRL list: Activos Registrados y sus LMR, con actualizaciones posteriores (Res. 934/2010).
The National of MRLs is rarely updated, e.g. 2008 Resolution 934/2010. Due to this fact the commercial data base HOMOLOGA was checked in addition to the official website of the authority.
(http://www.homologa-new.com/pls/apex/f?p=550:1)

The list of MRLs currently set in Argentina is given below.

Commodity	Pyraclostrobin MRLs in Argentina [mg/kg]
Garlic	0.05
Blueberry	0.5
Peach	0.2
Lemon	1
Orange	1
Soybean (oil)	0.1
Soybean (seed)	0.05
Wheat (seed)	0.2
Peanuts (shelled)	0.05
Mandarin	0.5
Beans (seed, fresh)	0.1
Tablegrape	2
Winegrape	2
Potato	0.04
Tomato	0.2
Corn (oil)	0.3
Corn (grain)	0.1
Sunflower (seed)	0.05
Sunflower (oil)	0.05
Barley	0.5 *
Strawberry	0.5 *
Pummelo/Pomelo/Shaddock	1 *
Sweet Pepper/Bell pepper	0.5 *
Radicchio	2 *
Peas (seed, fresh)	0.2 *

* MRL of the commercial data base HOMOLOGA

Australia

The list of current MRLs set for pyraclostrobin in Australia can be found on the Australian pesticides and veterinary medicines authority (APVMA) website:

(http://www.apvma.gov.au/residues/standard.php#tables)
MRL list: comlaw website (external site) - Agricultural and Veterinary Chemicals Code Instrument No. 4 (*MRL Standard*) 2012 - prepared 11 April 2014

The list of MRLs currently set in Australia (as of May 2014) is given below.

Commodity	**Pyraclostrobin MRLs in Australia [mg/kg]**
Banana	* 0.02
Blackberries	T 3
Blueberries	T 5
Brassica leafy vegetables	T 3
Broccoli, Chinese	T 1
Cereal grains	* 0.01
Cherries	T 1
Cloudberry	T 3
Custard apple	T 3
Dewberries (including Boysenberry and Loganberry)	T 3
Dried grapes	5
Edible offal (Mammalian)	0.1
Eggs	* 0.05
Fruiting vegetables, other than Cucurbits	0.3
Grapes	2
Litchi	T 2
Mango	0.1
Meat [mammalian][in the fat]	* 0.05
Milks	* 0.01
Mung bean (dry)	T 0.2
Papaya [pawpaw]	T 0.5
Passion fruit	T 1
Pistachio nut	T 1
Pome fruits	1
Poppy seed	* 0.05
Potato	* 0.02
Poultry, Edible offal of	* 0.05
Poultry meat [in the fat]	* 0.05
Raspberries, Red, Black	T 3

Commodity	Pyraclostrobin MRLs in Australia [mg/kg]
Silvanberries	T 3
Sunflower seed	T 0.3
Tree nuts [except Pistachio nut]	* 0.01
Youngberry	T 3
Almond hulls	3
Apple pomace, dry	25
Cereal forage, green	5
Grape pomace, dry	10
Mung bean fodder	T 2
Mung bean forage	T 2
Oat forage (green)[fresh weight]	* 0.05
Oat straw and fodder, dry	* 0.05
Straw and fodder of cereal grains, dry	0.5

* The MRL or the ERL is set at or about the limit of analytical quantitation.

T The MRL or ERL, residue definition or use is temporary to enable further experimental work to be carried out in Australia or overseas, and will be reconsidered at some future date. This symbol is also used in cases where an MRL or ERL is being phased out.

Brazil

The list of current MRLs set for pyraclostrobin in Brazil can be found on the Brazilian food safety agency (ANVISA) website:

(http://portal.anvisa.gov.br/wps/content/Anvisa+Portal/Anvisa/Inicio/Agrotoxicos+e+Toxicologia/Assuntos+de+Interesse/Monografias+de+Agrotoxicos/Monografias)

The list of MRLs currently set in Brazil (as of May 2014) is given below.

Commodity	Pyraclostrobin MRLs in Brazil [mg/kg]
Cotton	0.2
Garlic	0.1
Peanut	0.1
Rice	0,02
Oat	1.0
Banana	0.5
Potato	0.01
Coffee bean	0.5
Sugarcane	0.1
Onion	0.5
Carrot	0.2
Barley	1.0
Citrus fruits	0.5
Bean	0.1
Apple	2.0
Papaya	0.1
Mango	0.1
Watermelon	0.1
Melon	0.1
Corn	0.1
Cucumber	0.05
Pepper	1.0
Soybean	0.1
Sorghum	0.02
Tomato	0.2
Wheat	0.5
Grape	2.0

Canada

The list of current MRLs set for pyraclostrobin in Canada can be found on the Health Canada (PMRA) website:

(http://pr-rp.hc-sc.gc.ca/mrl-lrm/index-eng.php)

The list of MRLs currently set in Canada (as of May 2014) is given below.

Commodity	Pyraclostrobin MRLs in Canada [mg/kg]
Raisins	7
Grapes	2
Liver of cattle	1.5
Liver of goats	1.5
Liver of hogs	1.5
Liver of horses	1.5
Liver of sheep	1.5
Bell peppers	1
Eggplants	1
Groundcherries	1
Non-bell peppers	1
Pepinos	1
Pepper hybrids	1
Tomatillos	1
Tomatoes	1
Apricots	0.7
Nectarines	0.7
Peaches	0.7
Plumcots	0.7
Plums	0.7
Fresh prune plums	0.7
Sweet cherries	0.7
Tart cherries	0.7
Balsam apples	0.5
Balsam pears	0.5
Cantaloupes	0.5
Chayote fruit	0.5
Chinese cucumbers	0.5
Chinese waxgourds	0.5
Citron melons	0.5
Cucumbers	0.5
Dry adzuki beans	0.5
Dry beans	0.5

Commodity	Pyraclostrobin MRLs in Canada [mg/kg]
Dry blackeyed peas	0.5
Dry broad beans	0.5
Dry catjang seeds	0.5
Dry chickpeas	0.5
Dry field peas	0.5
Dry guar seeds	0.5
Dry kidney beans	0.5
Dry lablab beans	0.5
Dry lentils	0.5
Dry lima beans	0.5
Dry moth beans	0.5
Dry mung beans	0.5
Dry navy beans	0.5
Dry pigeon peas	0.5
Dry pink beans	0.5
Dry pinto beans	0.5
Dry rice beans	0.5
Dry southern peas	0.5
Dry tepary beans	0.5
Dry urd beans	0.5
Edible gourds (other than those listed in this item)	0.5
Grain lupin	0.5
Muskmelons (other than those listed in this item)	0.5
Pistachio nuts	0.5
Pumpkins	0.5
Summer squash	0.5
Watermelons	0.5
West Indian gherkins	0.5
Winter squash	0.5
Barley	0.4
Black salsify roots	0.4
Carrot roots	0.4
Celeriac roots	0.4
Chicory roots	0.4
Edible burdock roots	0.4
Edible-podded dwarf peas	0.4
Edible-podded jackbeans	0.4
Edible-podded moth beans	0.4
Edible-podded peas	0.4
Edible-podded pigeon peas	0.4
Edible-podded runner beans	0.4

Commodity	Pyraclostrobin MRLs in Canada [mg/kg]
Edible-podded snap beans	0.4
Edible-podded snow peas	0.4
Edible-podded soybeans	0.4
Edible-podded sugar snap peas	0.4
Edible-podded sword beans	0.4
Edible-podded wax beans	0.4
Edible-podded yardlong beans	0.4
Garden beet roots	0.4
Ginseng roots	0.4
Horseradish roots	0.4
Oriental radish roots	0.4
Parsnip roots	0.4
Radish roots	0.4
Rutabaga roots	0.4
Salsify roots	0.4
Skirret roots	0.4
Spanish salsify roots	0.4
Turnip-rooted chervil roots	0.4
Turnip roots	0.4
Turnip-rooted parsley roots	0.4
Succulent shelled blackeyed peas	0.35
Succulent shelled broad beans	0.35
Succulent shelled English peas	0.35
Succulent shelled garden peas	0.35
Succulent shelled green peas	0.35
Succulent shelled lima beans	0.35
Succulent shelled peas	0.35
Succulent shelled pigeon peas	0.35
Succulent shelled southern peas	0.35
Meat byproducts of cattle (except liver)	0.2
Meat byproducts of goats (except liver)	0.2
Meat byproducts of hogs (except liver)	0.2
Meat byproducts of horses (except liver)	0.2
Meat byproducts of sheep (except liver)	0.2
Wheat	0.2
Sugar beet roots	0.15
Fat of cattle	0.1
Fat of goats	0.1
Fat of hogs	0.1
Fat of horses	0.1
Fat of sheep	0.1
Meat of cattle	0.1

Commodity	Pyraclostrobin MRLs in Canada [mg/kg]
Meat of goats	0.1
Meat of hogs	0.1
Meat of horses	0.1
Meat of sheep	0.1
Milk	0.1
Peanut oil (refined)	0.1
Peanuts	0.05
Almond nuts	0.04
Arracacha	0.04
Arrowroot	0.04
Bananas	0.04
Beechnuts	0.04
Black walnuts	0.04
Brazil nuts	0.04
Butternuts	0.04
Cashew nuts	0.04
Cassava roots	0.04
Chayote roots	0.04
Chestnuts	0.04
Chinese artichokes	0.04
Chinquapin nuts	0.04
Chufa	0.04
Dry soybeans	0.04
Edible canna	0.04
English walnuts	0.04
Field corn	0.04
Hazelnuts	0.04
Ginger roots	0.04
Hickory nuts	0.04
Jerusalem artichokes	0.04
Lerens	0.04
Macadamia nuts	0.04
Pecan nuts	0.04
Popcorn grain	0.04
Potatoes	0.04
Rye	0.04
Sweet corn kernels plus cob with husks removed	0.04
Sweet potato roots	0.04
Tanier corms	0.04
Taro corms	0.04
True yam tubers	0.04
Turmeric roots	0.04

Commodity	Pyraclostrobin MRLs in Canada [mg/kg]
Yam bean roots	0.04
Blackberries	3.5
Blueberries	3.5
Currants	3.5
Elderberries	3.5
Gooseberries	3.5
Huckleberries	3.5
Loganberries	3.5
Raspberries	3.5
Chinese onions	0.9
Dry bulb onions	0.9
Garlic	0.9
Great headed garlic	0.9
Green onions	0.9
Leeks	0.9
Potato onions	0.9
Shallots	0.9
Tree onion tops	0.9
Welsh onion tops	0.9
Eggs	0.1
Meat byproducts of poultry	0.1
Meat of poultry	0.1
Amaranth	29
Arugula	29
Cardoon	29
Celery	29
Celtuce	29
Chinese celery	29
Corn salad	29
Dandelion leaves	29
Dock	29
Edible leaved chrysanthemum	29
Endives	29
Fresh chervil leaves	29
Fresh Florence fennel leaves and stalks	29
Fresh parsley leaves	29
Garden cress	29
Garden purslane	29
Garland chrysanthemum	29
Head lettuce	29
Leaf lettuce	29
Borage seeds	0.45

Commodity	Pyraclostrobin MRLs in Canada [mg/kg]
Cuphea seeds	0.45
Echium seeds	0.45
Flaxseeds	0.45
Gold of Pleasure seeds	0.45
Hare's ear mustard seeds	0.45
Milkweed seeds	0.45
Mustard seeds (oilseed type)	0.45
New Zealand spinach	29
Orach	29
Radicchio	29
Rhubarb	29
Spinach	29
Swiss chard	29
Upland cress	29
Malabar spinach	29
Winter purslane	29
Hops (dried)	23
Bok choy Chinese cabbages	16
Broccoli raab	16
Collards	16
Kales	16
Mustard greens	16
Mustard spinach	16
Rape leaves	16
Turnip tops	16
Citrus oil	9
Broccoli	5
Brussels sprouts	5
Cabbages	5
Cauliflowers	5
Chinese broccoli	5
Chinese mustard cabbages	5
Kohlrabies	5
Napa Chinese cabbages	5
Calamondins	2
Citrus citrons	2
Citrus hybrids	2
Grapefruits	2
Kumquats	2
Lemons	2
Limes	2
Oranges	2

Commodity	Pyraclostrobin MRLs in Canada [mg/kg]
Pummelos	2
Satsuma mandarins	2
Tangerines	2
Apples	1.5
Crabapples	1.5
Loquats	1.5
Mayhaws	1.5
Asian pears	1.5
Pears	1.5
Quinces	1.5
Strawberries	1.2
Sunflower seeds	0.45
Mangoes	0.1
Papayas	0.1
Oats	1.2
Saskatoon berries (juneberries)	3.5
Oil radish seeds	0.45
Poppy seeds	0.45
Rapeseeds (canola)	0.45
Sesame seeds	0.45
Sweet rocket seeds	0.45
Peppermint tops	8
Calendula seeds	0.45
Evening primrose seeds	0.45
Jojoba seeds	0.45
Niger seed seeds	0.45
Safflower seeds	0.45
Tallowwood seeds	0.45
Tea oil plant seeds	0.45
Mustard seeds (condiment type)	0.45
Spearmint tops	8
Triticale	0.2
Celeriac tops	16

Chile

The list of current MRLs set for pyraclostrobin in Chile can be found on the BCN website:

(http://www.bcn.cl/)
(*Link: search for* FIJA TOLERANCIAS MÁXIMAS DE RESIDUOS, Currently: link to LMR list dated 2010-02-05, additional norms: list from 2011-10-20 containing updates)

The list of MRLs currently set in Chile (as of May 2014) is given below.

Commodity	**Pyraclostrobin MRLs in Chile [mg/kg]**
Acerola	1
Almond	0.02
Apple	0.5
Apricot	1
Artichoke	0.02
Asparagus	0.02
Avocado	0.6
Banana	0.02
Bean, dry	0.2
Beetroot	0.02
Beetroot	0.1
Broad bean {Fava bean}	0.02
Cabbage	0.2
Carrot	0.5
Celery	29
Cherry	1
Cherry, Morello	1
Citrus fruits	1
Citrus fruits, other	1
Clementine	1
Corn, sweet	0.02
Cranberry	1
Cucumber {Sweet cucumber}	0.5
Custard apple {Cherimoya}	0.02
Endive {Escarole, Scarole}	2
Garlic, bulb	0.05
Grape	2
Grapefruit	1
Green bean	0.02
Kiwifruit	0.02
Lemon	1
Lentil	0.5

Commodity	**Pyraclostrobin MRLs in Chile [mg/kg]**
Lettuce	29
Lime	1
Lime, Key {West Indian lime, Mexican lime (Citrus aurantifolia)}	1
Mandarin	1
Mango	0.05
Melon	0.5
Melon	0.02
Nectarine	1
Oat	0.5
Olive	0.02
Onion, bulb	0.2
Orange	1
Orange, sweet	1
Orange, sweet & sour	1
Pea	0.02
Peach	1
Pear	1.5
Pepper, bell	0.5
Pepper, chili	0.5
Pineapple	0.02
Plum	1
Plumcot	1
Pluot	1
Pomelo {Pummelo, Shaddock}	1
Potato	0.02
Raspberry	2
Rice	0.02
Spinach	0.5
Squash, Summer {Zucchini}	0.3
Squash, Winter {Pumpkin, Giant pumpkin, Squash}	0.5
Squash, Winter {Pumpkin, Giant pumpkin, Squash}	0.02
Stone fruits	1
Stone fruits, other	1
Strawberry	0.5
Swiss chard {Chard}	0.5
Tangerine	1
Tomato	0.3
Walnut	0.04
Watermelon	0.02
Watermelon	0.5
Wheat	0.2

China

China's National Health and Family Planning Commission (NHFPC) published the updated National food safety standard – Maximum residue limits for pesticides in foods, GB2763-2014. This updated standard, GB2763-2014, enters into force on **August 1, 2014**. Upon implementation, GB2763-2014 will override the current MRL standard, GB2763-2012.

The commercial data base HOMOLOGA (http://www.homologa-new.com/pls/apex/f?p=550:1) and the New Zealand Food Safety Authority Pesticide MRL Database (https://pxmrl.maf.govt.nz/) were checked in addition to the official document of the authority.

The list of MRLs currently set in China (as of May 2014) is given below.

Commodity	Pyraclostrobin MRLs in China [mg/kg]
Apple	0.5
Banana	0.02
Head Cabbage	0.5
Chinese Cabbage	5
Cucumber	0.5
Grape	2
Lychee {Litchi}	0.05
Mango	0.05
Melon	0.5
Peanut, kernels	0.05
Pepper, green	0.5
Pepper, hot {Pepper, chili}	0.5
Potato	0.02
Watermelon	0.5

Israel

The list of current MRLs set for pyraclostrobin in Israel can be found on the Ministry of Agriculture Plant Protection an Inspection Services (PPIS) website (MARD (PPIS) Data Bank):

(http://www.hadbara.moag.gov.il/hadbara/english/)

The list of MRLs currently set in Israel (as of May 2014) is given below.

Commodity	Pyraclostrobin MRLs in Israel [mg/kg]
Almond	1.5
Apple	0.5
Basil	2.0
Cabbage	0.05
Carrot	0.1
Celeriac	0.2
Chick pea	0.05
Chives	0.2
Citrus fruits	0.03
Coriander	2.0
Cotton seed	0.2
Cucumber	2.0
Dill	2.0
Eggplant	0.03
Estragon/Tarragon	2.0
Garlic	0.3
Grapes	2.0
Lettuce	1.0
Marjoram	2.0
Melissa/Lemon balm	2.0
Melon	2.0
Mint	2.0
Nectarine	0.2
Onion	0.3
Parsley	2.0
Peach	0.2
Peanut	0.05
Peas	0.02
Pepper	0.4
Persimmon	0.02
Potato	0.03
Pumpkin	0.1

Commodity	Pyraclostrobin MRLs in Israel [mg/kg]
Rucola	2.0
Rosmarine	2.0
Sage	2.0
Scarlet runner bean	0.1
Squash	2.0
Strawberry	0.2
Sweet potato	0.5
Thyme	2.0
Tomato	0.05
Watermelon	0.05

Japan

The list of current MRLs set for pyraclostrobin in Japan can be found on the FFCR website:

(http://www.m5.ws001.squarestart.ne.jp/foundation/search.html)

The list of MRLs currently set in Japan (as of May 2014) is given below.

Commodity	Pyraclostrobin MRLs in Japan [mg/kg]	Note
Wheat	0.2	
Barley	1	
Rye	0.2	
Corn (maize, including pop corn and sweet corn)	0.02	
Other cereal grains	1	
Soybeans, dried	0.2	
Beans, dried	0.5	
Peas	0.3	
Broad beans	0.3	
Peanuts, dried	0.05	
Other legumes/pulses	0.3	
Potato	0.02	
Taro	0.04	
Sweet potato	0.04	
Yam	0.04	
Other Potatoes	0.04	
Sugar beet	0.2	
Sugarcane	0.1	
Japanese radish, roots (including radish)	0.5	
Japanese radish, leaves (including radish)	20	
Turnip, roots (including rutabaga)	0.4	
Turnip, leaves (including rutabaga)	16	
Horseradish	0.4	
Watercress	29	
Chinese cabbage	3	
Cabbage	0.2	
Brussels sprouts	0.3	
Kale	1	
KYONA	16	
Qing-geng-cai	5	
Cauliflower	5	
Broccoli	5	

Commodity	Pyraclostrobin MRLs in Japan [mg/kg]	Note
Other cruciferous vegetables	16	
Burdock	0.4	
Salsify	0.4	
Artichoke	2	
Chicory	29	
Endive	29	
Lettuce (including cos lettuce and leaf lettuce)	2	
Other composite vegetables	29	
Onion	2	
Welsh (including leek)	0.7	
Garlic	0.2	
Other liliaceous vegetables	2	
Carrot	0.5	
Parsnip	0.4	
Parsley	29	
Celery	29	
Other umbelliferous vegetables	29	
Tomato	0.5	
Pimento (sweet pepper)	1	
Egg plant	0.5	
Other solanaceous vegetables	3	
Cucumber (including gherkin)	0.5	
Pumpkin (including squash)	0.5	
Oriental pickling melon (vegetable)	0.5	
Water melon	0.5	
Melons	0.2	
MAKUWAURI melon	0.5	
Other cucurbitaceous vegetables	0.5	
Ginger	0.04	
Peas, immature (with pods)	0.02	
Kidney beans, immature (with pods)	0.5	
Green soybeans	0.5	
Other vegetables	16	
UNSHU orange, pulp	0.02	
Citrus NATSUDAIDAI, whole	1	
Lemon	2	
Orange (including navel orange)	2	
Grapefruit	2	
Lime	2	
Other citrus fruits	2	
Apple	1	

Commodity	Pyraclostrobin MRLs in Japan [mg/kg]	Note
Japanese pear	2	
Pear	2	
Quince	2	
Loquat	2	
Peach	0.02	
Nectarine	1	
Apricot	2	
Japanese plum (including prune)	0.8	Time limit for application: 1 (2014.09.09)
Mume plum	2	
Cherry	3	
Strawberry	2	
Raspberry	3	
Blackberry	3	
Blueberry	4	
Huckleberry	4	
Other berries	1	
Grape	3	
Japanese persimmon	0.7	
Banana	0.02	
Papaya	0.2	
Mango	0.05	
Other Fruits	0.02	
Sunflower seeds	0.5	
Sesam seeds	0.5	
Safflower seeds	0.5	
Cotton seeds	0.4	
Rapeseeds	0.5	
Other oil seeds	0.5	
Ginkgo nut	0.02	
Chestnut	0.04	
Pecan	0.02	
Almond	0.02	
Walnut	0.04	
Other nuts	1	
Tea	5	
Coffee beans	0.3	
Hop	15	
Other spices	29	
Other herbs	29	
Cattle, muscle	0.5	

Commodity	Pyraclostrobin MRLs in Japan [mg/kg]	Note
Pig, muscle	0.5	
Other terrestrial mammals, muscle	0.5	
Cattle, fat	0.5	
Pig, fat	0.5	
Other terrestrial mammals, fat	0.5	
Cattle, liver	0.05	
Pig, liver	0.05	
Other terrestrial mammals, liver	0.05	
Cattle, kidney	0.05	
Pig, kidney	0.05	
Other terrestrial mammals, kidney	0.05	
Cattle, edible offal	0.05	
Pig, edible offal	0.05	
Other terrestrial mammals, edible offal	0.05	
Milk	0.03	
Chicken, muscle	0.05	
Other poultry, muscle	0.05	
Chicken, fat	0.05	
Other poultry, fat	0.05	
Chicken, liver	0.05	
Other poultry, liver	0.05	
Chicken, kidney	0.05	
Other poultry, kidney	0.05	
Chicken, edible offal	0.05	
Other poultry, edible offal	0.05	
Chicken eggs	0.05	
Other poultry, eggs	0.05	
Raisin	5	

New Zealand

The list of current MRLs set for pyraclostrobin in New Zealand can be found on the New Zealand food safety authority website:

(http://www.foodsafety.govt.nz/industry/sectors/plant-products/pesticide-mrl/)

The list of MRLs currently set in New Zealand (as of May 2014) is given below.

Commodity	Pyraclostrobin MRLs in New Zealand [mg/kg]
Apple	0.02 (*)
Barley	0.02 (*)
Cherries	1
Grapes	3
Kiwifruit	0.02 (*)
Mammalian fat	0.02 (*)
Mammalian kidney	0.02 (*)
Mammalian liver	0.02 (*)
Mammalian meat	0.02 (*)
Milk	0.02 (*)
Pears	0.02 (*)
Stone fruits (except cherries)	0.02 (*)
Wheat	0.02 (*)

(*) The MRL is set at or about the limit of analytical quantitation.

Russian Federation

The list of current MRLs set for pyraclostrobin in Russia can be found on the following websites:

(http://ec.europa.eu/food/international/trade/ru_requirements_MRLs_pesticides_en.htm)
(http://35.rospotrebnadzor.ru/Default.aspx?ig=c2066b3dcde6474ba85c689c956f6c91&&et=4&&mnu=3b9c05540de84077875422afed45d537)
Russian website without English translation (site is only in Russian) and on the following document (inofficial English translation) from the USDA Foreign Agricultural Service:
(http://gain.fas.usda.gov/Recent%20GAIN%20Publications/New%20Russian%20MRLs%20for%20Pesticides%20in%20Agricultural%20and%20Food%20Products_Moscow_Russian%20Federation_1-10-2014.pdf)

The list of MRLs currently set in Russia (as of May 2014) is given below.

Commodity	Pyraclostrobin MRLs in Russia [mg/kg]
Grapes	2.0
Fruits (seed type)	0.5
Cereal grains	0.5
Corn (grain, oil)	0.02
Soybean oil	0.02
Soybean (bean)	0.05
Sunflower seed (seed, oil)	0.3
Almonds (in shell)	20 *,**
Almond (shelled)	0.02 *,**
Lettuce/Salad (headed)	20 *,**
Raspberry (red, black)	20 *,**
Banana	0.02 *,**
Peanut (in shell)	0.02 *,**
Peas (pods, not-ripened seeds)	0.02 *,**
Pecan	0.02 *,**
Beans (dry)	0.3 *,**
Cabbages (all types)	0.3 *,**
Cantaloupe melon	0.2 *,**
Onion (bulb)	0.2 *,**
Sugar beet	0.2 *,**
Potato	0.2
Tomato	0.3
Cucumber	0.5
Carrot	0.5
Blueberry	1.0 *,**
Citrus fruits	1.0 *,**
Pistachios	1.0 *,**

Commodity	Pyraclostrobin MRLs in Russia [mg/kg]
Fruits (stone type)	1.0 *,**
Coffee beans	0.5 *,**
Eggplants	0.5 *,**
Peas (dry)	0.5 *,**
Pumpkin (ordinary)	0.5 *,**
Lentils (dry)	0.5 *,**
Meat of mammals (except sea mammals)	0.5 *,**
Pepper	0.5 *,**
Radish	0.5 *,**
Strawberry	0.5 *,**
Dried grapes (raisins)	5.0 *,**
Offal of mammals	0.05 *,**
Poultry meat and offal	0.05 *,**
Eggs	0.05 *,**
Garlic	0.05 *,**
Mango	0.05 *,**
Papaya	0.05 *,**
Hop (dry)	15 *,**
Leek	0.7 *,**
Milk	0.03

* Temporary MRL
** Import tolerance

South Africa

The list of current MRLs set for pyraclostrobin in South Africa can be found on the Agri-Intel website:

(http://www.agri-intel.com/):

The commercial data bases HOMOLOGA (http://www.homologa-new.com/pls/apex/f?p=550:1) and the New Zealand Food Safety Authority Pesticide MRL Database (https://pxmrl.maf.govt.nz/) were checked in addition.

The list of MRLs currently set in South Africa (as of May 2014) is given below.

Commodity	Pyraclostrobin MRLs in South Africa [mg/kg]
Apple	0.02
Barley	1.0 *,**
Corn/Maize	0.03
Citrus fruits	0.5 *,**
Grapefruit	0.5
Lemon	0.5
Orange	0.5
Grapes	0.5 *,**
Pear	0.02
Potato	0.02
Soybean	0.03 *
Table grape	0.5
Tomato	0.01

* MRL of the commercial data base HOMOLOGA
** MRLs of New Zealand Food Safety Authority Pesticide MRL Database

South Korea

The list of current MRLs set for pyraclostrobin in South Korea can be found on the KFDA MRL website:

(http://fse.foodnara.go.kr/residue/mrl/mrl_search.jsp)

The list of MRLs currently set in Korea (as of May 2014) is given below.

Commodity	Pyraclostrobin MRLs in Korea [mg/kg]
Apple	0.2
Chinese magnolia vine	5
Crimson glory vine	3
Cucumber	0.5
Garlic	0.05
Ginseng	2
Grape	3
Jujube	2
Korean cabbage	0.5
Korean cabbage, head	0.2
Leek	10
Mandarin {Tangerine}	1
Melon, Korean	0.5
Onion	0.05
Onion, Welsh, tops	1
Parsley	7
Parsley	7
Peach {Prunus persica}	1
Pear	1
Pepper, green & red	0.5
Pepper, sweet	0.5
Perilla, leaves	10
Persimmon	0.1
Plum	1
Plum, Korean	3
Potato	0.5
Rubi Fructus {Raspberry, palmleaf}	1
Strawberry	1
Tomato	1
Watermelon	0.1

Taiwan

The list of current MRLs set for pyraclostrobin in Taiwan can be found on the FDA Food legislation website:

(https://consumer.fda.gov.tw/Law/Detail.aspx?nodeID=518&lang=1&lawid=127)

The list of MRLs currently set in Taiwan (as of May 2014) is given below.

Commodity	Pyraclostrobin MRLs in Taiwan [mg/kg]
Almonds	0.02
Apples	1.0
Apricots	1.0
Avocado	0.5
Banana	0.02
Barley	0.4
Beetroot	0.2
Bitter melon	0.5
Blueberries	1.0
Bottle gourd	0.5
Broad bean (fresh)	0.5
Camphorweed	2.0
Cantaloups	0.5
Carambola	1.0
Carrot	0.4
Celery	5.0
Chayote shoots	2.0
Cherries	1.0
Chickpea	0.5
Chinese chive	2.0
Chive flower	2.0
Chrysanthemum	5.0
Citrus fruit	1.0
Coffee bean	0.3
Corn	0.1
Cos lettuce	2.0
Cowpea (fresh)	0.5
Cruciferous vegetables (Leaf vegetables with small leaves) (except leaf -mustard)	2.0
Cruciferous vegetables (Leaf vegetables with wrapped leaves)	2.0

Commodity	Pyraclostrobin MRLs in Taiwan [mg/kg]
Edible podded pea, English pea, Green pea	0.5
Eggplant	0.5
English pea (Dry), Green pea (Dry), Field pea (Dry)	0.1
Fig	1.0
Fireweed	2.0
Garland chrysanthemum	2.0
Goa bean	0.5
Grapes	2.0
Grapefruits	1.0
Green garlic	2.0
Guava	1.0
Gynura's Deux Couleurs	2.0
Gynura Oralis Hay	2.0
Head lettuce	2.0
Herbs and spices (Dry)	0.05 *
Hot pepper	0.5
Hyacinth bean	0.5
Indian jujube	1.0
Leaf-mustard	5.0
Leafy pea	2.0
Lycii fructus	0.5
Kidney bean, Field bean	0.5
Kidney bean (Dry), Field bean (Dry)	0.1
Leaf lettuce	2.0
Leaved chrysanthemum	2.0
Leek sprout	2.0
Lemons	1.0
Lima bean	0.5
Litchi	0.5
Longan	0.5
Loquat	1.0
Luffa	0.5
Mangos	0.5
Marigold flower	0.05
Melon	0.5
Mint	5.0
Nectarines	1.0
Oriental pickling melon	0.5
Papaya	0.5
Passion fruit	0.5

Commodity	Pyraclostrobin MRLs in Taiwan [mg/kg]
Peaches	1.0
Pears	1.0
Pepper	0.5
Persimmons	1.0
Pigeon pea (fresh)	0.5
Pitaya	0.5
Plums	1.0
Potatoes	0.02
Prune	1.0
Pumpkin	0.5
Rambutan	0.5
Rye	0.04
Soybeans	0.04
Spinach	5.0
Spring onion	2.0
Strawberries	0.5
Summer squash	0.5
Sunflower seed	0.3
Sweet sop	0.5
Sword bean	0.5
Tea	5.0
Tomato	1.5
Vegetable pear	0.5
Vegetable soybean	0.5
Watermelon	0.5
Wax apples	1.0
Wax gourd	0.5
Wheat	0.02
White dutch runner bean (fresh)	0.5
Yam bean	0.4
Others	0.01 *

* limit of determination of the analytical method

USA

The list of current MRLs set for pyraclostrobin in the USA can be found on the Electronic Code of Federal Regulations (e-CFR) website:

(http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&SID=07b96fcbec7602f5f0fbdc6dfe01a430&tpl=/ecfrbrowse/Title40/40cfr180_main_02.tpl)

The list of MRLs currently set in the USA (as of May 2014) is given below.

Commodity	Pyraclostrobin MRLs in USA [mg/kg]
Alfalfa, forage	10
Alfalfa, hay	30
Almond, hulls	7.0
Apple, wet pomace	8.0
Artichoke, globe	3.0
Avocado	0.6
Banana	0.04
Barley, grain	1.4
Barley, hay	25
Barley, straw	6.0
Bean, succulent shelled	0.5
Beet, sugar, dried pulp	1.0
Beet, sugar, roots	0.2
Beet, sugar, tops	8.0
Berry, low growing, subgroup 13-07G, except cranberry	1.2
Brassica, head and stem, subgroup 5A	5.0
Brassica, leafy greens, subgroup 5B	16.0
Bushberry subgroup 13-07B	4.0
Caneberry subgroup 13-07A	4.0
Canistel	0.6
Citrus, dried pulp	12.5
Citrus, oil	9.0
Coffee, green bean	0.3
Corn, field, forage	5.0
Corn, field, grain	0.1
Corn, field, refined oil	0.2
Corn, field, stover	17.0
Corn, pop, grain	0.1
Corn, pop, stover	17.0
Corn, sweet, forage	5.0

Commodity	Pyraclostrobin MRLs in USA [mg/kg]
Corn, sweet, kernel plus cob with husks removed	0.04
Corn, sweet, stover	23.0
Cotton, gin byproducts	30
Endive, belgium	4.0
Fruit, citrus, group 10-10	2.0
Fruit, pome, group 11-10	1.5
Fruit, small vine climbing, except fuzzy kiwifruit, subgroup 13-07F	2.0
Fruit, stone, group 12	2.5
Grain, aspirated fractions	2.5
Grape, raisin	7.0
Grass, forage	10
Grass, hay	4.5
Grass, seed screenings	27
Grass, straw	14
Hop, dried cones	23.0
Mango	0.6
Nut, tree, group 14	0.04
Oat, grain	1.2
Oat, hay	18
Oat, straw	15
Oilseed group 20	0.45
Papaya	0.6
Pea, succulent	0.2
Pea and bean, dried shelled, except soybean, subgroup 6C	0.5
Peanut	0.05
Peanut, refined oil	0.1
Peppermint, tops	8.0
Persimmon	3.0
Pistachio	0.7
Radish, tops	16
Rye, grain	0.04
Rye, straw	0.5
Sapodilla	0.6
Sapote, black	0.6
Sapote, mamey	0.6
Sorghum, grain, forage	5.0
Sorghum, grain, grain	0.60
Sorghum, grain, stover	0.80
Soybean, forage	11
Soybean, hay	14

Commodity	Pyraclostrobin MRLs in USA [mg/kg]
Soybean, hulls	0.06
Soybean, seed	0.04
Spearmint, tops	8.0
Star apple	0.6
Sugarcane, cane	0.20
Vegetable, bulb, group 3-07	0.9
Vegetable, cucurbit, group 9	0.5
Vegetable, foliage of legume, except soybean, subgroup 7A	25.0
Vegetable, fruiting, group 8-10	1.4
Vegetable, leafy, except brassica, group 4	29.0
Vegetable, leaves of root and tuber, group 2, except sugar beet	16.0
Vegetable, legume, edible podded, subgroup 6A	0.5
Vegetable, root, except sugar beet, subgroup 1B	0.4
Vegetable, tuberous and corm, subgroup 1C	0.04
Vegetables, foliage of legume, group 7	25
Wheat, grain	0.02
Wheat, hay	6.0
Wheat, straw	8.5
Cattle, fat	0.1
Cattle, liver	1.5
Cattle, meat	0.1
Cattle, meat byproducts, except liver	0.2
Goat, fat	0.1
Goat, liver	1.5
Goat, meat	0.1
Goat, meat byproducts, except liver	0.2
Hog, fat	0.1
Hog, liver	1.5
Hog, meat	0.1
Hog, meat byproducts, except liver	0.2
Horse, fat	0.1
Horse, liver	0.1
Horse, meat	0.1
Horse, meat byproducts, except liver	0.2
Milk	0.1
Poultry, eggs	0.10
Poultry, fat	0.10
Poultry, meat	0.10
Poultry, meat byproducts	0.10

Commodity	Pyraclostrobin MRLs in USA [mg/kg]
Sheep, fat	0.1
Sheep, liver	1.5
Sheep, meat	0.1
Sheep, meat byproducts, except liver	0.2

BASF
The Chemical Company

Pyraclostrobin

Document F

Notification submitted to the Commission

Compiled by:

[redacted]

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Telephone [redacted]
Telefax
E-mail

APPLICATION FOR RENEWAL OF
THE ACTIVE SUBSTANCE

PYRACLOSTROBIN

according to Annex of Regulation (EU) No. 844/2012

NOTIFICATION OF AN ACTIVE SUBSTANCE ACCORDING TO ARTICLE 2

BASF DocID 2013/1401473

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22 January 2014

Table of Contents

1 Information concerning the applicant

1.1 Name and address of the applicant

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1.2 Contact details

1.2.1 Applicant

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1.2.2 Contact

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Telefax:
e-mail address:

2 Information to facilitate identification

2.1 Common name (proposed or ISO-accepted)

Pyraclostrobin (ISO-accepted)

2.2 Chemical name (IUPAC and CAS nomenclature)

IUPAC: Methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-methoxy carbamate

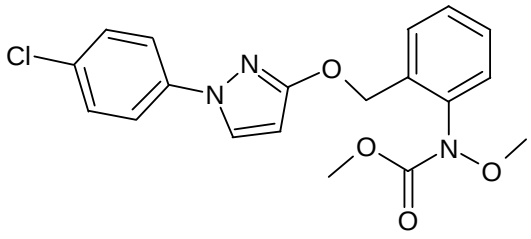
CAS: Carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester

2.3 CAS, CIPAC and EEC numbers (if available)

CAS: 175013-18-0
CIPAC: 657
EINECS: not assigned

2.4 Empirical and structural formula, molecular mass

Structural formula:



Molecular formula: $C_{19}H_{18}ClN_3O_4$
Molecular mass: 387.82

2.5 Specification of purity of the active substance in g/kg

Minimum purity: 975 g/kg

2.6 Classification and labelling

The classification and labelling according to Table 3.1 of Regulation (EC) No. 1272/2008 as amended by Commission Regulation (EC) No. 790/2009 is given as follows:

Classification:

Hazard class and Category:	Acute Tox. 3 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1
H-statements:	H331, H315, H400, H410

Labelling:

Pictogram:	GHS06, GHS09
Signal word:	Danger
H-statements:	H331, H315, H410

3 New information

3.1 List of new information

References are given for the active substance pyraclostrobin (BAS 500 F), its metabolites and the representative formulations BAS 500 06 F and BAS 516 07 F.

These references are intended to be submitted together with a justification showing that they are considered necessary, in accordance with Article 15(2) of Regulation (EC) No 1107/2009.

As literature search is ongoing, only a few results are considered in the list.

Studies to determine the analytical profile of the active substance and the toxicity of its impurities are listed in a separate document, because for these studies confidentiality is claimed according to art 63 of 1107/2009.

3.1.1 Active substance pyraclostrobin (BAS 500 F)

Section 1: Identity of the active substance

New studies on the identity of the active substance are presented in the confidential part of the application.

Section 2: Physical and chemical properties of the active substance

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 2.3/1	Kroehl T.	2013 a	Appearance of Pyraclostrobin technical material TC 2013/1399332 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New information	BASF
KCA 2.3/2	Kroehl T.	2009 a	Storage stability of Pyraclostrobin (BAS 500 F, Reg.No. 304 428) TC crystalline when stored for 2 years at 25°C in big bag material 2009/1084158 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New technical material	BASF
KCA 2.4/1	Kroehl T., Doetzer R.	2014	Mass, NMR, IR and UV/Vis spectra of dimethylsulfate – Minor component in technical grade pyraclostrobin 2014/1001441 BASF SE Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New requirement	BASF
KCA 2.8/1	Daum A.	2000 a	Determination of the dissociation constant of Reg.No. 304 428 (BAS 500 F) 2000/1012252 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New requirement	BASF
KCA 2.9/1	Achhammer G.	2013 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2013/1002641 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New information	BASF
KCA 2.9/2	Loehr S.	2011 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2011/1000601 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New technical material	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 2.9/3	Loehr S.	2011 b	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) 2011/1048145 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New technical material	BASF
KCA 2.11/1	Achhammer G.	2013 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2013/1002641 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New information	BASF
KCA 2.11/2	Loehr S.	2011 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2011/1000601 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New technical material	BASF
KCA 2.11/3	Loehr S.	2011 b	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) 2011/1048145 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New technical material	BASF
KCA 2.13/1	Achhammer G.	2013 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2013/1002641 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New information	BASF
KCA 2.13/2	Loehr S.	2011 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2011/1000601 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New technical material	BASF
KCA 2.13/3	Loehr S.	2011 b	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) 2011/1048145 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New technical material	BASF

Section 4: Analytical methods

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 4.1.1/1	Anonymous	2007 a	Determination of Pyraclostrobin in Pyraclostrobin technical, Pyraclostrobin technical concentrate, Pyraclostrobin emulsifiable concentrates and Pyraclostrobin water dispersible granules 2007/1017547 CIPAC - Collaborative International Pesticides Analytical Council, Harpenden Hertfordshire AL5 2HG, United Kingdom No Published	No	No	New CIPAC method (used in the sub-mitted 5 batch analyses)	CIPAC
KCA 4.1.2/1	Leite R.	2005 a	Validation study of the SOP-PA.0243 for determination of Pyraclostrobin and its metabolite (BF 500-3) residues in coffee (grain), soybean (grain) and wheat (grain) 2005/1037978 BASF SA, Resende, Brazil Yes Unpublished	No	Yes	Addressed as data gap during Article 12 evaluation in 2011	BASF
KCA 4.1.2/2	Eilers B.	2014	Method for the determination of 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS 2014/1001641 No Unpublished	No	Yes	New analytical method supporting dietary risk assessment	BASF
KCA 4.1.2/3	N.N.	2014	Validation of the Method for the determination of 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS 2014/1001721 Yes Unpublished	No	Yes	New analytical method supporting dietary risk assessment	BASF
KCA 4.1.2/4	Eilers B.	2014	Validation of the analytical method 446/2 (L0058/03) for the determination of BAS 500 F (Reg.No. 304428) and its metabolites 500M04 (Reg.No. 298327) and 500M85 2013/1400972 Yes Unpublished	No	Yes	New analytical method supporting dietary risk assessment	BASF
KCA 4.1.2/5	Hopf B.	2010 a	Validation of analytical method L0151/01: Method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices 2010/1018944 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New analytical method fulfilling data requirements of OECD and SANCO 825	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 4.1.2/6	Hopf B.	2011 a	Technical procedure: Method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices - BASF Method Number L0151/01 2011/1018046 BASF SE, Limburgerhof, Germany Fed.Rep. No Unpublished	No	Yes	New analytical method fulfilling data requirements of OECD and SANCO 825	BASF
KCA 4.1.2/7	Malinsky D.S., Riley M.E.	2000 a	Method validation of BASF analytical method D9902: Method for determination of residues of BAS 500F and its metabolite BF 500-16 in hen tissues using LC/MS/MS 2000/5004 BASF Corp. Agricultural Products Center, Research Triangle Park NC, United States of America Yes Unpublished	No	Yes	Supplemental information to hen feeding study	BASF
KCA 4.1.2/8	Tilting N., Sopena-Vazquez F.	2014	Validation of analytical method L0166/01: Determination of BAS 500 F (Pyraclostrobin) and its metabolites Reg. No. 364380 (500M01), Reg. No. 369315 (500M02) and Reg No. 340266 (500M07) in soil using LC/MS/MS 2013/1184817 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New requirements	BASF
KCA 4.1.2/9	Tilting N.	2012 a	Validation of method L0182/01: Determination of BAS 500 F and its metabolites Reg.No. 412053 (500M59), Reg.No. 411847 (500M60), Reg.No. 412785 (500M62), Reg.No. 413038, and Reg.No. 377613 in ground-surface- and tapwater using LC-MS/MS 2012/1009641 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New requirements	BASF
KCA 4.1.2/10	N.N.	2014	Validation of Analytical Method L0182/02 for the Determination of Reg.No.298327 - 500M04, metabolite of BAS 500 F in Water by LC-MS/MS 2014/1004891 Yes Unpublished	No	Yes	New requirements	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 4.1.2/11	N.N.	2014	Independent Laboratory Methods Validation of BASF Method Number L0182/01: Determination of BAS 500 F (Pyraclostrobin) and its metabolites Reg No. 412053 (500M59), Reg No. 411847 (500M60), Reg No. 412785 (500M62), Reg No. 413038 (500M76), and Reg No. 377613 (500M78) in ground-, surface- and tap water by LC-MS/MS 2014/7000022 Yes Unpublished	No	Yes	New requirements	BASF
KCA 4.1.2/12	N.N.	2014	Independent Laboratory Validation of Analytical Method L0182/02: Determination of Reg.No.298327 500M04, metabolite of BAS 500 F in Water by LC-MS/MS 2014/7000107 Yes Unpublished	No	Yes	New requirements	BASF
KCA 4.1.2/13	Penning H.	2012 a	Validation of analytical method L0197/01: Method for the determination of BAS 500 F (Pyraclostrobin) in air by LC-MS/MS 2012/1220256 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New requirements	BASF
KCA 4.2/1	Scherthan D.	2011 a	Independent laboratory validation of the BASF analytical method 421/0: Method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using LC/MS/MS 2011/1268146 RLP AgroScience GmbH, Neustadt/Weinstrasse, Germany Fed.Rep. Yes Unpublished	No	Yes	Addressed as data gap during Article 12 evaluation in 2011	BASF
KCA 4.2/2	Schacherl A.	2010 a	Independent laboratory validation (ILV) of an analytical method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices 2010/1123694 Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New analytical method fulfilling data requirements of OECD and SANCO 825	BASF

Section 5: Toxicology and metabolism studies

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 5.1/1	N.N.	2014	Comparative In-vitro-metabolism with ^{14}C-BAS 500 F 2014/1001562 Yes Unpublished	No	Yes	New data requirement according to Reg. 283/2013	BASF
KCA 5.2.3/1	[redacted]	2001 a	BAS 500 F 40% in Solvesso (technical active ingredient) - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2001/1010625 [redacted]. Yes Unpublished	Yes	Yes	New data (submitted to US-EPA)	BASF
KCA 5.2.3/2	[redacted]	2002 a	BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2002/1012053 [redacted] Yes Unpublished	Yes	Yes	New data	BASF
KCA 5.2.3/3	[redacted]	2003 a	Amendment No. 1 to the report: BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2003/1009200 [redacted] Yes Unpublished	Yes	Yes	New data	BASF
KCA 5.2.3/4	[redacted]	2003 b	Amendment No. 2 to the report: BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2003/1009427 [redacted] Yes Unpublished	Yes	Yes	New data	BASF
KCA 5.2.7/1	Cetto V., Landsiedel R.	2012 a	BAS 500 F (Pyraclostrobin) - In vitro 3T3 NRU phototoxicity test 2012/1189936 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New data requirement	BASF
KCA 5.3.2/1	[redacted]	1999 a	BAS 500 F - Subchronic oral toxicity study in Wistar rats. Administration in the diet for 3 months 1999/10195 [redacted] Yes Unpublished	Yes	No	Previously evaluated study submitted for completeness (basis for the assessment of the amendments listed below)	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 5.3.2/2	[redacted]	1999 b	Amendment No. 1 to the report: BAS 500 F - Subchronic oral toxicity study in Wistar rats. Administration in the diet for 3 months 1999/11899 [redacted] Yes Unpublished	Yes	No	Correction of clerical errors (see page 29)	BASF
KCA 5.3.2/3	[redacted]	2000 a	Amendment No. 2 to the report: BAS 500 F - Subchronic oral toxicity study in Wistar rats administration in the diet for 3 months 2000/1012360 [redacted] Yes Unpublished	Yes	No	Correction of clerical errors	BASF
KCA 5.3.2/4	[redacted]	2003 a	Amendment No. 3 to the report: BAS 500 F - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months 2003/1013399 [redacted] Yes Unpublished	Yes	No	Additional histopatho-logical evaluation	BASF
KCA 5.3.2/5	[redacted]	2004 a	Amendment to the report for Japanese registration: BAS 500 F - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months 2004/1027673 [redacted] Yes Unpublished	Yes	No	Description of duodenum trimming procedure (requested by Japanese authorities)	BASF
KCA 5.3.2/6	[redacted]	1998 a	BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl BR mice. Administration in the diet for 3 months 1998/11345 [redacted] Yes Unpublished	Yes	No	Previously evaluated study submitted for completeness (basis for the assessment of the amendments listed below)	BASF
KCA 5.3.2/7	[redacted]	1999 c	Amendment No. 1 to the report: BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl mice. Administration in the diet for 3 months 1999/11900 [redacted] Yes Unpublished	Yes	No	Correction of clerical errors and addition of new data	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 5.3.2/8	[redacted]	2003 b	Amendment No. 2 to the report: BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl mice - Administration in the diet for 3 months 2003/1013400 [redacted] Yes Unpublished	Yes	No	Additional histopatho-logical evaluation	BASF
KCA 5.3.3/1	[redacted]	2005 a	BAS 500 F - Subacute inhalation study in Wistar rats - 20 aerosol exposures during 4 weeks 2005/1013950 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 5.3.3/2	[redacted]	2014	Supplementary subacute inhalation study in Wistar rats 2014/1003946 [redacted] Yes Unpublished	Yes	Yes	Additional concentrations needed for risk assessment	BASF
KCA 5.5/1	[redacted]	1999 d	BAS 500 F - Chronic toxicity study in Wistar rats. Administration in the diet for 24 months 1999/11672 [redacted] Yes Unpublished	Yes	No	Previously evaluated study submitted for completeness (basis for the assessment of the amendment listed below)	BASF
KCA 5.5/2	[redacted]	2002 a	Amendment No. 1 to the report: BAS 500 F - Chronic toxicity study in Wistar rats. Administration in the diet for 24 months 2002/1005113 [redacted] Yes Unpublished	Yes	No	Additional histopatho-logical evaluation of intermediate dose group males (requested by US-EPA)	BASF
KCA 5.5/3	[redacted]	1999 e	BAS 500 F - Carcinogenicity study in Wistar rats. Administration in the diet for 24 months 1999/11868 [redacted] Yes Unpublished	Yes	No	Previously evaluated study submitted for completeness (basis for the assessment of the amendment listed below)	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 5.5/4	[redacted]	2002 b	Amendment No. 1 to the report: BAS 500 F - Carcinogenicity study in Wistar rats. Administration in the diet for 24 months 2002/1005114 [redacted] Yes Unpublished	Yes	No	Additional histopatho-logical evaluation of intermediate dose group males (requested by US-EPA)	BASF
KCA 5.5/6	[redacted]	2002 c	BAS 500 F - First supplementary chronic toxicity study in Wistar rats - Administration in the diet for 24 months 2002/1004125 [redacted] Yes Unpublished	Yes	Yes	New data (submitted to US-EPA)	BASF
KCA 5.5/7	[redacted]	2002 d	BAS 500 F - Second supplementary chronic toxicity study in female Wistar rats - Administration in the diet for 24 months 2002/1004126 [redacted] Yes Unpublished	Yes	Yes	New data (submitted to US-EPA)	BASF
KCA 5.5/8	[redacted]	2002 e	BAS 500 F - First supplementary carcinogenicity study in Wistar rats - Administration in the diet for 24 months 2002/1004123 [redacted] Yes Unpublished	Yes	Yes	New data (submitted to US-EPA)	BASF
KCA 5.5/9	[redacted]	2002 f	BAS 500 F - Second supplementary carcinogenicity study in female Wistar rats - Administration in the diet for 24 months 2002/1004124 [redacted] Yes Unpublished	Yes	Yes	New data (submitted to US-EPA)	BASF
KCA 5.5/10	[redacted]	2005 a	BAS 500 F: Terminated carcinogenicity study in female B6C3F1 mice; administration in the diet for 7 months 2005/1026477 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 5.5/11	[redacted]	2002 a	Chronic and oncogenicity study with BAS 500 F: Further evaluations of body weight, food consumption and food efficiency 2002/5002875 [redacted] No Unpublished	Yes	No	New evaluation of BW, FC and Food eff. data from the rat and mouse long-term studies (submitted to US-EPA)	BASF
KCA 5.8.1/1	Woitkowiak C.	2013 a	Reg.No. 78810 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2013/1255749 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/2	Schulz M., Landsiedel R.	2014	Reg.No. 78810 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells - (cytokinesis block method) 2013/1361922 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/3	Schulz M., Landsiedel R.	2014	Reg.No. 78810 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells (Tk+/- locus assay, microwell version) 2013/1298448 BASF SE, Ludwigshafen/Rhein, Germany Fed. Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/4	[redacted]	1997 a	Study on the acute oral toxicity of Pyrazolon in rats 1997/10963 [redacted] Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/5	[redacted]	1997 b	Study on the acute dermal irritation/corrosion of Pyrazolon in the rabbit 1997/10964 [redacted] Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/6	[redacted]	1997 c	Study on the acute eye irritation of Pyrazolon in the rabbit 1997/10965 [redacted] Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 5.8.1/7	[redacted]	1997 a	Pyrazolon - Maximization test in guinea pigs 1997/10968 [redacted] Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/8	[redacted]	2013 a	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Repeated dose 90-day oral toxicity study in Wistar rats - Administration via the diet 2013/1042164 [redacted] Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/9	Woitkowiak C.	2012 a	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2012/1220416 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/10	Schulz M., Landsiedel R.	2012 a	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro chromosome aberration assay in V79 cells 2012/1185707 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/11	Schulz M., Landsiedel R.	2012 b	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in CHO cells (HPRT locus assay) 2012/1272482 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/12	[redacted]	2013 a	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2013/1026779 [redacted] Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/13	[redacted]	2013 a	^{14}C-Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Study on the kinetics in mice 2012/1278425 [redacted] Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 5.8.1/14	Engelhardt G., Leibold E.	2003 a	In vitro chromosome aberration assay with Reg.No. 411847 in V79 cells 2003/1004383 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/15	Schulz M., Landsiedel R.	2012 c	Reg.No. 411847 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in CHO cells (HPRT locus assay) 2012/1148607 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/16	[redacted]	2012 d	Reg.No. 411847 (metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2012/1218557 [redacted] Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/17	Schulz M., Landsiedel R.	2013 b	Reg.No. 413038 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro chromosome aberration assay in V79 cells 2012/1044766 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/18	Engelhardt G., Leibold E.	2003 b	In vitro gene mutation test with Reg.No. 413 038 in CHO cells (HPRT locus assay) 2003/1004384 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/19	[redacted]	2012 e	Reg.No. 413038 (Metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2012/1220183 [redacted] Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/20	Woitkowiak C.	2013 a	Reg.No. 5916420 (Metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2013/1281928 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 5.8.1/21	Schulz M., Landsiedel R.	2014	Reg.No. 5916420 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells (Mixed population method) 2013/1361921 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/22	Schulz M., Landsiedel R.	2014	Reg.No. 5916420 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells ($TK^{+/-}$ locus assay, microwell version) 2013/1298447 BASF SE, Ludwigshafen/Rhein, Germany Fed. Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/23	Woitkowiak C.	2014	Reg.No. 5916421 (Metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2013/1323364 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/24	Schulz M., Landsiedel R.	2014	Reg.No. 5916421 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells (Mixed population method) 2013/1363549 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/25	Schulz M., Landsiedel R.	2014	Reg.No. 5916421 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells ($TK^{+/-}$ locus assay, microwell version) 2013/1298449 BASF SE, Ludwigshafen/Rhein, Germany Fed. Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/26	[redacted]	2014	Reg.No. 5916421 (Metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus Assay in Bone Marrow Cells of the Mouse 2013/1389659 [redacted] Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 5.8.1/27	Esdaile D.J.	2012 a	Plant and animal metabolites of BAS 500 F: Structure-activity hazard identification screen using DEREK 2011/1022602 CiToxLAB Hungary Ltd., Veszprem, Hungary No Unpublished	No	No	New data for the risk assessment of metabolites	BASF
KCA 5.8.2/1	[redacted]	2003 a	BAS 500 F - Determination of iron in urine and serum of Wistar rats - Administration in the diet over 14 days 2003/1009534 [redacted] No Unpublished	Yes	No	New data (submitted to US-EPA)	BASF
KCA 5.8.2/2	[redacted]	2011 a	A 28-Day Oral (Dietary) Range Finding Study of BAS 500 F in B6C3F1 Mice 2011/1194286 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 5.8.2/3	[redacted]	2012 a	A 28-day oral (dietary) natural killer cell immunotoxicity study of BAS 500 F in female B6C3F1 mice 2011/1035857 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 5.8.2/4	[redacted]	2012 b	A 28-day oral (dietary) antibody forming cell immunotoxicity study of BAS 500 F in female B6C3F1 mice 2012/1020986 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 5.8.2/5	[redacted]	2012 c	A 28-day oral (dietary) antibody forming cell immunotoxicity study of BAS 500 F in female B6C3F1 mice 2012/1084176 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF

Section 6: Residues in or on treated products, food and feed and plant metabolism

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 6.2.1/1	Hamm R.T.	1998 a	Metabolism of BAS 500 F in grapes 1998/10988 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	No	Previously evaluated study submitted for completeness (basis for the assessment of the amendment listed below	BASF
KCA 6.2.1/2	Hamm R.T.	2000 a	Report Amendment No. 1 to final report: Metabolism of BAS 500 F in grapes 2000/1000201 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	No	Previously evaluated amendment submitted for completeness	BASF
KCA 6.2.1/3	Bross M.	2004 a	Pyraclostrobin (BAS 500 F) - Grape metabolism: Additional information on the investigations of grape leaves 2004/1000758 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New data (request of Japanese authorities)	BASF
KCA 6.2.1/4	Sato K.	2000 a	Metabolic fate of BAS 500 F in Chinese cabbage 2000/1018512 The Institute of Environmental Toxicology, Mitsukaido-shi Ibaraki 303-0043, Japan Yes Unpublished	No	Yes	New data (request of Japanese authorities)	BASF
KCA 6.2.1/5	Rabe U.	2014	Metabolism of ^{14}C-Pyraclostrobin in rice 2013/1134958 Yes Unpublished	No	Yes	Submission of all meta-bolism data as agreed with RMS	BASF
KCA 6.2.1/6	Birk B., Kloeppner U.	2013 a	Metabolism of ^{14}C-Pyraclostrobin (^{14}C-BAS 500 F) in wheat after seed treatment 2012/1158148 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Submission of all meta-bolism data as agreed with RMS	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 6.2.3/1	Bross M., Lutz T.	2009 a	In vitro investigations of the metabolism of BAS 500 F in goat and cow 2009/1067176 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Submission of all metabolism data as agreed with RMS	BASF
KCA 6.2.5/1	[redacted]	1999 a	Bioaccumulation and metabolism of (^{14}C)-BAS 500 F in bluegill sunfish 1999/11348 [redacted] Yes Unpublished	Yes	No	Submission of all metabolism data as agreed with RMS (study previously evaluated within ecotox)	BASF
KCA 6.2.5/2	[redacted]	2014	The Metabolism of ^{14}C-Pyraclostrobin (^{14}C-BAS 500 F) in rainbow trout (Oncorhynchus mykiss) 2014/1001601 Yes Unpublished	Yes	Yes	New data requirement according to Reg. 283/2013	BASF
KCA 6.2.5/3	Madden S.	2014	The Comparative Metabolism of [^{14}C]-Pyraclostrobin ([^{14}C]-BAS 500 F) in Rainbow Trout Cryopreserved Hepatocytes and Microsomes 2014/1001602 Yes Unpublished	No	Yes	Supplemental information supporting metabolism behaviour in fish	BASF
KCA 6.3.1/1	Schulz H.	2004 a	Study on the residue behaviour of BAS 510 F and BAS 500 F in potatoes after application of BAS 516 00 F under field conditions in The Netherlands, Germany, United Kingdom, Denmark, France North and South, 2003 2004/1015948 Institut Fresenius Chemische und Biologische Laboratorien AG, Taunusstein, Germany Fed.Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 6.3.1/2	Schulz H.	2006 a	Study on the residue behaviour of Dimethomorph and Pyraclostrobin in potatoes after treatment with BAS 536 01 F under field conditions in France (N & S), Denmark, Germany, Belgium, Italy, Spain and Greece, 2005 2006/1000581 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF
KCA 6.3.2/1	Plier S.	2013 a	Determination of residues of BAS 500 F in barley after two applications of BAS 500 06 F in Germany, Netherlands, France (North), France (South), Greece, Italy and Spain, 2011 2012/1067587 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF
KCA 6.3.2/2	Plier S.	2011 a	Determination of residues of BAS 500 F in barley after two applications of BAS 500 06 F in Germany, United Kingdom, Denmark, France (North), France (South), Greece, Italy and Spain, 2010 2011/1135916 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF
KCA 6.3.2/3	Erdmann H.-P.	2010 a	Study on the residue behaviour of BAS 700 F, Epoxiconazole and Pyraclostrobin in barley after application of BAS 702 01 F, BAS 700 00 F, BAS 500 06 F and BAS 480 38 F under field condition in France, Spain, Italy and Germany, 2009 2010/1006342 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 6.3.2/4	Tandy R.	2012 a	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in winter barley after treatment with either BAS 556 03 F, BAS 500 06 F or BAS 555 00 F in Northern and Southern Europe during 2011 2012/1194990 Eurofins Agroscience Services, Melbourne Derbyshire DE73 8AG, United Kingdom Yes Unpublished	No	Yes	Residue study with representative use	BASF
KCA 6.3.2/5	Meyer M.	2013 a	Study on the residue behaviour of Fluxapyroxad (BAS 700 F) and Pyraclostrobin (BAS 500 F) in barley after treatment with either BAS 703 04 F or BAS 700 00 F or BAS 500 06 F under field conditions in Germany, N-France, Spain and Greece, 2012 2013/1282605 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF
KCA 6.3.2/6	Plier S.	2011 b	Determination of residues of BAS 500 F in wheat after two applications of BAS 500 06 F in Germany, United Kingdom, France (North), Denmark, France (South), Greece, Italy and Spain, 2010 2011/1135915 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF
KCA 6.3.2/7	Plier S.	2013 b	Determination of residues of BAS 500 F in wheat after two applications of BAS 500 06 F in Germany, United Kingdom, Netherlands, France (South), Greece, Italy and Spain, 2011 2012/1067588 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 6.3.2/8	Tandy R.	2012 b	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in winter wheat after treatment with either BAS 556 03 F, BAS 500 06 F or BAS 555 00 F in Northern andSouthern Europe during 2011 2012/1194991 Eurofins Agroscience Services, Melbourne Derbyshire DE73 8AG, United Kingdom Yes Unpublished	No	Yes	Residue study with representa-tive use	BASF
KCA 6.3.2/9	Meyer M.	2013 b	Study on residue behaviour of Fluxapyroxad (BAS 700 F), Pyraclostrobin (BAS 500 F) in wheat after treatment with either BAS 703 04 F, BAS 700 00 F or BAS 500 06 F under field conditions, Germany, United Kingdom, Spain, Southern France, 2012 2013/1336790 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. Yes Unpublished	No	Yes	Residue study with representa-tive use	BASF
KCA 6.3.3/1	Aitken A.	2013 a	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) in maize following one application of BAS 500 06 F to 8 trials in 2012 - SEU and NEU 2013/1308888 Charles River, Tranent EH33 2NE, United Kingdom Yes Unpublished	No	Yes	Residue study with representa-tive use	BASF
KCA 6.3.3/2	Aitken A.	2014	Study on the Residue Behaviour of Pyraclostrobin (BAS 500 F) in Maize Following One Application of BAS 500 06 F to 1 Trial in 2013 – NEU 2014/1001741 Yes Unpublished	No	Yes	Residue study with representa-tive use	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 6.3.3/3	Schulz H., Ziske J.	2010 a	Study on the residue behaviour of Pyraclostrobin and Epoxiconazole in maize after treatment with BAS 512 04 F under field conditions in Germany, Northern France, United Kingdom, the Netherlands, Italy, Greece, Southern France and Spain 2008 2010/1025690 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF
KCA 6.3.3/4	Schulz H.	2010 a	1st addendum to the report - Study on the residue behaviour of Pyraclostrobin and Epoxiconazole in maize after treatment with BAS 512 04 F under field conditions in DE, N-FR, UK, NL, IT, GR, S-FR and ES, 2008 2010/1080941 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF
KCA 6.3.3/5	Fleischer G.	2013 a	Study on the residue behaviour of BAS 480 F, BAS 500 F and BF 500-3 (500M07) in corn after treatment with BAS 512 04 F under field conditions in Germany, 2011 2013/1065883 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF
KCA 6.3.3/6	Schulz H.	2010 b	Residue behaviour of Epoxiconazole and Pyraclostrobin in maize after treatment with BAS 512 04 F and BAS 500 06 F under field conditions in Germany Northern France United Kingdom the Netherlands Italy Greece Southern France and Spain 2009 2010/1039144 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. Yes Unpublished	No	Yes	Residue study with representative use	BASF
KCA 6.3.4/1	Courtois J.	2014	Determination of 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS 2014/1001661 Yes Unpublished	No	Yes	Residue analysis supporting dietary risk assessment	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 6.4.1/1	[redacted]	2000 a	A meat and egg magnitude of the residue study with BAS 500 F in laying hens 2000/5005 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 6.5.3/1	Plier S.	2013 c	Determination of residues of BAS 500 F (Pyraclostrobin) in wheat and its processed products after two applications of BAS 500 06 F in Germany 2012/1067586 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	Processing study with representa-tive use	BASF
KCA 6.5.3/2	Plier S.	2013 d	Determination of residues of BAS 700 F (Fluxapyroxad) and BAS 500 F (Pyraclostrobin) in oat and its processed products after two applications of BAS 703 04 F in Germany, 2012 2013/1037950 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	Processing study with representa-tive use	BASF
KCA 6.5.3/3	Braun D.	2011 a	Determination of residues of BAS 500 F (Pyraclostrobin) and BAS 480 F (Epoxiconazole) in maize and its processed products after one application of BAS 512 04 F in Germany 2010/1144336 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	Processing study with representa-tive use	BASF
KCA 6.6.1/1	Rabe U.	2014	Confined Rotational Crop Study with ^{14}C Pyraclostrobin 2014/1001761 Yes Unpublished	No	Yes	New study addressing formation of metabolites	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 6.9/1	Bross M.	2014	Pyraclostrobin (BAS 500 F) - Refinement of the dietary exposure assessment of pyraclostrobin metabolites in plant and animal commodities 2014/1001541 No Unpublished	No	Yes	New evaluation	BASF

Section 7: Fate and behaviour in the environment

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 7.1.1.1 /1	Hassink J.	2014	Metabolism of (pyrazole-3-C14) BAS 500 F in soil under aerobic conditions 2013/1348620 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Additional data (information on third label)	BASF
KCA 7.1.1.3 /1	Hassink J.	2014	Soil photolysis of (pyrazole-3-C14) BAS 500 F 2013/1348621 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Additional data (information on third label)	BASF
KCA 7.1.2.1 .1/1	Staudenmaier H., Kuhnke G.	2013 a	Rate of degradation of ^{14}C-Pyraclostrobin (BAS 500 F) in aerobic soil 2011/1102370 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Old study insufficient	BASF
KCA 7.1.2.1 .2/1	Tornisielo A., Sacchi R.R.	2011 a	Rate of degradation of BF 500-6 on European soils under aerobic conditions 2011/1142307 BASF SA, Guaratingueta, Brazil Yes Unpublished	No	Yes	New data requirement	BASF
KCA 7.1.2.1 .2/2	Tornisielo A., Sacchi R.R.	2011 b	Rate of degradation of BF 500-7 on European soils under aerobic conditions 2011/1142308 BASF SA, Guaratingueta, Brazil Yes Unpublished	No	Yes	New data requirement	BASF
KCA 7.1.2.1 .2/3	N.N.	2014	Rate of degradation of BF 500-4 2013/1294779 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New data requirement	BASF
KCA 7.1.2.1 .2/4	N.N.	2014	Rate of degradation of BF 500-5 2013/1294780 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New data requirement	BASF
KCA 7.1.2.1 .2/5	Gericke D., Bastiansen F.	2010 a	Kinetic evaluation of aerobic soil degradation studies for BAS 500 F - Pyraclostrobin and metabolites BF 500-6 and BF 500-7 according to FOCUS 2010/1197290 BASF SE, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New guidance for kinetic evaluation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 7.1.2.1 .4/1	Eickler B.	2014	Kinetic evaluation of anaerobic soil degradation studies for BAS 500 F – pyraclostrobin according to FOCUS 2014/1000701 BASF SE, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New guidance for kinetic evaluation	BASF
KCA 7.1.2.2 .1/1	Wiedemann G.	2011 a	Normalisation of the degradation rate constants of BAS 500 F - Pyraclostrobin in the field to a reference temperature of 20°C and reference soil moisture at pF 2 according to EFSA 2011 2011/1142490 BASF SE, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New guidance for kinetic evaluation	BASF
KCA 7.1.2.2 .1/2	Bayer H.	2014	Field soil dissipation study of BAS 500 F (Pyraclostrobin) in the formulation BAS 500 14 F on bare soil at four different sites in Europe, 2011-2012 2013/1348661 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New guideline	BASF
KCA 7.1.2.2 .1/3	Tilting N.	2014	Stability of residues of BAS 500 F (Pyraclostrobin, Reg. No 304428) and its metabolites M500F01 (Reg. No. 364380), M500F02 (Reg.No. 369315) and M500F07 (Reg.No. 340266) in various soils 2014/1000723 Yes Unpublished	No	Yes	New guideline	BASF
KCA 7.1.3.1 .2/1	N.N.	2014	Study on the adsorption behaviour of BF 500-6 (Reg.No. 364380) on different soils 2014/1000624 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Old study insufficient	BASF
KCA 7.1.3.1 .2/2	N.N.	2014	Study on the adsorption behaviour of BF 500-7 (Reg.No. 369315) on different soils 2014/1000625 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Old study insufficient	BASF
KCA 7.1.3.1 .2/3	N.N.	2014	Adsorption/desorption of BF 500-4 (Reg.No. 358672) on soil 2014/1000721 Yes Unpublished	No	Yes	New data requirement	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 7.1.3.1 .2/4	N.N.	2014	Adsorption/desorption of BF 500-5 (Reg.No. 298327) on soil 2014/1000722 Yes Unpublished	No	Yes	New data requirement	BASF
KCA 7.2.1.1 /1	Scharf J.	1999	Hydrolysis of BAS 500 F 1999/10060 BASF AG, Agrarzentrum Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	No	Previously evaluated study submitted for completeness (basis for the assessment of the amendment listed below)	BASF
KCA 7.2.1.1 /2	Ebert D.	2011 a	Report Amendment Hydrolysis of BAS 500 F 2011/1201705 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	No	Report amendment not yet peer-reviewed	BASF
KCA 7.2.2.2 /1	Ebert D., Possienke M.	2013 a	^{14}C-BAS 500 F (Pyraclostrobin): Aerobic mineralisation in surface water 2013/1002741 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New data requirement	BASF
KCA 7.2.2.3 /1	Wiedemann G.	2013 a	Kinetic evaluation of BAS 500 F - Pyraclostrobin in water/sediment systems under aerobic conditions 2012/1165029 BASF SE, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New guidance for kinetic evaluation	BASF
KCA 7.2.2.3 /2	Miles B.	2012 a	Kinetic evaluation of BAS 500 F in water/sediment systems under aerobic conditions 2012/1021122 BASF SE, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New guidance for kinetic evaluation	BASF
KCA 7.2.2.4 /1	Ebert D.	2012 a	Degradation of BAS 500 F in water/sediment under irradiated conditions 2011/1101715 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Old study insufficient (missing material balance)	BASF
KCA 7.3.1/1	Hassink J.	2014	Photochemical oxidative degradation of BAS 500 F (QSAR estimates) 2013/1350648 BASF SE, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New calculation according newest model version	BASF

Section 8: Ecotoxicology

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 8.1.1.1/ 1	[redacted]	2014	BAS 500 F - Acute Toxicity in the Canary after single oral administration 2013/1400375 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 8.2.1/1	[redacted]	2000 a	Flow-through acute toxicity of BAS 500 F to the rainbow trout, Oncorhynchus mykiss 2000/5034 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 8.2.1/2	[redacted]	2000 b	Flow-through acute toxicity of BAS 500 F to the bluegill sunfish, Lepomis macrochirus 2000/5033 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 8.2.1/3	[redacted]	2000 c	Flow-through acute toxicity of BAS 500 F to the sheepshead minnow, Cyprinodon variegatus 2000/5032 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 8.2.1/4	[redacted]	2007 a	Reg.No. 340266 (metabolite BF 500-3 of BAS 500 F): Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in a static system over 96 hours 2007/1010836 [redacted] Yes Unpublished	Yes	Yes	New data (request of Canadian authorities)	BASF
KCA 8.2.1/5	N.N.	2014	study in preparation (Acute fish (trout) study with pyraclostrobin metabolite BF 500-5) 2013/1308543 Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 8.2.2/1	[redacted]	2000 d	Early life stage toxicity of BAS 500 F to the fathead minnow, Pimephales promelas 2000/5053 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 8.2.2/2	[redacted]	2001 a	Early life stage toxicity of BAS 500 F to the Sheepshead Minnow, Cyprinodon variegatus 2000/5247 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 8.2.4.1/1	Bergtold M., Janson G.	2006 a	Acute toxicity of Reg.No. 340 266 (Metabolite of BAS 500 F) to Daphnia magna STRAUS in a 48 hour static test 2006/1038907 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New data (request of Canadian authorities)	BASF
KCA 8.2.4.1/2	N.N.	2014	study in preparation (acute Daphnia study with pyraclostrobin metabolite BF 500-5) 2013/1308542 Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.2.4.2/1	Boeri R.L. et al.	2000 a	Flow-Through Acute Toxicity of BAS 500 F to the Mysid, Americanysis bahia 2000/5031 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America Yes Unpublished	No	Yes	New data (request of US-EPA)	BASF
KCA 8.2.4.2/2	Magazu J.P.	2000 a	Flow-Through Mollusc Shell Deposition Test with BAS 500 F 2000/5042 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America Yes Unpublished	No	Yes	New data (request of US-EPA)	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 8.2.5/1	Wyskiel D.C. et al.	2004 a	BAS 500 F: A flow-through life-cycle toxicity test with the Saltwater Mysid Americamysis bahia 2004/5000004 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America Yes Unpublished	No	Yes	New data (request of US-EPA)	BASF
KCA 8.2.5/2	Dinehart S.	2013 a	BAS 500 F: Life-cycle toxicity test of the saltwater mysid, Americamysis bahia, conducted under flow-through test conditions 2013/7002075 ABC Laboratories Inc., Columbia MO, United States of America Yes Unpublished	No	Yes	New data (request of US-EPA)	BASF
KCA 8.2.5.3/1	Kuhl R., Wydra V.	2013 a	Effects of BAS 500 F (Pyraclostrobin) on the development of sediment dwelling larvae of Chironomus riparius in a sediment-water system - Exposed via spiked sediment 2012/1185699 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for sediment risk assessment	BASF
KCA 8.2.5.3/2	Kuhl R., Wydra V.	2013 b	Effects of Reg.No. 340266 (metabolite of BAS 500 F (Pyraclostrobin), synonymous: 500M07, BF 500-3) on the development of sediment dwelling larvae of Chironomus riparius in a sediment-water system - exposed via spiked sediment 2013/1237446 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.2.5.3/3	N.N.	2014	study in preparation (chronic Chironomus study with pyraclostrobin metabolite BF 500-6) 2014/1001481 Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.2.5.3/4	N.N.	2014	study in preparation (chronic Chironomus study with pyraclostrobin metabolite BF 500-7) 2014/1001482 Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 8.2.5.4/1	Gaertner K.	2013 a	BAS 500 F: Whole sediment acute toxicity to a marine amphipod (Leptocheirus plumulosus) 2013/7000055 ABC Laboratories Inc., Columbia MO, United States of America Yes Unpublished	No	Yes	New data (request of US-EPA)	BASF
KCA 8.2.6.1/1	Dohmen G.P.	1999 a	Effect of BAS 500 F on the growth of the green alga Pseudokirchneriella subcapitata 1999/11020 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	No	Previously evaluated study submitted for completeness (basis for the assessment of the reference listed below)	BASF
KCA 8.2.6.1/2	Hoffmann F.	2009 a	Effect of BAS 500 F on the growth of the green alga Pseudokirchneriella subcapitata - Additional calculation of the inhibition values for growth rate and yield data after a test period of 72 h 2009/1037148 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	Recalculation of study endpoints	BASF
KCA 8.2.6.1/3	Hoffmann F.	2006 a	Effect of BF 500-3 (Reg.No. 340 266, metabolite of BAS 500 F) on the growth of the green alga Pseudokirchneriella subcapitata 2006/1038445 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New data (request of Canadian authorities)	BASF
KCA 8.2.6.1/4	N.N.	2014	study in preparation (algae study with pyraclostrobin metabolite BF 500-5) 2013/1349201 Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.2.6.2/1	Boeri R.L. et al.	2000 b	Growth and Reproduction Toxicity Test with BAS 500F and the Freshwater Alga, Navicula pelliculosa 2000/5046 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America Yes Unpublished	No	Yes	New data (request of US-EPA)	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 8.2.6.2/2	Boeri R.L. et al.	2000 c	Growth and reproduction toxicity test with BAS 500 F and the freshwater alga, Anabaena flos-aquae 2000/5036 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America Yes Unpublished	No	Yes	New data (request of US-EPA)	BASF
KCA 8.2.6.2/3	Boeri R.L. et al.	2000 e	Growth and reproduction toxicity test with BAS 500 F and the marine alga, Skeletonema costatum 2000/5035 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America Yes Unpublished	No	Yes	New data (request of US-EPA)	BASF
KCA 8.2.7/1	Boeri R.L. et al.	2000 d	Growth and reproduction toxicity test with BAS 500 F and the duckweed, Lemna gibba G3 2000/5037 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America Yes Unpublished	No	Yes	New data (request of US-EPA)	BASF
KCA 8.2.8/1	Dohmen G.P.	2000 a	The effect of BAS 500 00 F on aquatic ecosystems - an outdoor mesocosm investigation 2000/1000011 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	No	Previously evaluated study submitted for completeness (basis for the reference listed below)	BASF
KCA 8.2.8/2	Dohmen G.P.	2013 a	Mesocosm study evaluation (BAS 500 00 F) - The effect of BAS 500 00 F on aquatic ecosystems - An outdoor mesocosm investigation 2012/1357084 BASF SE, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	Evaluation of mesocosm study according to current guidance	BASF
KCA 8.3.1/1	N.N.	2014	study in preparation (residues in honey bee feed items) 2014/1000204 Yes Unpublished	No	Yes	New data requirement	BASF
KCA 8.3.1/2	N.N.	2014	study in preparation (residues in honey bee feed items) 2014/1000182 Yes Unpublished	No	Yes	New data requirement	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 8.3.1.1.1/1	Sekine T.	2013 a	Effects of BAS 500 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory 2013/1003210 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	Old study showed weakness with respect to reference compound	BASF
KCA 8.3.1.1.2/1	Sekine T.	2013 a	Effects of BAS 500 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory 2013/1003210 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	Old study showed weakness with respect to reference compound	BASF
KCA 8.3.1.2/1	N.N.	2014	study in preparation (chronic honey bee study with BAS 500 06 F) 2013/1235056 Yes Unpublished	No	Yes	New data requirement	BASF
KCA 8.3.1.3/1	Barth M.	2012 a	Effects of BAS 500 06 F on the honeybee Apis mellifera L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development 2011/1112669 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	New data requirement	BASF
KCA 8.4.1/1	N.N.	2014	study in preparation (earthworm reproduction study with pyraclostrobin) 2014/1000461 Yes Unpublished	No	Yes	New data for earthworm risk assessment	BASF
KCA 8.4.1/2	Ganssmann M.	2013 a	Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms Eisenia fetida in artificial soil 2013/1003174 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 8.4.1/3	Ganssmann M.	2013 b	Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms Eisenia fetida in artificial soil with 10% peat 2013/1224029 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.4.2/1	Ganssmann M.	2013 c	Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction of the collembola Folsomia candida in artificial soil with 5% peat 2013/1068054 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.4.2/2	Ganssmann M.	2013 d	Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction of the collembola Folsomia candida in artificial soil with 5% peat 2013/1224030 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.4.2/3	Friedrich S.	2008 a	Effects of BAS 500 06 F on the reproduction of the collembolans Folsomia candida in artificial soil with 5% peat 2008/1037495 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	Data needed for risk assessment	BASF
KCA 8.4.2/4	Schulz L.	2012 a	BAS 500 06 F - Effects of BAS 500 06 F on the reproduction of the predatory mite Hypoaspis aculeifer 2012/1129444 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	Data needed for risk assessment	BASF

3.1.2 Representative formulation BAS 500 06 F

Section 2: Physical and chemical properties of the plant protection product

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 2.1/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.2/1	Loeffler U.	2004 a	Evaluation of physical and chemical properties according to Directive 92/69/EC, Annex A9-A17 2004/1004104 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.3/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.3/2	Loeffler U.	2004 a	Evaluation of physical and chemical properties according to Directive 92/69/EC, Annex A9-A17 2004/1004104 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.4/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.5/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.6/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 2.7/1	Kroehl T.	2006 a	Shelf life at 23°C in original container of the formulation BAS 500 06 F, 24 month storage, analytical results and physical properties 2006/1019717 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.8.2/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.8.2/2	Kroehl T.	2008 a	Emulsion stability, persistent foaming and pH value of Pyraclostrobin 200 g/L EC (BAS 500 06 F) 2008/1086283 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.8.6/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 500 06 F 2004/1010381 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.8.6/2	Kroehl T.	2008 a	Emulsion stability, persistent foaming and pH value of Pyraclostrobin 200 g/L EC (BAS 500 06 F) 2008/1086283 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.9/1	Rudoll B., Schneider K.-H.	2004 a	Physical and chemical compatibility in aqueous tank mixtures of BAS 500 06 F 2004/1008462 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New representative formulation	BASF

Section 4: Further information on the plant protection product

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 4.4/1	Schreiner B.	2005 a	EU performance tests of BAS 500 06 F (Standard-Coex-Bottle, 1 L, Spec.-No. 775 5108) 2005/1025057 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. No Unpublished	No	No	New representative formulation	BASF

Section 5: Analytical methods

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 5.1.1/1	Ziegler H., Machauer B.	2003 a	Analytical method CF-A 669: Quantitative determination of the active ingredient Pyraclostrobin in BAS 500 06 F by HPLC 2003/1022228 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New representative formulation	BASF
KCP 5.1.1/2	Ziegler H.	2004 a	Validation of the analytical method CF-A 669: Determination of the active ingredient Pyraclostrobin in BAS 500 06 F by HPLC 2004/1004041 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 5.1.1/3	Stegmaier W.	2011 a	Gas chromatographic determination of dimethyl sulfate in BAS 500 06 F 2011/1009063 BASF SE - GMC Competence Center Analytics, Ludwigshafen, Germany Fed.Rep. No Unpublished	No	No	New representative formulation	BASF
KCP 5.1.1/4	Stegmaier W.	2011 b	Validation of an analytical method for the determination of dimethyl sulfate in BAS 500 06 F 2011/1009064 BASF SE - GMC Competence Center Analytics, Ludwigshafen, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Section 7: Toxicology

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 7.1.1/1	[redacted]	2008 a	BAS 500 06 F: Acute oral toxicity study in rats 2007/1053390 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.1.2/1	[redacted]	2009 a	BAS 500 06 F: Acute dermal toxicity study in rats 2009/1084157 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.1.3/1	[redacted]	2010 a	BAS 500 06 F - Acute inhalation toxicity study in Wistar rats - 4-Hour liquid aerosol (head-nose only) 2009/1122167 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.1.4/1	[redacted]	2010 a	BAS 500 06 F - Acute dermal irritation / corrosion in rabbits 2009/1100358 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.1.5/1	[redacted]	2010 b	BAS 500 06 F - Acute eye irritation in rabbits 2009/1100359 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.1.6/1	[redacted]	2008 b	BAS 500 06 F: Murine local lymph node assay (LLNA) 2007/1053391 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.1.6/2	[redacted]	2009 a	BAS 500 06 F - Maximization test in guinea pigs 2009/1018498 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.3/1	Fabian E., Landsiedel R.	2014	^{14}C-BAS 500 F in BAS 500 06 F - Study of penetration through human skin in vitro 2014/1001501 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New data	BASF

Section 9: Fate and behaviour in the environment

A full set of exposure calculations will be provided for pyraclostrobin and its metabolites for which environmental risk assessments are triggered. The list of PEC calculation reports given below should thus be considered as preliminary. Additional PEC calculation reports might be provided as seen appropriate.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 9.1.3/1	Eickler B.	2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.2.4/1	Eickler B.	2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.2.5/1	Eickler B.	2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF

Section 10: Ecotoxicology

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.1.1. 1/1	[redacted]	2008 a	BAS 500 06 F - Acute toxicity in the bobwhite quail (Colinus virginianus) after single oral administration (LD50) 2008/1078602 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 10.1.2. 1/1	[redacted]	2009 a	BAS 500 06 F: Acute toxicity testing study - Repeated dose oral toxicity study in Wistar rats 2009/1108893 [redacted] Yes Unpublished	Yes	Yes	Data needed for risk assessment	BASF
KCP 10.1.2. 1/2	[redacted]	2011 a	BAS 500 06 F - Repeated dose oral toxicity study in Wistar rats - Administration via the diet over 5 days 2011/1146588 [redacted] No Unpublished	Yes	No	Data needed for risk assessment	BASF
KCP 10.1.2. 2/1	N.N.	2014	Field study acute and long-term effects-BAS 500 06 F small mammals in Central Europe 2014/1000041 Yes Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2. 2/2	N.N.	2014	Study to assess avoidance and effects in small mammals upon dietary exposure to a pyraclostrobin-formulation - 2012/1129348 Yes Unpublished	Yes	Yes	Data needed for risk assessment	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.1.2. 2/3	Moreno S.	2013 a	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) on wheat (young plants) after treatment with BAS 500 06 F under field conditions in North and South Europe, season 2012 2013/1045207 Agricultura y Ensayo SL, Alcala de Guadaira, Spain Yes Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2. 2/4	Martin T.	2013 a	Study on the residue behavior of Pyraclostrobin (BAS 500 F) on pea (young plants) after the application of BAS 500 06 F under field conditions in France (North), Germany, United Kingdom, Italy and Spain, 2012 2013/1044539 Agrologia SL, Utrera, Spain Yes Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2. 2/5	Mastitsky S.E.	2014	BAS 500F: Body-burden modelling to assess the acute risk of formulated pyraclostrobin to small mammals 2014/1001603 No Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2. 2/6	N.N.	2014	BAS 500 06 F: Acute risk to mammals of the formulated product 2014/1010735 No Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2. 2/7	N.N.	2014	BAS 500 F - Pyraclostrobin: Ecologically relevant chronic toxicity endpoint for the wild mammal reproductive risk assessment (Ecotoxicology) 2014/1010736 No Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2. 2/8	Barfknecht R.	2006 a	Generic field monitoring of mammals in cereal fields in spring and summer in Germany 2007/1042674 Bayer CropScience AG, Monheim, Germany Fed.Rep. Yes Unpublished	No	No	Data needed for risk assessment	Bayer Crop Science

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.1.2. 2/9	Anonymous	2007 b	Letter of Access for Generic Behavioural Ecology Data - Study report Bayer CropScience AG DocID BAR/FS030 2007/1044667 Bayer CropScience AG, Monheim, Germany Fed.Rep. No Unpublished	No	No	Data needed for risk assessment	Bayer Crop Science
KCP 10.1.2. 2/10	Schroeer A., Grimm T.	2011 a	Field monitoring of hares and rabbits in cereal fields 2011/1112612 RIFCon GmbH, Heidelberg, Germany Fed.Rep. Yes Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2. 2/11	Schroeer A., Grimm T.	2012 a	First amendment to final report - Field monitoring of hares and rabbits in cereals fields 2012/1105899 RIFCon GmbH, Heidelberg, Germany Fed.Rep. Yes Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2. 2/12	Hecht-Rost S., Münderle M., Grimm T.	2013 a	Habitat use of wood mice (Apodemus sylvaticus) in maize in Germany 2013/1298445 RIFCon GmbH, Hirschberg, Germany Fed.Rep. No Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2. 2/13	Zaccaroni M., Dessi Fulgheri F.	2012 a	Generic field monitoring of hares in a mixed landscape in Italy 2011/1089129 University of Florence, Firenze, Italy No Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.3/ 1	Belden et al.	2010 a	Acute toxicity of fungicide formulations to amphibians at environmentally relevant concentrations Literature report in: *Environmental Toxicology and Chemistry*, 29(11):2477-2480 No Published	No	No	New requirement	public

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.1.3/ 2	Brühl et al.	2013 a	Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline? Literature report in: *Scientific Reports*, 3: 1135; DOI: 10.1038 /srep01335 (2013) No Published	No	No	New requirement	public
KCP 10.1.3/ 3	[redacted]	2014	Report in preparation (laboratory test with amphibians) 2013/1375098 Yes Unpublished	Yes	Yes	New requirement	BASF
KCP 10.1.3/ 4	[redacted]	2014	Report in preparation (semi-field test with amphibians) 2013/1375099 Yes Unpublished	Yes	Yes	New requirement	BASF
KCP 10.2.1/ 1	[redacted]	2008 a	BAS 500 06 F - Acute toxicity study with the rainbow trout (Oncorhynchus mykiss) 2008/1018046 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 10.2.1/ 2	[redacted]	2012 a	BAS 500 06 F - Acute toxicity study in the common carp (Cyprinus carpio) 2012/1250190 [redacted] Yes Unpublished	Yes	Yes	New data (request of Japanese authorities)	BASF
KCP 10.2.1/ 3	Funk M.	2004 a	Effect of BAS 500 06 F on the immobility of Daphnia magna STRAUS in a 48 hours static, acute toxicity test 2004/1004393 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.2.1/ 4	Hoffmann F.	2008 a	Effect of BAS 500 06 F on the growth of the green alga Pseudokirchneriella subcapitata 2008/1009325 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.3.1. 1.1/1	Bocksch S.	2004 a	Assessment of side effects of BAS 500 06 F to the honey bee, Apis mellifera L. in the laboratory 2004/1015008 GAB Biotechnologie GmbH & GAB Analytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.1. 1.2/1	Bocksch S.	2004 a	Assessment of side effects of BAS 500 06 F to the honey bee, Apis mellifera L. in the laboratory 2004/1015008 GAB Biotechnologie GmbH & GAB Analytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.1. 5/1	Barth M.	2012 a	Effects of BAS 500 06 F on the honeybee Apis mellifera L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development 2011/1112669 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	New data requirement	BASF
KCP 10.3.2. 1/1	Sipos K.	2007 a	Effect of BAS 500 06 F on the predatory mite (Typhlodromus pyri) in a laboratory trial 2007/1035599 LAB International Research Centre Hungary Ltd., Veszprem, Hungary Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2. 1/2	Sipos K.	2007 b	Effect of BAS 500 06 F on the parasitic wasp (Aphidius rhopalosiphi) in a laboratory trial 2007/1035600 LAB International Research Centre Hungary Ltd., Veszprem, Hungary Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.3.2. 1/3	Sipos K.	2007 c	Amendment to the final report: Effect of BAS 500 06 F on the parasitic wasp (Aphidius rhopalosiphi) in a laboratory trial 2007/1050841 LAB International Research Centre Hungary Ltd., Veszprem, Hungary Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2. 2/1	Vaughan R.	2008 a	A rate-response extended laboratory test to determine the effects of BAS 500 06 F on the predatory mite, Typhlodromus pyri (Acari: Phytoseiidae) 2008/1010712 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2. 2/2	Stevens J.	2008 a	A rate-response extended laboratory test to determine the effects of BAS 500 06 F on the parasitic wasp, Aphidius rhopalosiphi (Hymenoptera, Braconidae) 2008/1010713 Mambo-Tox Ltd., Southampton SO16 7NP, United Kingdom Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2. 2/3	Roehlig U.	2008 a	Effects of BAS 500 06 F on the green lacewing Chrysoperla carnea STEPH. under extended laboratory conditions - Rate-response test 2008/1032666 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.3.2. 2/4	Roehlig U.	2008 b	Effects of BAS 500 06 F on the green lacewing Chrysoperla carnea STEPH. in an extended laboratory test (under semi-field conditions aged residues on bean plants) 2008/1042190 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2. 2/5	Schmitzer S.	2008 a	Effects of BAS 500 06 F on the reproduction of rove beetles (Aleochara bilineata) - Extended laboratory study 2008/1010700 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.4.1. 1/1	Luehrs U.	2008 a	Effects of BAS 500 06 F on reproduction and growth of earthworms Eisenia fetida in artificial soil with 5% peat 2008/1036409 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.4.1. 2/1	Luehrs U.	2010 a	Field study to evaluate the effects of BAS 500 06 F on earthworms 2010/1000056 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.4.2/ 1	Friedrich S.	2008 a	Effects of BAS 500 06 F on the reproduction of the collembolans Folsomia candida in artificial soil with 5% peat 2008/1037495 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.4.2/ 2	Schulz L.	2012 a	BAS 500 06 F - Effects of BAS 500 06 F on the reproduction of the predatory mite Hypoaspis aculeifer 2012/1129444 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.5/1	Schulz L.	2012 b	Effects of BAS 500 06 F on the activity of soil microflora (Nitrogen transformation test) 2012/1129443 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.6.2/ 1	Stroemel C. et al.	2013 a	Effect of BAS 500 06 F on vegetative vigour of ten species of terrestrial plants under greenhouse conditions 2012/1115894 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.6.2/ 2	Stroemel C. et al.	2012 a	Effect of BAS 500 06 F on seedling emergence and seedling growth of ten species of terrestrial plants under greenhouse conditions 2012/1115895 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.7/1	Fleischer G.	2004 a	Effect of BAS 500 06 F on the mortality of the earthworm Eisenia fetida 2004/1004367 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.7/2	Luehrs U., Schabio S.	2010 a	Effects of BAS 500 06 F on the breakdown of organic matter in litter bags in the field 2010/1000081 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

3.1.3 Representative formulation BAS 516 07 F

Section 2: Physical and chemical properties of the plant protection product

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 2.1/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.2/1	Bitterlich S.	2005 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Directive 92/69/EC: Annex A.9-A.17) 2005/1011601 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.2/2	Loehr S.	2011 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) 2011/1065573 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.3/1	Bitterlich S.	2005 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Directive 92/69/EC: Annex A.9-A.17) 2005/1011601 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.3/2	Loehr S.	2011 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) 2011/1065573 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.4/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 2.6/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.7/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.7/2	Keller M.	2011 a	Chemical and physical stability of formula BAS 516 07 F when stored at 23°C up to 3 years in commercial packs (HDPE-bottle) 2011/1102466 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.8.1/ 1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.8.2/ 1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.8.3/ 1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.8.5. 1/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 2.8.5. 2/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.8.5. 3/1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.8.7/ 1	Kaestel R.	2004 a	Physical and chemical properties of the formulation BAS 516 07 F 2004/1024836 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 2.9/1	Schneider K.-H.	2001 a	Physical and chemical compatibility in aqueous tank mixtures of BAS 516 00 F with other products 2001/1001884 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New representative formulation	BASF

Section 4: Further information on the plant protection product

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 4.4/1	Schreiner B.	2009 a	EU-Performance-Test of BAS 516 07 F, Bottle, rectangular, 1 Kg (PE-HD) 2009/1052799 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. No Unpublished	No	No	New representative formulation	BASF
KCP 4.4/2	Schreiner B.	2009 b	EU-Performance-Test of BAS 516 07 F (Form Fill and Seal Bag (FFS bag), 1 Kg 2009/1052800 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. No Unpublished	No	No	New representative formulation	BASF

Section 5: Analytical methods

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 5.1.1/1	Ziegler H.	2000 a	The determination of the amounts of active ingredients Reg.No. 300 355 and Pyraclostrobin in BAS 516 00 F by HPLC 2000/1014140 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New representative formulation	BASF
KCP 5.1.1/2	Ziegler H.	2000 b	Development and validation of the analytical method CF-A 598. Determination of Reg.No. 300 355 and Pyraclostrobin in water dispersible granules (WG) (BAS 516 00 F) 2000/1012385 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 5.1.1/3	Euler K.	2009 a	Final report: Supplement to the development and validation of the analytical method CF-A 598, Study No. 09L00088 2009/1050295 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 5.1.1/4	Stegmaier W.	2009 a	Analytical method AM/01120/01e, gas chromatographic determination of dimethyl sulfate in BAS 516 07 F 2009/1105184 BASF SE - GKA Competence Center Analytics, Ludwigshafen, Germany Fed.Rep. No Unpublished	No	No	New representative formulation	BASF
KCP 5.1.1/5	Stegmaier W.	2012 a	Validation of an analytical method for the determination of dimethyl sulfate in BAS 516 07 F 2012/1213598 BASF SE - GMC Competence Center Analytics, Ludwigshafen, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Section 7: Toxicology

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 7.1.1/1	[redacted]	2008 a	Acute oral toxicity in rats - Acute toxic class method 2008/1004838 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.1.2/1	[redacted]	2001 a	BAS 516 00 F - Acute dermal toxicity study in rats 2001/1003723 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.1.3/1	[redacted]	2001 b	BAS 516 00 F - Acute inhalation toxicity study in Wistar rats 4-hour dust exposure 2001/1001824 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.1.4/1	[redacted]	2007 a	BAS 516 07 F: Acute dermal irritation / corrosion in rabbits 2007/1056989 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.1.5/1	[redacted]	2007 b	BAS 516 07 F: Acute eye irritation in rabbits 2007/1056988 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 7.1.6/1	[redacted]	2014	BAS 516 07 F: Skin Sensitization: Local Lymph Node Assay 2014/1001403 [redacted] Yes Unpublished	Yes	Yes	New data	BASF
KCP 7.3/1	Hassler S.	2014	BAS 510 F: Percutaneous Penetration of ^{14}C BAS 510 F formulated as BAS 516 07 F through human split-thickness skin membranes (in-vitro) 2014/1001401 Harlan Laboratories Ltd., Itingen, Switzerland Yes Unpublished	No	Yes	New data	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 7.3/2	Hassler S.	2014	BAS 500 F: Percutaneous Penetration of ^{14}C BAS 500 F formulated as BAS 516 07 F through human split-thickness skin membranes (in-vitro) 2014/1001401 Harlan Laboratories Ltd., Itingen, Switzerland Yes Unpublished	No	Yes	New data	BASF

Section 9: Fate and behaviour in the environment

A full set of exposure calculations will be provided for pyraclostrobin and its metabolites for which environmental risk assessments are triggered. Exposure calculations will be also provided for the second active substance boscalid in formulation BAS 516 07 F. The list of PEC calculation reports given below should thus be considered as preliminary. Additional PEC calculation reports might be provided as seen appropriate.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 9.1.3/1	Eickler B.	2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.1.3/2	Eickler B.	2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of boscalid after application of BAS 516 07 F to potatoes 2014/1001461 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.2.4/1	Eickler B.	2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.2.4/2	Eickler B.	2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of boscalid after application of BAS 516 07 F to potatoes 2014/1001461 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.2.5/1	Eickler B.	2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 9.2.5/2	Eickler B.	2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of boscalid after application of BAS 516 07 F to potatoes 2014/1001461 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF

Section 10: Ecotoxicology

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.1.1.1/1	[redacted]	2006 a	BAS 516 07 F - Acute toxicity in the bobwhite quail (Colinus virginianus) after single oral administration (LD50) 2006/1030309 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 10.2.1/1	[redacted]	2000 a	BAS 516 00 F - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a static system (96 hours) 2000/1018726 [redacted] Yes Unpublished	Yes	Yes	New representative formulation	BASF
KCP 10.2.1/2	Janson G.-M.	2007 a	Acute toxicity of BAS 516 07 F to Daphnia magna STRAUS in a 48 hour static test 2007/1008605 BASF AG Agrarzentrum Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.2.1/3	Janson G.-M.	2009 a	Acute toxicity of BAS 516 07 F to Daphnia magna STRAUS in a 48 hour static test 2009/1117877 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.2.1/4	Hoffmann F.	2007 a	Effect of BAS 516 07 F on the growth of the green alga Pseudokirchneriella subcapitata 2007/1005074 BASF AG Agrarzentrum Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.3.1.1.1/1	Kling A.	2001 a	Assessment of side effects of BAS 516 00 F to the honey bee, Apis mellifera L. in the laboratory 2001/1000868 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.1.1.2/1	Kling A.	2001 a	Assessment of side effects of BAS 516 00 F to the honey bee, Apis mellifera L. in the laboratory 2001/1000868 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2.1/1	Buehler A.	2000 a	Effect of BAS 516 00 F on the ground dwelling predator Poecilus cupreus (Coleoptera, Carabidae) in a laboratory trial 2000/1012478 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2.1/2	Adelberger I.	2001 a	BAS 516 00 F: Toxicity to the predatory mite, Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) in the laboratory 2001/1000880 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2.1/3	Ufer A.	2001 a	Effect of BAS 516 00 F on the green lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) in a laboratory trial 2001/1001860 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.3.2.1/4	Schuld M.	2001 a	BAS 516 00 F: Toxicity to the aphid parasitoid, Aphidius rhopalosiphi (Hymenoptera, Braconidae) DeStefani-Perez in the laboratory 2001/1001864 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2.1/5	Hermann P.	2001 a	BAS 516 00 F: Toxicity to the staphylinid beetle, Aleochara bilineata GYLL. (Coleoptera, Staphylinidae) in the laboratory 2001/1001863 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2.1/6	Schmitzer S.	2001 a	Effects of BAS 516 00 F on the wolf spider Pardosa spec. in the laboratory 2001/1005964 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2.2/1	Ufer A.	2001 b	Effect of BAS 516 00 F on the green lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) in an extended laboratory trial 2001/1001874 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2.4/1	Muether J.	2001 a	A field study to evaluate the effects of BAS 516 00 F against predatory mites in damson plum 2001/1000905 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.3.2.4/2	Gossmann A.	2001 a	Effects of BAS 516 00 F on predatory mites Typhlodromus pyri SCHEUTEN (Acari, Phytoseiidae) in apple orchards (field experiments) 2001/1001866 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.3.2.4/3	Nienstedt K.M.	2001 a	A field test to determine the effect of BAS 516 00 F on predatory mite populations (Acari: Phytoseiidae) on stone fruit trees (cherry, Prunus avium) 2001/1001870 Springborn Laboratories (Europe) AG, Horn, Switzerland Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.4.1.1/1	Friedrich S.	2006 a	Sublethal toxicity of BAS 516 07 F to the earthworm Eisenia fetida in artificial soil with 5% peat 2006/1015860 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.4.1.2/1	Hamberger A.	2006 a	Field study to evaluate the effects of BAS 516 07 F on earthworms in Southern Germany 2011/1043488 Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.4.2/1	Friedrich S.	2006 b	Effects of BAS 516 07 F on the reproduction of the collembolans Folsomia candida in artificial soil with 5% peat 2006/1015861 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.4.2/2	N.N.	2014	study in preparation (effects on reproduction of soil mites Hypoaspis aculeifer) 2014/1010834 Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.5/1	Wachter S.	2001 a	Assessment of the side effects of BAS 516 00 F on the activity of the soil microflora; nitrogen turnover 2001/1005959 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.6.2/1	Oberwalder C., Schmidt O.	2001 a	BAS 516 00 F: Effects on non-target plants in the greenhouse - a limit test 2001/1005953 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.6.2/2	N.N.	2014	study in preparation (vegetative vigor of non-target plants) 2014/1010836 Yes Unpublished	No	Yes	Old study has no analytic included	BASF
KCP 10.6.2/3	N.N.	2014	study in preparation (seedling emergence of non-target plants) 2014/1010835 Yes Unpublished	No	Yes	New representative formulation	BASF
KCP 10.7/1	Wachter S.	2001 b	Acute toxicity of BAS 516 00 F on earthworms, Eisenia foetida using an artificial soil test 2001/1001837 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
10.7/2	Wachter S.	2001 c	Assessment of the side effects of BAS 516 00 F on the activity of the soil microflora; short-term respiration 2001/1005960 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. Yes Unpublished	No	Yes	New representative formulation	BASF

3.2 List of new studies on vertebrate animals

References of studies on vertebrate animals are given for the active substance pyraclostrobin (BAS 500 F), its metabolites and the representative formulations BAS 500 06 F and BAS 516 07 F.

3.2.1 Active substance pyraclostrobin (BAS 500 F)

Section 5: Toxicology and metabolism studies - active substance and its metabolites

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCA 5.2.3/1	[redacted]	2001 a	BAS 500 F 40% in Solvesso (technical active ingredient) - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2001/1010625 [redacted] Yes Unpublished	New data (submitted to US-EPA)	Yes	BASF
KCA 5.2.3/2	[redacted]	2002 a	BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2002/1012053 [redacted] Yes Unpublished	New data	Yes	BASF
KCA 5.2.3/3	[redacted]	2003 a	Amendment No. 1 to the report: BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2003/1009200 [redacted] Yes Unpublished	New data	Yes	BASF
KCA 5.2.3/4	[redacted]	2003 b	Amendment No. 2 to the report: BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2003/1009427 [redacted] Yes Unpublished	New data	Yes	BASF
KCA 5.3.2/1	[redacted]	1999 a	BAS 500 F - Subchronic oral toxicity study in Wistar rats. Administration in the diet for 3 months - Volume I of III 1999/10195 [redacted] Yes Unpublished	Previously evaluated study submitted for completeness (basis for the assessment of the amendments listed below)	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCA 5.3.2/2	[redacted]	1999 b	Amendment No. 1 to the report: BAS 500 F - Subchronic oral toxicity study in Wistar rats. Administration in the diet for 3 months 1999/11899 [redacted] Yes Unpublished	Correction of clerical errors (see page 29)	No	BASF
KCA 5.3.2/3	[redacted]	2000 a	Amendment No. 2 to the report: BAS 500 F - Subchronic oral toxicity study in Wistar rats administration in the diet for 3 months 2000/1012360 [redacted] Yes Unpublished	Correction of clerical errors	No	BASF
KCA 5.3.2/4	[redacted]	2003 a	Amendment No. 3 to the report: BAS 500 F - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months 2003/1013399 [redacted] Yes Unpublished	Additional histopatho-logical evaluation	No	BASF
KCA 5.3.2/5	[redacted]	2004 a	Amendment to the report for Japanese registration: BAS 500 F - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months 2004/1027673 [redacted] Yes Unpublished	Description of duodenum trimming procedure (requested by Japanese authorities)	No	BASF
KCA 5.3.2/6	[redacted]	1998 a	BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl BR mice. Administration in the diet for 3 months 1998/11345 [redacted] Yes Unpublished	Previously evaluated study submitted for completeness (basis for the assessment of the amendments listed below)	No	BASF
KCA 5.3.2/7	[redacted]	1999 c	Amendment No. 1 to the report: BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl mice. Administration in the diet for 3 months 1999/11900 [redacted] Yes Unpublished	Correction of clerical errors and addition of new data	No	BASF
KCA 5.3.2/8	[redacted]	2003 b	Amendment No. 2 to the report: BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl mice - Administration in the diet for 3 months 2003/1013400 [redacted] Yes Unpublished	Additional histopatho-logical evaluation	No	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCA 5.3.3/1	[redacted]	2005 a	BAS 500 F - Subacute inhalation study in Wistar rats - 20 aerosol exposures during 4 weeks 2005/1013950 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF
KCA 5.3.3/2	[redacted]	2014	Supplementary subacute inhalation study in Wistar rats 2014/1003946 [redacted] Yes Unpublished	Additional concentrations needed for risk assessment	Yes	BASF
KCA 5.5/1	[redacted]	1999 d	BAS 500 F - Chronic toxicity study in Wistar rats. Administration in the diet for 24 months 1999/11672 [redacted] Yes Unpublished	Previously evaluated study submitted for completeness (basis for the assessment of the amendment listed below)	No	BASF
KCA 5.5/2	[redacted]	2002 a	Amendment No. 1 to the report: BAS 500 F - Chronic toxicity study in Wistar rats. Administration in the diet for 24 months 2002/1005113 [redacted] Yes Unpublished	Additional histopatho-logical evaluation of intermediate dose group males (requested by US-EPA)	No	BASF
KCA 5.5/3	[redacted]	1999 e	BAS 500 F - Carcinogenicity study in Wistar rats. Administration in the diet for 24 months 1999/11868 [redacted] Yes Unpublished	Previously evaluated study submitted for completeness (basis for the assessment of the amendment listed below)	No	BASF
KCA 5.5/4	[redacted]	2002 b	Amendment No. 1 to the report: BAS 500 F - Carcinogenicity study in Wistar rats. Administration in the diet for 24 months 2002/1005114 [redacted] Yes Unpublished	Additional histopatho-logical evaluation of intermediate dose group males (requested by US-EPA)	No	BASF
KCA 5.5/6	[redacted]	2002 c	BAS 500 F - First supplementary chronic toxicity study in Wistar rats - Administration in the diet for 24 months 2002/1004125 [redacted] Yes Unpublished	New data (submitted to US-EPA)	Yes	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCA 5.5/7	[redacted]	2002 d	BAS 500 F - Second supplementary chronic toxicity study in female Wistar rats - Administration in the diet for 24 months 2002/1004126 [redacted] Yes Unpublished	New data (submitted to US-EPA)	Yes	BASF
KCA 5.5/8	[redacted]	2002 e	BAS 500 F - First supplementary carcinogenicity study in Wistar rats - Administration in the diet for 24 months 2002/1004123 [redacted] Yes Unpublished	New data (submitted to US-EPA)	Yes	BASF
KCA 5.5/9	[redacted]	2002 f	BAS 500 F - Second supplementary carcinogenicity study in female Wistar rats - Administration in the diet for 24 months 2002/1004124 [redacted] Yes Unpublished	New data (submitted to US-EPA)	Yes	BASF
KCA 5.5/10	[redacted]	2005 a	BAS 500 F: Terminated carcinogenicity study in female B6C3F1 mice; administration in the diet for 7 months 2005/1026477 [redacted] Yes Unpublished	New data (submitted to US-EPA)	Yes	BASF
KCA 5.5/11	[redacted]	2002 a	Chronic and oncogenicity study with BAS 500 F: Further evaluations of body weight, food consumption and food efficiency 2002/5002875 [redacted] No Unpublished	New evaluation of BW, FC and Food eff. data from the rat and mouse long-term studies (evaluation submitted to US-EPA)	No	BASF
KCA 5.8.1/4	[redacted]	1997 a	Study on the acute oral toxicity of Pyrazolon in rats 1997/10963 [redacted] Yes Unpublished	New data for the risk assessment of metabolites	Yes	BASF
KCA 5.8.1/5	[redacted]	1997 b	Study on the acute dermal irritation/corrosion of Pyrazolon in the rabbit 1997/10964 [redacted] Yes Unpublished	New data for the risk assessment of metabolites	Yes	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCA 5.8.1/6	[redacted]	1997 c	Study on the acute eye irritation of Pyrazolon in the rabbit 1997/10965 [redacted] Yes Unpublished	New data for the risk assessment of metabolites	Yes	BASF
KCA 5.8.1/7	[redacted]	1997 a	Pyrazolon - Maximization test in guinea pigs 1997/10968 [redacted] Yes Unpublished	New data for the risk assessment of metabolites	Yes	BASF
KCA 5.8.1/8	[redacted]	2013 a	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Repeated dose 90-day oral toxicity study in Wistar rats - Administration via the diet 2013/1042164 [redacted] Yes Unpublished	New data for the risk assessment of metabolites	Yes	BASF
KCA 5.8.1/12	[redacted]	2013 a	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2013/1026779 [redacted] Yes Unpublished	New data for the risk assessment of metabolites	Yes	BASF
KCA 5.8.1/13	[redacted]	2013 a	^{14}C-Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Study on the kinetics in mice 2012/1278425 [redacted] Yes Unpublished	New data for the risk assessment of metabolites	Yes	BASF
KCA 5.8.1/16	[redacted].	2012 d	Reg.No. 411847 (metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2012/1218557 [redacted] Yes Unpublished	New data for the risk assessment of metabolites	Yes	BASF
KCA 5.8.1/19	[redacted]	2012 e	Reg.No. 413038 (Metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2012/1220183 [redacted]. Yes Unpublished	New data for the risk assessment of metabolites	Yes	BASF
KCA 5.8.1/26	[redacted]	2014	Reg.No. 5916421 (Metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus Assay in Bone Marrow Cells of the Mouse 2013/1389659 [redacted] Yes Unpublished	New data for the risk assessment of metabolites	Yes	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCA 5.8.2/1	[redacted]	2003 a	BAS 500 F - Determination of iron in urine and serum of Wistar rats - Administration in the diet over 14 days 2003/1009534 [redacted] No Unpublished	New data (submitted to US-EPA)	No	BASF
KCA 5.8.2/2	[redacted]	2011 a	A 28-Day Oral (Dietary) Range Finding Study of BAS 500 F in B6C3F1 Mice 2011/1194286 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF
KCA 5.8.2/3	[redacted]	2012 a	A 28-day oral (dietary) natural killer cell immunotoxicity study of BAS 500 F in female B6C3F1 mice 2011/1035857 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF
KCA 5.8.2/4	[redacted]	2012 b	A 28-day oral (dietary) antibody forming cell immunotoxicity study of BAS 500 F in female B6C3F1 mice 2012/1020986 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF
KCA 5.8.2/5	[redacted]	2012 c	A 28-day oral (dietary) antibody forming cell immunotoxicity study of BAS 500 F in female B6C3F1 mice 2012/1084176 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF

Section 6: Residues in or on treated products, food and feed and plant metabolism

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCA 6.2.5/1	[redacted]	1999 a	Bioaccumulation and metabolism of (^{14}C)-BAS 500 F in bluegill sunfish 1999/11348 [redacted] Yes Unpublished	Submission of all meta-bolism data as agreed with RMS (study previously evaluated within ecotox)	No	BASF
KCA 6.2.5/2	[redacted]	2014	The Metabolism of ^{14}C-Pyraclostrobin (^{14}C-BAS 500 F) in rainbow trout (Oncorhynchus mykiss) 2014/1001601 Yes Unpublished	New data requirement according to Reg. 283/2013	Yes	BASF
KCA 6.4.1/1	[redacted]	2000 a	A meat and egg magnitude of the residue study with BAS 500 F in laying hens 2000/5005 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF

Section 8: Ecotoxicology - active substance and its metabolites

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCA 8.1.1.1/1	[redacted]	2014	BAS 500 F - Acute Toxicity in the Canary after single oral administration 2013/1400375 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF
KCA 8.2.1/1	[redacted]	2000 a	Flow-through acute toxicity of BAS 500 F to the rainbow trout, Oncorhynchus mykiss 2000/5034 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF
KCA 8.2.1/2	[redacted]	2000 b	Flow-through acute toxicity of BAS 500 F to the bluegill sunfish, Lepomis macrochirus 2000/5033 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF
KCA 8.2.1/3	[redacted]	2000 c	Flow-through acute toxicity of BAS 500F to the sheepshead minnow, Cyprinodon variegatus 2000/5032 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF
KCA 8.2.1/4	[redacted]	2007 a	Reg.No. 340266 (metabolite BF 500-3 of BAS 500 F): Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in a static system over 96 hours 2007/1010836 [redacted] Yes Unpublished	New data (request of Canadian authorities)	Yes	BASF
KCA 8.2.1/5	N.N.	2014	study in preparation (Acute fish (trout) study with pyraclostrobin metabolite BF 500-5) 2013/1308543 Yes Unpublished	New data for the risk assessment of metabolites	Yes	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCA 8.2.2/1	[redacted]	2000 d	Early life stage toxicity of BAS 500 F to the fathead minnow, Pimephales promelas 2000/5053 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF
KCA 8.2.2/2	[redacted]	2001 a	Early life stage toxicity of BAS 500 F to the Sheepshead Minnow, Cyprinodon variegatus 2000/5247 [redacted] Yes Unpublished	New data (request of US-EPA)	Yes	BASF

3.2.2 Representative formulation BAS 500 06 F

Section 7: Toxicology

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCP 7.1.1/1	[redacted]	2008 a	BAS 500 06 F: Acute oral toxicity study in rats 2007/1053390 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 7.1.2/1	[redacted]	2009 a	BAS 500 06 F: Acute dermal toxicity study in rats 2009/1084157 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 7.1.3/1	[redacted]	2010 a	BAS 500 06 F - Acute inhalation toxicity study in Wistar rats - 4-Hour liquid aerosol (head-nose only) 2009/1122167 [redacted]. Yes Unpublished	New representative formulation	Yes	BASF
KCP 7.1.4/1	[redacted]	2010 a	BAS 500 06 F - Acute dermal irritation / corrosion in rabbits 2009/1100358 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 7.1.5/1	[redacted]	2010 b	BAS 500 06 F - Acute eye irritation in rabbits 2009/1100359 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 7.1.6/1	[redacted]	2008 b	BAS 500 06 F: Murine local lymph node assay (LLNA) 2007/1053391 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 7.1.6/2	[redacted]	2009 a	BAS 500 06 F - Maximization test in guinea pigs 2009/1018498 [redacted] Yes Unpublished	New representative formulation	Yes	BASF

Section 10: Ecotoxicology

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCP 10.1.1.1/1	[redacted]	2008 a	BAS 500 06 F - Acute toxicity in the bobwhite quail (Colinus virginianus) after single oral administration (LD50) 2008/1078602 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 10.1.2.1/1	[redacted]	2009 a	BAS 500 06 F: Acute toxicity testing study - Repeated dose oral toxicity study in Wistar rats 2009/1108893 [redacted] Yes Unpublished	Data needed for risk assessment	Yes	BASF
KCP 10.1.2.1/2	[redacted].	2011 a	BAS 500 06 F - Repeated dose oral toxicity study in Wistar rats - Administration via the diet over 5 days 2011/1146588 [redacted] No Unpublished	Data needed for risk assessment	No	BASF
KCP 10.1.2.2/2	N.N.	2014	Study to assess avoidance and effects in small mammals upon dietary exposure to a pyraclostrobin-formulation 2012/1129348 Yes Unpublished	Data needed for risk assessment	Yes	BASF
KCP 10.1.3/1	[redacted]	2014	Report in preparation (laboratory test with amphibians) 2013/1375098 Yes Unpublished	New requirement	Yes	BASF
KCP 10.1.3/2	[redacted]	2014	Report in preparation (semi-field test with amphibians) 2013/1375099 Yes Unpublished	New requirement	Yes	BASF
KCP 10.2.1/1	[redacted]	2008 a	BAS 500 06 F - Acute toxicity study with the rainbow trout (Oncorhynchus mykiss) 2008/1018046 [redacted] Yes Unpublished	New representative formulation	Yes	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCP 10.2.1/2	[redacted]	2012 a	BAS 500 06 F - Acute toxicity study in the common carp (Cyprinus carpio) 2012/1250190 [redacted] Yes Unpublished	New data (request of Japanese authorities)	Yes	BASF

3.2.3 Representative formulation BAS 516 07 F

Section 7: Toxicology

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCP 7.1.1/1	[redacted]	2008 a	Acute oral toxicity in rats - Acute toxic class method 2008/1004838 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 7.1.2/1	[redacted]	2001 a	BAS 516 00 F - Acute dermal toxicity study in rats 2001/1003723 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 7.1.3/1	[redacted]	2001 b	BAS 516 00 F - Acute inhalation toxicity study in Wistar rats 4-hour dust exposure 2001/1001824 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 7.1.4/1	[redacted]	2007 a	BAS 516 07 F: Acute dermal irritation / corrosion in rabbits 2007/1056989 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 7.1.5/1	[redacted]	2007 b	BAS 516 07 F: Acute eye irritation in rabbits 2007/1056988 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 7.1.6/1	[redacted]	2014	BAS 516 07 F: Skin Sensitization: Local Lymph Node Assay 2014/1001403 [redacted] Yes Unpublished	New data	Yes	BASF

Section 10: Ecotoxicology

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Justification for submission of study	Data protection claimed Y/N	Owner
KCP 10.1.1.1/ 1	[redacted]	2006 a	BAS 516 07 F - Acute toxicity in the bobwhite quail (Colinus virginianus) after single oral administration (LD50) 2006/1030309 [redacted] Yes Unpublished	New representative formulation	Yes	BASF
KCP 10.2.1/1	[redacted]	2000 a	BAS 516 00 F - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a static system (96 hours) 2000/1018726 [redacted] Yes Unpublished	Data needed for risk assessment	Yes	BASF

3.3 Timetable of any new and ongoing studies

References of documents and study reports that were not finalized on Dec 31st 2013 are given for the active substance pyraclostrobin (BAS 500 F), its metabolites and the representative formulations BAS 500 06 F and BAS 516 07 F.

3.3.1 Active substance pyraclostrobin (BAS 500 F)

Section 2: Physical and chemical properties of the active substance

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 2.4/1	Kroehl T., Doetzer R.	03/2014	Mass, NMR, IR and UV/Vis spectra of dimethylsulfate – Minor component in technical grade pyraclostrobin 2014/1001441 BASF SE Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New requirement	BASF

Section 4: Analytical methods

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 4.1.2/2	Eilers B.	04/2014	Method for the determination of 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS 2014/1001641 No Unpublished	No	Yes	New analytical method supporting dietary risk assessment	BASF
KCA 4.1.2/3	N.N.	04/2014	Validation of the Method for the determination of 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS 2014/1001721 Yes Unpublished	No	Yes	New analytical method supporting dietary risk assessment	BASF
KCA 4.1.2/4	Eilers B.	04/2014	Validation of the analytical method 446/2 (L0058/03) for the determination of BAS 500 F (Reg.No. 304428) and its metabolites 500M04 (Reg.No. 298327) and 500M85 2013/1400972 Yes Unpublished	No	Yes	New analytical method supporting dietary risk assessment	BASF
KCA 4.1.2/8	Tilting N., Sopena-Vazquez F.	03/2014	Validation of analytical method L0166/01: Determination of BAS 500 F (Pyraclostrobin) and its metabolites Reg. No. 364380 (500M01), Reg. No. 369315 (500M02) and Reg No. 340266 (500M07) in soil using LC/MS/MS 2013/1184817 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New requirements	BASF
KCA 4.1.2/10	N.N.	04/2014	Validation of Analytical Method L0182/02 for the Determination of Reg.No.298327 - 500M04, metabolite of BAS 500 F in Water by LC-MS/MS 2014/1004891 Yes Unpublished	No	Yes	New requirements	BASF

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 4.1.2/11	N.N.	04/2014	Independent Laboratory Methods Validation of BASF Method Number L0182/01: Determination of BAS 500 F (Pyraclostrobin) and its metabolites Reg No. 412053 (500M59), Reg No. 411847 (500M60), Reg No. 412785 (500M62), Reg No. 413038 (500M76), and Reg No. 377613 (500M78) in ground-, surface- and tap water by LC-MS/MS 2014/7000022 Yes Unpublished	No	Yes	New requirements	BASF
KCA 4.1.2/12	N.N.	04/2014	Independent Laboratory Validation of Analytical Method L0182/02: Determination of Reg.No.298327 500M04, metabolite of BAS 500 F in Water by LC-MS/MS 2014/7000107 Yes Unpublished	No	Yes	New requirements	BASF

Section 5: Toxicology and metabolism studies - active substance and its metabolites

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 5.1/1	N.N.	04/2014	Comparative In-vitro-metabolism with ^{14}C-BAS 500 F 2014/1001562 Yes Unpublished	No	Yes	New data requirement according to Reg. 283/2013	BASF
KCA 5.3.3/2	[redacted]	07/2014	Supplementary subacute inhalation study in Wistar rats 2014/1003946 [redacted] Yes Unpublished	Yes	Yes	Additional concentrations needed for risk assessment	BASF
KCA 5.8.1/2	Schulz M., Landsiedel R.	03/2014	Reg.No. 78810 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells - (cytokinesis block method) 2013/1361922 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/3	Schulz M., Landsiedel R.	03/2014	Reg.No. 78810 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells (Tk+/- locus assay, microwell version) 2013/1298448 BASF SE, Ludwigshafen/Rhein, Germany Fed. Rep Yes Unpublished	No	Yes	New Data for the risk assessment of metabolites	BASF
KCA 5.8.1/21	Schulz M., Landsiedel R.	03/2014	Reg.No. 5916420 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells (Mixed population method) 2013/1361921 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 5.8.1/22	Schulz M., Landsiedel R.	03/2014	Reg.No. 5916420 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells ($TK^{+/-}$ locus assay, microwell version) 2013/1298447 BASF SE, Ludwigshafen/Rhein, Germany Fed. Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/23	Woitkowiak C.	03/2014	Reg.No. 5916421 (Metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2013/1323364 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/24	Schulz M., Landsiedel R.	03/2014	Reg.No. 5916421 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells (Mixed population method) 2013/1363549 BASF SE, Ludwigshafen/Rhein, Germany Fed. Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/25	Schulz M., Landsiedel R.	06/2014	Reg.No. 5916421 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells ($TK^{+/-}$ locus assay, microwell version) 2013/1298449 BASF SE, Ludwigshafen/Rhein, Germany Fed. Rep Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 5.8.1/26	[redacted]	03/2014	Reg.No. 5916421 (Metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus Assay in Bone Marrow Cells of the Mouse 2013/1389659 [redacted] Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF

Section 6: Residues in or on treated products, food and feed and plant metabolism

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 6.2.1/5	Rabe U.	04/2014	Metabolism of ^{14}C-Pyraclostrobin in rice 2013/1134958 Yes Unpublished	No	Yes	Submission of all metabolism data as agreed with RMS	BASF
KCA 6.2.5/2	[redacted]	04/2014	The Metabolism of ^{14}C-Pyraclostrobin (^{14}C-BAS 500 F) in rainbow trout (Oncorhynchus mykiss) 2014/1001601 Yes Unpublished	Yes	Yes	New data requirement according to Reg. 283/2013	BASF
KCA 6.2.5/3	Madden S.	04/2014	The Comparative Metabolism of [^{14}C]-Pyraclostrobin ([^{14}C]-BAS 500 F) in Rainbow Trout Cryopreserved Hepatocytes and Microsomes 2014/1001602 Yes Unpublished	No	Yes	Supplemental information supporting metabolism behaviour in fish	BASF
KCA 6.3.3/2	Aitken A.	04/2014	Study on the Residue Behaviour of Pyraclostrobin (BAS 500 F) in Maize Following One Application of BAS 500 06 F to 1 Trial in 2013 – NEU 2014/1001741 Yes Unpublished	No	Yes	Residue study with representative use	BASF
KCA 6.3.4/1	Courtois J.	04/2014	Determination of 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS 2014/1001661 Yes Unpublished	No	Yes	Residue analysis supporting dietary risk assessment	BASF
KCA 6.6.1/1	Rabe U.	04/2014	Confined Rotational Crop Study with ^{14}C Pyraclostrobin 2014/1001761 Yes Unpublished	No	Yes	New study addressing formation of metabolites	BASF

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 6.9/1	Bross M.	04/2014	Pyraclostrobin (BAS 500 F) - Refinement of the dietary exposure assessment of pyraclostrobin metabolites in plant and animal commodities 2014/1001541 No Unpublished	No	Yes	New evaluation	BASF

Section 7: Fate and behaviour in the environment

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 7.1.1.1/1	Hassink J.	02/2014	Metabolism of (pyrazole-3-C14) BAS 500 F in soil under aerobic conditions 2013/1348620 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Additional data (information on third label)	BASF
KCA 7.1.1.3/1	Hassink J.	02/2014	Soil photolysis of (pyrazole-3-C14) BAS 500 F 2013/1348621 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Additional data (information on third label)	BASF
KCA 7.1.2.1.2/3	N.N.	04/2014	Rate of degradation of BF 500-4 2013/1294779 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New data requirement	BASF
KCA 7.1.2.1.2/4	N.N.	04/2014	Rate of degradation of BF 500-5 2013/1294780 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New data requirement	BASF
KCA 7.1.2.1.4/1	Eickler B.	01/2014	Kinetic evaluation of anaerobic soil degradation studies for BAS 500 F – pyraclostrobin according to FOCUS 2014/1000701 BASF SE, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New guidance for kinetic evaluation	BASF
KCA 7.1.2.2.1/2	Bayer H.	01/2014	Field soil dissipation study of BAS 500 F (Pyraclostrobin) in the formulation BAS 500 14 F on bare soil at four different sites in Europe, 2011-2012 2013/1348661 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	New guideline	BASF

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 7.1.2.2.1/3	Tilting N.	04/2014	Stability of residues of BAS 500 F (Pyraclostrobin, Reg. No 304428) and its metabolites M500F01 (Reg. No. 364380), M500F02 (Reg.No. 369315) and M500F07 (Reg.No. 340266) in various soils 2014/1000723 Yes Unpublished	No	Yes	New guideline	BASF
KCA 7.1.3.1.2/1	N.N.	02/2014	Study on the adsorption behaviour of BF 500-6 (Reg.No. 364380) on different soils 2014/1000624 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Old study insufficient	BASF
KCA 7.1.3.1.2/2	N.N.	03/2014	Study on the adsorption behaviour of BF 500-7 (Reg.No. 369315) on different soils 2014/1000625 BASF SE, Limburgerhof, Germany Fed.Rep. Yes Unpublished	No	Yes	Old study insufficient	BASF
KCA 7.1.3.1.2/3	N.N.	02/2014	Adsorption/desorption of BF 500-4 (Reg.No. 358672) on soil 2014/1000721 Yes Unpublished	No	Yes	New data requirement	BASF
KCA 7.1.3.1.2/4	N.N.	02/2014	Adsorption/desorption of BF 500-5 (Reg.No. 298327) on soil 2014/1000722 Yes Unpublished	No	Yes	New data requirement	BASF
KCA 7.3.1/1	Hassink J.	04/2014	Photochemical oxidative degradation of BAS 500 F (QSAR estimates) 2013/1350648 BASF SE, Limburgerhof, Germany Fed.Rep. No Unpublished	No	No	New calculation according newest model version	BASF

Section 8: Ecotoxicology - active substance and its metabolites

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 8.1.1.1/1	[redacted]	03/2014	BAS 500 F - Acute Toxicity in the Canary after single oral administration 2013/1400375 [redacted] Yes Unpublished	Yes	Yes	New data (request of US-EPA)	BASF
KCA 8.2.1/5	N.N.	03/2014	study in preparation (Acute fish (trout) study with pyraclostrobin metabolite BF 500-5) 2013/1308543 Yes Unpublished	Yes	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.2.4.1/2	N.N.	02/2014	study in preparation (acute Daphnia study with pyraclostrobin metabolite BF 500-5) 2013/1308542 Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.2.5.3/3	N.N.	04/2014	study in preparation (chronic Chironomus study with pyraclostrobin metabolite BF 500-6) 2014/1001481 Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.2.5.3/4	N.N.	04/2014	study in preparation (chronic Chironomus study with pyraclostrobin metabolite BF 500-7) 2014/1001482 Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.2.6.1/4	N.N.	02/2014	study in preparation (algae study with pyraclostrobin metabolite BF 500-5) 2013/1349201 Yes Unpublished	No	Yes	New data for the risk assessment of metabolites	BASF
KCA 8.3.1/1	N.N.	03/2014	study in preparation (residues in honey bee feed items) 2014/1000204 Yes Unpublished Unpublished	No	Yes	New data requirement	BASF

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCA 8.3.1/2	N.N.	03/2014	study in preparation (residues in honey bee feed items) 2014/1000182 Yes Unpublished	No	Yes	New data requirement	BASF
KCA 8.3.1.2/1	N.N.	04/2014	study in preparation (chronic honey bee study with BAS 500 06 F) 2013/1235056 Yes Unpublished	No	Yes	New data requirement	BASF
KCA 8.4.1/1	N.N.	04/2014	study in preparation (earthworm reproduction study with pyraclostrobin) 2014/1000461 Yes Unpublished	No	Yes	New data for earthworm risk assessment	BASF

3.3.2 Representative formulation BAS 500 06 F

Section 7: Toxicology

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 7.3/1	Fabian E., Landsiedel R	06/2014	^{14}C-BAS 500 F in BAS 500 06 F - Study of penetration through human skin in vitro 2014/1001501 BASF SE, Ludwigshafen/Rhein, Germany Fed. Rep. Yes Unpublished	No	Yes	New Data	BASF

Section 9: Fate and behaviour in the environment

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 9.1.3/1	Eickler B.	04/2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	new exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.2.4/1	Eickler B.	04/2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	new exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.2.5/1	Eickler B.	04/2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	new exposure assessments according to newest guidelines and guidance documents	BASF

Section 10: Ecotoxicology

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.1.2.2/1	N.N.	04/2014	Field study acute and long-term effects-BAS 500 06 F small mammals in Central Europe 2014/1000041 Yes Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2.2/2	N.N.	04/2014	Study to assess avoidance and effects in small mammals upon dietary exposure to a pyraclostrobin-formulation - 2012/1129348 Yes Unpublished	Yes	Yes	Data needed for risk assessment	BASF
KCP 10.1.2.2/5	Mastitsky S.E.	04/2014	BAS 500F: Body-burden modelling to assess the acute risk of formulated pyraclostrobin to small mammals 2014/1001603 No Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2.2/6	N.N.	04/2014	BAS 500 06 F: Acute risk to mammals of the formulated product 2014/1010735 No Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.2.2/7	N.N.	04/2014	BAS 500 F - Pyraclostrobin: Ecologically relevant chronic toxicity endpoint for the wild mammal reproductive risk assessment (Ecotoxicology) 2014/1010736 No Unpublished	No	Yes	Data needed for risk assessment	BASF
KCP 10.1.3/1	[redacted]	03/2014	Report in preparation (laboratory test with amphibians) 2013/1375098 Yes Unpublished	Yes	Yes	New requirement	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.1.3/2	[redacted]	04/2014	Report in preparation (semi-field test with amphibians) 2013/1375099 Yes Unpublished	Yes	Yes	New requirement	BASF

3.3.3 Representative formulation BAS 516 07 F

Section 7: Toxicology

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 7.1.6/1	[redacted]	05/2014	BAS 516 07 F: Skin Sensitization: Local Lymph Node Assay 2014/1001403 [redacted] Yes Unpublished	Yes	Yes	New data	BASF
KCP 7.3/1	Hassler S.	06/2014	BAS 510 F: Percutaneous Penetration of ^{14}C BAS 510 F formulated as BAS 516 07 F through human split-thickness skin membranes (in-vitro) 2014/1001401 Harlan Laboratories Ltd., Itingen, Switzerland Yes Unpublished	No	Yes	New data	BASF
KCP 7.3/2	Hassler S	06/2014	BAS 500 F: Percutaneous Penetration of ^{14}C BAS 500 F formulated as BAS 516 07 F through human split-thickness skin membranes (in-vitro) 2014/1001401 Harlan Laboratories Ltd., Itingen, Switzerland Yes Unpublished	No	Yes	New data	BASF

Section 9: Fate and behaviour in the environment

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 9.1.3/1	Eickler B.	04/2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	new exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.1.3/2	Eickler B.	04/2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of boscalid after application of BAS 516 07 F to potatoes 2014/1001461 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.2.4/1	Eickler B.	04/2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	new exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.2.4/2	Eickler B.	04/2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of boscalid after application of BAS 516 07 F to potatoes 2014/1001461 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.2.5/1	Eickler B.	04/2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of pyraclostrobin and its metabolites after application of its representative formulations 2014/1000685 No Unpublished	No	No	new exposure assessments according to newest guidelines and guidance documents	BASF
KCP 9.2.5/2	Eickler B.	04/2014	Predicted environmental concentrations in soil, groundwater, surface water and sediment of boscalid after application of BAS 516 07 F to potatoes 2014/1001461 No Unpublished	No	No	New exposure assessments according to newest guidelines and guidance documents	BASF

Section 10: Ecotoxicology

Data point	Author(s)	Estimated date for final report	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification for submission of study	Owner
KCP 10.4.2/2	N.N.	05/2014	study in preparation (effects on reproduction of soil mites Hypoaspis aculeifer) 2014/1010834 Yes Unpublished	No	Yes	New representa-tive formulation	BASF
KCP 10.6.2/2	N.N.	04/2014	study in preparation (vegetative vigor of non-target plants) 2014/1010836 Yes Unpublished	No	Yes	Old study has no analytic included	BASF
KCP 10.6.2/3	N.N.	04/2014	study in preparation (seedling emergence of non-target plants) 2014/1010835 Yes Unpublished	No	Yes	New representa-tive formulation	BASF

The applicant confirms that the above information included in the application is correct.

Limburgerhof, 22nd January 2014

[redacted]

[redacted]

[redacted]

[redacted]

APPLICATION FOR RENEWAL OF
THE ACTIVE SUBSTANCE

PYRACLOSTROBIN

according to Annex of Regulation (EU) No. 844/2012

NEW INFORMATION ACCORDING TO SANCO/2012/11251 rev. 1.2

BASF DocID 2013/1401475

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22 January 2014

Table of Contents

1 BACKGROUND

Pyraclostrobin is a broad spectrum fungicide, which is active against fungal development stages both on the plant surface and within the tissues. It has a protective as well as an eradicative/curative action. Pyraclostrobin belongs to the group of active substances which is collectively known as strobilurins. The biochemical mode of action of the strobilurins is the inhibition of mitochondrial respiration.
In addition to the fungicidal effects plant physiology is also affected by the application of pyraclostrobin. Among these effects higher yield and better product quality in absence of diseases, improvement of the assimilation rate and delayed senescence have been reported. Furthermore, some studies showed better stress tolerance to abiotic stresses (e.g. drought or frost).
Pyraclostrobin is used world-wide in a large variety of crops including cereals, oilseeds, legumes, fruits and vegetables.

BASF submitted a dossier to include pyraclostrobin in Annex I on 28 February 2000. The Rapporteur Member State (RMS) was Germany.

Monograph and Addenda

Monograph
Pyraclostrobin, 1 August 2001

1st Addendum
C.1 Confidential information, 21 May 2003, 6 pages, 3 references

2nd Addendum
B.6 Toxicology and metabolism, 25 June 2003, 13 pages, 8 references

3rd Addendum
B.7 Residue data, 14 October 2003, 16 pages, 6 references

4th Addendum
B.4 Proposals for the classification and labelling and
B.6.6.2 Developmental toxicity, 18 December 2003, 3 pages, no reference list

5th Addendum
C.1 Addendum Confidential information, 12 February 2004, 5 pages, no reference list

Pyraclostrobin was included in Annex I of Directive 91/414/EEC on 1 June 2004 (entry into force) under Inclusion Directive 2004/30/EC for the use as fungicide. This use was amended on 22 April 2009 (entry into force) by Inclusion Directive 2009/25/EC to the use as fungicide or plant growth regulator. Both Annex I inclusion decisions were based on the same endpoints and provisions.

The Review Report (Pyraclostrobin - SANCO/1420/2001-Final) is dated 08 September 2004 and provides endpoints agreed during first inclusion evaluation (Appendix II to the Review Report).

Member States were required to pay particular attention to the following areas:
- should pay particular attention to the protection of aquatic organisms, especially fish,
- should pay particular attention to the protection of terrestrial arthropods and earthworms

Due to the improvement of physical and chemical properties, the representative formulation will be changed to a 200 g/L EC solo formulation (coded BAS 500 06 F) representing the use in important and widely grown “row crops” (cereals and maize). In addition a second (new) representative formulation (coded BAS 516 07 F and containing boscalid as second active substance) has been chosen to represent the many uses in “specialty crops” were typically lower application rates are used.

The intended use patterns (GAPs) of the representative crops for which data and risk assessments will be provided in the supplemental dossier are already registered in several EU Member States for BAS 500 06 F and BAS 516 07 F. Details of the use patterns are shown in the Appendix of this document.

List of endpoints agreed at approval (Annex I inclusion) and proposed changes, by scientific area:

Toxicology and metabolism

The endpoints as given in the EU Review Report of pyraclostrobin in 2004 are listed in the tables below. In case new information on certain endpoints is available, a comment is made in the additional right table column. Further explanations are given in the text below the table.

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of absorption:	About rapid absorption: Tmax ~ 1 hour: 50% (based on urinary and biliary excretion within 48 hours)	
Distribution:	Widely, highest concentrations in the liver	
Potential for accumulation:	None	
Rate and extent of excretion:	Complete within 5 d; mainly via faeces (80-90%, biliary excretion amounting to 35%), via urine 11-15%	
Toxicologically significant compounds:	Parent compound and metabolites	
Metabolism in animals:	Extensive (>95%) with nearly 50 metabolites occurring Main metabolic pathways included N-demethoxylation, hydroxylation, cleavage of ester bond and further oxidation of the resulting molecule parts, conjugation with glucoronic acid or sulphate	

Acute toxicity

Rat LD_{50} oral:	> 5000 mg/kg bw (Mouse: mortality at ≥ 300 mg/kg bw)	
Rat LD_{50} dermal:	> 2000 mg/kg bw	
Rat LC_{50} inhalation:	0.69 mg/L	0.58 mg/L (see comment below)
Skin irritation:	Irritating	
Eye irritation:	Not irritating	
Skin sensitisation (test method used and result):	Not sensitising (M&K maximisation test)	

Short term toxicity

Target / critical effect:	Reduced body weight, gastrointestinal tract, red blood cells, diarrhoea (dog), hepatocellular hypertrophy (rat), white blood cells and lymphatic organs (mouse)	
Lowest relevant oral NOAEL / NOEL:	90-day mouse[3]: 30 ppm (4 mg/kg bw/d)	
Lowest relevant dermal NOAEL / NOEL:	28-day rat: > 250 mg/kg bw/d (systemic)	
Lowest relevant inhalation NOAEL / NOEL:	No data - not required (because of physical and chemical properties)	New data available, respectively in the process of generation (see below)

[3] based on effects on body weight after 90 days in the carcinogenicity study in male mice

Genotoxicity

No genotoxic potential	

Long term toxicity and carcinogenicity

Target / critical effect:	Reduced body weight (rat & mouse); liver cell necrosis (rat)	
Lowest relevant NOAEL:	Chronic rat (75 ppm) 3 mg/kg bw/d	
Carcinogenicity:	No carcinogenic potential	

Reproductive toxicity

Target / critical effect - Reproduction:	Reduced pup body weight gain in the presence of parental toxicity	
Lowest relevant reproductive NOAEL / NOEL:	75 ppm (8.2 mg/kg bw/d)	
Target / critical effect - Developmental toxicity:	Developmental effects in rats and embryotoxicity (including malformations) in rabbits at maternally toxic doses	
Lowest relevant developmental NOAEL / NOEL:	5 mg/kg bw/d (rabbit)	
Lowest relevant maternal NOAEL / NOEL:	3 mg/kg bw/d (rabbit)	

Delayed neurotoxicity

No neurotoxic potential (rat, acute and 13wk studies)	

Other toxicological studies

Three water metabolites (BF 500-11, BF 500-13, BF 500-14) proved negative in the Ames test	New data available (see below)

Medical data

Limited data (new compound); no human health problems identified	After around 10 years of production, literature and company surveys did not identify human health problems.

Summary

	Value	Study	Safety factor	
ADI:	0.03 mg/kg bw	Chronic rat study	100	
AOEL systemic:	0.015 mg/kg bw	Rabbit, developmental toxicity study (maternal toxicity); 50 % oral absorption	100	
ARfD (acute reference dose):	0.03 mg/kg bw	Rabbit, developmental toxicity study (maternal toxicity)	100	

Dermal absorption

EC formulation: 2.6% (rat, in vivo); in vitro data suggest much lower permeability of human skin; 1% used for calculation based on in vitro/in vivo data	new representative formulations, studies ongoing (see below)

Toxicology and metabolism – comments to endpoints

Acute inhalation toxicity
A new acute inhalation toxicity study with pyraclostrobin dissolved in acetone was conducted for a more accurate determination of the LC_{50}. This study was performed at actual concentrations of 0.52, 0.65 and 0.85 mg/L. This study revealed a LC_{50} of 0.58 mg/L.

Subchronic inhalation toxicity
A 28-day inhalation toxicity study was performend upon request of US EPA at concentrations of 0, 1, 30 and 300 mg/m³. A 28-day inhalation study with intermediate dose levels (3 and 10 mg/m³) is currently conducted.

Other toxicological studies
Additional in-vitro and in-vivo mutagenicity studies were performed for the water metabolites BF 500-11, BF500-13 and BF 500-14. Furthermore, in-vivo and/or in-vitro mutagenicity studies were conducted for the metabolites BF 500-5 (synonym: 500M04), 500M24, 500M49 and 500M51. For all mentioned metabolites the weight of evidence showed the absence of a mutagenic potential.
For the metabolite BF 500-5 (500M04) a set of acute toxicity studies as well as a subchronic 90-day study in rats is available. All studies indicate that the metabolite has a lower toxicological potential than pyraclostrobin.

Dermal absorption
While the dermal penetration values were derived from studies with the former representative formulation BAS 500 00 F, new in-vitro dermal penetration studies with human skin are currently conducted. The studies will be evaluated according to the latest EFSA Guidance Document on dermal penetration.

Fate and behaviour in the environment

The endpoints as given in the EU Review Report of pyraclostrobin in 2004 are listed in the tables below. Endpoints for risk assessments which were used up to now and which differed from the EU Review Report are presented below in the third table column. Since the endpoints which will be derived from the new studies are not finally known yet, an indication on possible changes for soil, water, and air assessments is given in the text below the respective table sections.

Fate and behaviour in soil

Route of degradation

Aerobic

Mineralization after 100 days:	4% after 87 d (tolyl-label, route study) 5% after 91 d (chlorophenyl-label, route study)	
Non-extractable residues after 100 days:	54.3% after 87 d (tolyl-label, route study) 56.1% after 91 d (chlorophenyl-label, route study)	
Major metabolites above 10 % of applied active substance: name and/or code % of applied rate (range and maximum)	BF 500-6, max. 31% after 120 days (rate studies) BF 500-7, max. 13% after 62 days (rate studies)	

Supplemental studies

Anaerobic:	no residues of the parent after 120 days, bound residues: 61% (tolyl-label), 37% (chlorophenyl-label). Major metabolite BF 500-3: max 95.8% after 14 d (tolyl-label), 80% after 14 d (chlorophenyl-label)	
Soil photolysis:	after 15 days: 64-74% parent, 12% bound residues, 2% CO_2, no major metabolites (>10%)	
Remarks:	degradation in soil is mainly depending on microbial activity	

Rate of degradation

Laboratory studies

DT_{50lab} (20 °C, aerobic):	DT_{50lab} **as** (20°C, aerobic): 12-101 days (5 soils) DT_{50lab} BF 500-6 (tolyl-label, route study): 129 d DT_{50lab} BF 500-6 (chlorphenyl-label, route study): 166 d DT_{50lab} BF 500-7 (tolyl-label, route study): 112 d DT_{50lab} BF 500-7 (chlorphenyl-label, route study): 159 d	
DT_{90lab} (20 °C, aerobic):	DT_{90lab} **as** (20°C, aerobic): 143-163 days (5 soils) DT_{90lab} BF 500-6 (tolyl-label, route study): 428 d DT_{90lab} BF 500-6 (chlorphenyl-label, route study): 552 d DT_{90lab} BF 500-7 (tolyl-label, route study): 372 d DT_{90lab} BF 500-7 (chlorphenyl-label, route study): 529 d	
DT_{50lab} (10 °C, aerobic):	DT_{50lab} (5°C, aerobic): > 120 days	
DT_{50lab} (20 °C, anaerobic):	DT_{50lab} (20°C, anaerobic): 3 days	

Field studies (country or region)

DT_{50f} from soil dissipation studies:	DT_{50f}: 8 – 55 days, 6 locations (3 Germany, 2 Spain, 1 Sweden)	error in Review Report; highest observed DT_{50} in peer reviewed field diss. studies = 37 days
DT_{90f} from soil dissipation studies:	DT_{90f} : 83-230 days	
Soil accumulation studies:	not required	
Soil residue studies:	not required	

Remarks

e.g. effect of soil pH on degradation rate	no pH-effect, degradation in soil mainly depending on microbial activity, metabolites BF 500-6 and BF 500-7 were not found in field studies	

Adsorption/desorption

	Soils: 3 German, 2 US, 1 Canadian		
K_f / K_{oc}:	K_{oc}	Active substance (^{14}C-Chlorphenol-ring) 6000 – 16000 (no average value calculated because of high variance)	
K_d	K_d	30 – 368	
	1/n	0.861 – 1.025	
pH dependence:	none		

BF 500-6	K_{oc}	3160 – 71300	
	K_d	79 – 610	
	1/n	not available. Due to low water solubility only one concentration considered.	

BF 500-7	K_{oc}	3920 – 147600	
	K_d	98 – 738	
	1/n	not available. Due to low water solubility only one concentration considered.	

Mobility

Laboratory studies

Column leaching:	0% in leachate, all radioactivity in top soil layer	
Aged residue leaching:	0% in leachate, all radioactivity in top soil layer	

Field studies

Lysimeter/Field leaching studies:	based on K_{oc} and DT_{50} values, no leaching expected Studies are not required.	

Remarks

None	

Fate and behaviour in soil – comments to endpoints

Concerning route of degradation in soil, an aerobic soil metabolism study as well as a soil photolysis study was performed with the up to now not investigated ^{14}C-pyrazole-label of pyraclostrobin. This was done to confirm that no additional metabolite consisting solely of the pyrazole- moiety is formed during soil degradation although the chlorophenyl-label and tolyl-label produced high amounts of bound residues and also showed mineralization. No new metabolites occured with the pyrazole-label and thus the route of degradation as proposed in the original dossier is confirmed.

New DT_{50} endpoints in aerobic soil under laboratory conditions are being generated for parent, for the minor metabolite BF 500-3, and for the major soil metabolites BF 500-6 and BF 500-7. New DT_{50} endpoints in soil will be also produced for metabolites BF 500-4 and BF 500-5 which appear in measurable amounts only under strict anaerobic soil conditions, but which need now to be considered for risk assessment under Regulation 1107/2009.

A new field dissipation study addresses the degradation behaviour of pyraclostrobin in soil according to the new requirements of 1107/2009, i.e. dissipation processes like photolysis are excluded. New endpoints are expected.

New adsorption values will be generated for metabolites BF 500-6 and BF 500-7 as well as for BF 500-4 and BF 500-5.

Fate and behaviour in water

Abiotic degradation

Hydrolytic degradation:	pH 5 – 9, 25°C: stable	
Major metabolites:		
Photolytic degradation:	DT_{50}: 1 – 2 days	
Major metabolites:	not determined	

Biological degradation

Readily biodegradable:	not readily biodegradable	
Water/sediment study: DT_{50} water: DT_{90} water: DT_{50} whole system: DT_{90} whole system: DT_{50} water: DT_{50} whole system: DT_{50} sediment: DT_{90} sediment:	best fit pond: 3 days; river: 1 day pond: 41 days; river: 9 days pond: 27 days; river: 29 days** pond: 89 days; river: 96 days** ** = low r^2 value (0.5593) 1st-order pond: 8.7 days; river: 1 day pond: 29 days; river: not extrap. pond: 33 days; river: 9 days pond: 105 days; river: no calc. possible	
Distribution in water / sediment systems (active substance)	pond system: sediment max. 53% after 14 days, decreasing to 7% after 100 days river system: sediment max. 62% after 2 days, decreasing to 10% after 100 days	
(metabolites)	BF 500-3: in water: max. 2% in sediment: max. 12% after 100 days (pond); max. 66% after 14 days (river), decreasing to 29% after 100 days BF 500-6: (only in pond system) in sediment max. 7% after 61 days BF 500-7: (only in pond system) in sediment max. 6% after 61 days	
Mineralisation	0.7 – 7.5 % in 100 days	
Bound residues	51 – 66.2 % in 100 days	
Accumulation in water and/or sediment:	not expected, only bound residues	
Metabolites from additional water/sediment study with irradiation:	BF 500-11: max. 11% after 21 days BF 500-13: max. 16% after 62 days BF 500-14: max. 11% after 10 days	

Degradation in the saturated zone

not relevant	

Remarks

results from water/sediment study with irradiation only useful as additional information concerning possible metabolites, not accepted as higher tier study for the determination of degradation rates	

Fate and behaviour in water – comments to endpoints

Since irradiation is an important degradation/dissipation route for pyraclostrobin in water, a new irradiated water/sediment study including full material balance was performed. The route of degradation in surface waters was confirmed. In addition, an aerobic mineralization study according to guideline OECD 309, Aerobic Mineralization in Surface Water, was performed due to the new data requirement in regulation 1107/2009. The only metabolite formed was the BF 500-5 (max. 7-8%), a hydrolytic cleavage product, which will be considered for aquatic risk assessment.

Fate and behaviour in air

Volatility

Vapour pressure:	2.6 x 10^{-8} Pa, 20°C	
Henry's law constant:	5.307 x 10^{-6} Pa m^3 mol^{-1}	

Photolytic degradation

Direct photolysis in air:	see photochemical oxidative degradation	
Photochemical oxidative degradation in air DT_{50}:	< 2 hours (24- hours day, according to Atkinson, AOP)	
Volatilisation:	from plant surfaces: about 3% in 24 hours	

Remarks

no short or long range transport in air expected	

Fate and behaviour in air – comments to endpoints

No new experimental data will be generated. A new Atkinson calculation will be performed according to the newest calculation procedure.

Ecotoxicology

A significant number of new studies was performed following Annex I inclusion. These studies were conducted
a) to support registrations outside the EU,
b) to fulfill new data requirements,
c) to cover further metabolites identified as potentially relevant in additional e-fate investigations,
d) to address the risk assessment for the new representative formulations.

The endpoints given in the Review Report from 2004 are shown in the tables below. Comments are made in the last column as appropriate. All additional study endpoints not previously evaluated during the Annex I inclusion process are presented in the tables as well (endpoint values are given in the last column). Further comments on possible changes for endpoints and risk assessments are given below the tables if appropriate.

The test substance is coded "as" for pyraclostrobin, "BAS … F" for the representative formulations and "BF 500 -.." for the metabolites.

Terrestrial vertebrates

Acute toxicity to mammals:	LD_{50} > 5000 mg/kg bw (rat)	
Acute toxicity to birds:	LD_{50} > 2000 mg/kg bw (bobwhite quail)	
	LD_{50} (canary)	Study ongoing
Dietary toxicity to birds:	LC_{50} > 5000 ppm (bobwhite quail and mallard duck) LC_{50} > 1176 mg/kg bw/d (bobwhite quail) LC_{50} > 1320 mg/kg bw/d (mallard duck)	No longer part of the core data package
Reproductive toxicity to birds:	NOEL 1000 ppm (bobwhite quail and mallard duck) NOEL 105 mg/kg bw/d (bobwhite quail) NOEL 128 mg/kg bw/d (mallard duck)	
Reproductive toxicity to mammals:		Tier 1: NOEL = 3 mg as/kg bw/d (rabbit, developmental toxicity) Higher tier: NOEL = 8.2 mg as/kg bw/d (rat, two-generation study)

Aquatic organisms

	Group	Test substance	Timescale	Endpoint	Toxicity (mg/L)	New Information / comments
Acute toxicity fish:	*Oncorhyn-chus mykiss*	as	96 h, static	LC_{50}	0.006	0.00616 is the correct value, which was rounded in the Review Report to 0.006
	O. mykiss	as	96 h, flow-through	LC_{50}		0.0062 (study performed as flow-through test for US registration)
	Lepomis macrochirus	as	96 h, flow-through	LC_{50}		0.0114 (study performed as flow-through test for US registration)
	Cyprinodon variegatus	as	96 h, flow-through	LC_{50}		0.0769 (study for US salt water/ estuarine risk assessment, not required in the EU)
	O. mykiss	BAS 500 00 F	96 h, static	LC_{50}	0.017	old representative formulation (given for completeness)
	O. mykiss	BAS 500 06 F	96 h, static	LC_{50}		0.046 new representative formulation
	Cyprinus carpio	BAS 500 06 F	96 h, static	LC_{50}		0.098 new representative formulation (study for registration in Japan)
	O. mykiss	BAS 516 07 F *	96 h, static	LC_{50}		0.088 new representative formulation
	O. mykiss	BF 500-13	96 h, static	LC_{50}	> 50 - < 100	
	O. mykiss	BF 500-14	96 h, static	LC_{50}	> 39 - < 83	
	O. mykiss	BF 500-11	96 h, static	LC_{50}	> 100	
	O. mykiss	BF 500-3	96 h, static	LC_{50}		> 0.095 soil/sediment metabolite (study for registration in Canada)
	O. mykiss	BF 500-5	96 h, static	LC_{50}		study ongoing (to address an additional aquatic metabolite)

Long term toxicity fish:	*O. mykiss*	as	28 d, flow-through	NOEC	0.005	
	O. mykiss	as	98 d, ELS flow-through	NOEC	0.002	0.002349 is the correct value, which was rounded to 0.0023 (study summary and DAR) and 0.002 (Review Report)
	Pimephales promelas	as	36 d, ELS flow-through	NOEC		0.00414 (study for US registration)
	Cyprinodon variegatus	as	36 d, ELS flow-through	NOEC		0.0108 (study for US salt water/ estuarine risk assessment, not required in the EU)
Bioaccumulation fish:	675 (whole fish, chlorophenyl label) 736 (whole fish, tolyl label)					
Acute toxicity invertebrate:	*Daphnia magna*	as	48 h, static	EC_{50}	0.016	
	Americamysis bahia	as	96 h, flow-through	LC_{50}		0.00416 (study for US salt water/ estuarine risk assessment, not required in the EU)
	Crassostrea virginica	as	96 h, flow-through	LC_{50}		0.0125 (study for US salt water/ estuarine risk assessment, not required in the EU)
	D. magna	BAS 500 00 F	48 h, static	EC_{50}	0.065	old representative formulation (given for completeness)
	D. magna	BAS 500 06 F	48 h, static	EC_{50}		0.065 new representative formulation
	D. magna	BAS 516 07 F	48 h, static	EC_{50}		0.210 new representative formulation
	D. magna	BF 500-13	48 h, static	EC_{50}	> 100	
	D. magna	BF 500-14	48 h, static	EC_{50}	61	
	D. magna	BF 500-11	48 h, static	EC_{50}	> 100	
	D. magna	BF 500-3	48 h, static	EC_{50}		> 0.100 soil/sediment metabolite (study for registration in Canada)
	D. magna	BF 500-5	48 h, static	EC_{50}		study ongoing (to address an additional aquatic metabolite)

Chronic toxicity invertebrate:	*D. magna*	as	21 d, semi-static	NOEC	0.004	
	A. bahia	as	31 d, flow-through	NOEC		0.00128 (study for US salt water/ estuarine risk assessment, not required in the EU)
Acute toxicity algae:	*Pseudokirch-neriella subcapitata*	as	72 h, static	E_rC_{50} E_bC_{50}	> 0.843 0.152	E_bC_{50} = 0.148 (based on recalculation of endpoints)
	Navicula pelliculosa	as	120 h, static	E_rC_{50} E_bC_{50}		> 0.0184 0.0015 (study for US registration, not required in the EU)
	Anabaena flos-aquae	as	120 h, static	E_rC_{50} E_bC_{50}		> 1.78 > 1.78 (study for US registration, not required in the EU)
	Skeletonema costatum	as	120 h, static	E_rC_{50} E_bC_{50}		> 0.159 0.0647 (study for US salt water/ estuarine risk assessment, not required in the EU)
	P. subcapitata	BAS 500 00F	96 h, static	E_rC_{50} E_bC_{50}	3.32 1.370	old representative formulation (given for completeness)
	P. subcapitata	BAS 500 06 F	72 h, static	E_rC_{50} E_bC_{50}		14.2 2.4 new representative formulation
	P. subcapitata	BAS 516 07 F	72 h, static	E_rC_{50} E_bC_{50}		10.8 3.0 new representative formulation
	Scendedsmus subspicatus	BF 500-13	72 h, static	E_rC_{50} E_bC_{50}	> 100 66	
	S. subspicatus	BF 500-14	72 h, static	E_rC_{50} E_bC_{50}	> 100 46	
	P. subcapitata	BF 500-11	72 h, static	E_rC_{50} E_bC_{50}	> 100	
	S. subspicatus	BF 500-3	72 h, static	E_rC_{50} E_bC_{50}		> 1.172 > 1.172 soil/sediment metabolite (study for registration in Canada)
	P. subcapitata	BF 500-5	72 h, static	E_rC_{50} E_bC_{50}		study ongoing (to address an additional aquatic metabolite)
Acute toxicity	*Lemna gibba*	as	14 d, static	EC_{50}		1.72

aquatic plants:				(dry weight)		(study for US registration, not required in the EU)
Chronic toxicity sediment dwelling organism:	*Chironomus riparius*	as	28 d, spiked water	NOEC	0.040	
	C. riparius	as	28 d, spiked sediment	NOEC		> 2.4 mg/kg dry sediment (new study for risk assessment)
	Leptocheirus plumulosus	as	10 d, spiked sediment	LC_{50}		4.412 mg/kg dry sediment (study for US registration, not required in the EU)
	C. riparius	BF 500-3	28 d, spiked sediment	NOEC		≥ 16.0 mg/kg dry sediment (new study for risk assessment)
	C. riparius	BF 500-6	spiked sediment	NOEC		study ongoing (for sediment risk assessment)
	C. riparius	BF 500-7	spiked sediment	NOEC		study ongoing (for sediment risk assessment)
Activated sludge:		as	0.5 h, static	EC_{20}	> 1000	
Mesocosm: #	ecosystem	BAS 500 00 F	c. 6 month, multiple applica-tions	NOEC		8 Study evaluated by RMS, but not included in the Review Report

* Study was conducted with the minor change formulation BAS 516 00 F.

\# A mesocosm study was conducted with the formulated product BAS 500 00 F and was evaluated according to current requirements. Four concentration levels ranging from 0.9 µg as/L to 24 µg as/L simulating a vineyard situation with 8 applications in 14 d intervals were investigated. Approximately 260 different taxa of aquatic invertebrates were determined in the study. In most cases only insignifcant transient effects were observed at the highest concentrations and affected populations usually recovered until the end of the study. The NOEAEC (No Observed Ecologically Adverse Effect Concentration) was determined to be > 8 < 24 µg as/L and the NOEC was 8 µg as/L.

Aquatic organisms – comments to endpoints

A significant number of new studies has been generated. However, the new data are largely in line with previous results and will not significantly change the overall aquatic risk assessment. This is also true regarding the new endpoints for the new representative formulations. The new endpoints for additional metabolites tested confirm the low risk of these metabolites, too. There are several new endpoints for marine organisms, which are partly lower (e.g. for mysids) than available data or which are higher or in line (e.g. fish data) with previous endpoints. However, the extensive information available for fresh water organisms including a mesocosm study with a wide range of taxa and more realistic exposure conditions provide the relevant information for the risk assessment of edge of field freshwater ecosystems.

Honeybees

Acute oral toxicity:	LD_{50} > 73.1 µg as/bee	LD_{50} > 110.0 µg as/bee [1)] New representative formulations: BAS 500 06 F: LD_{50} = 381.8 µg/bee BAS 516 07 F: LD_{50} > 258.7 µg/bee
Acute contact toxicity:	LD_{50} > 100 µg as/bee	LD_{50} > 100.0 µg as/bee [1)] New representative formulations: BAS 500 06 F: LD_{50} = 368.2 µg/bee BAS 516 07 F: LD_{50} > 299.4 µg/bee
Chronic toxicity:		Study with as ongoing
Semi-field cage test:		No unacceptable lethal or sublethal effects on honeybee colonies and bee brood development were found up to 1.25 L BAS 500 06 F/ha (250 g as/ha)

[1)] new study since old study showed weakness with respect to reference compound

Other arthropod species

In the table below the data for the previous representative formulation BAS 500 00 F as given in the EU Review Report are shown for completeness and to allow for comparison with the data of the new representative formulations BAS 500 06 F and BAS 516 07 F, which are shown below in a separate table.

Test species	Test substance	Dose (kg as/ha)	Endpoint	Effect %
T. pyri (protonymphs)	BAS 500 00 F	0.320	Mortality / Reproduction	47.3 / 99
A. rhopalosiphi (adults)	BAS 500 00 F	0.320	Mortality / Reproduction	30 / 80
A. rhopalosiphi (adults, extended study)	BAS 500 00 F	0.320	Mortality / Reproduction	0 / 0
C. carnea (larvae)	BAS 500 00 F	0.320	Mortality / Reproduction	79 / 0
C. carnea (adult/ LC, extended study)	BAS 500 00 F	0.160	Mortality / Reproduction	27 / 80
C. carnea (reproduction study)	BAS 500 00 F	0.04	Mortality / Reproduction	0
C. septempunctata (larvae)	BAS 500 00 F	0.320	Mortality	100
C. septempunctata (adults/LC, ext. study)	BAS 500 00 F	0.064	Mortality / Fertility	0 / 3.1
P. cupreus (adults)	BAS 500 00 F	0.320	Mortality / Food uptake	0 / 11
Pardosa spp (adults)	BAS 500 00 F	0.320	Mortality / Food uptake	0 / 10
*T. pyri (*nymphs, adults*)*	BAS 500 00 F	2.64 kg product/ha/year	Abundance	0.0 / 0.0 [1)]
T. pyri (nymphs, adults)	BAS 500 00 F	3.14 kg product/ha/year	Abundance	0.0 / 12 [1)]
T. pyri (nymphs, adults)	BAS 500 00 F	12 kg product/ha/year	Abundance	58/ 0.0 [1)]

[1)] after 1 week / 4 weeks

Data of the new representative formulations BAS 500 06 F and BAS 516 07 F.

Test species	Test substance	Endpoint	Endpoint value
T. pyri (protonymphs)	BAS 500 06 F	Mortality: LR_{50}	0.87 L/ha
	BAS 516 07 F [1)]	Mortality: LR_{50}	> 5.4 kg/ha
T. pyri (protonymphs, extended study)	BAS 500 06 F	Mortality: LR_{50} Reproduction: ER_{50}	2.45 L/ha > 2.5 L/ha
A. rhopalosiphi (adults)	BAS 500 06 F	Mortality: LR_{50}	0.04 L/ha
	BAS 516 07 F [1)]	Mortality: LR_{50}	> 5.4 kg/ha
A. rhopalosiphi (adults, extended study)	BAS 500 06 F	Mortality: LR_{50} Reproduction: ER_{50}	> 2.5 L/ha > 2.5 L/ha
C. carnea (larvae)	BAS 516 07 F [1)]	Mortality: LR_{50} Reproduction: ER_{50}	> 3.6 kg/ha > 3.6 kg/ha
C. carnea (adult/ LC, extended study)	BAS 500 06 F	Mortality: LR_{50} Reproduction: ER_{50}	0.72 L/ha > 0.63 L/ha
	BAS 516 07 F [1)]	Mortality: LR_{50} Reproduction: ER_{50}	> 2 x 1.8 kg/ha > 2 x 1.8 kg/ha
C. carnea (aged residue)	BAS 500 06 F	DAT 0 bioassay: Mortality: LR_{50} Reproduction: ER_{50} DAT 7 bioassay: Mortality: LR_{50} Reproduction: ER_{50} DAT 14 bioassay: Mortality: LR_{50} Reproduction: ER_{50}	 < 2.5 L/ha > 1.25 L/ha > 2.5 L/ha > 2.5 L/ha > 2.5 L/ha > 2.5 L/ha
A. bilineata (adults)	BAS 516 07 F [1)]	Reproduction: ER_{50}	> 3.6 kg/ha
A. bilineata (adults, extended)	BAS 500 06 F	Reproduction: ER_{50}	> 3.75 L/ha
Pardosa spec (adults)	BAS 516 07 F [1)]	Mortality: LR_{50} Feeding capacity: ER_{50}	> 3.6 kg/ha > 3.6 kg/ha
P. cupreus	BAS 516 07 F [1)]	Mortality: LR_{50} Feeding rate: ER_{50}	> 3.6 kg/ha > 3.6 kg/ha
T. pyri (nymphs, adults) field study in damson plum	BAS 516 07 F [1)]	Population development	no unacceptable effects up to 5 x 0.75 kg/ha
T. pyri (nymphs, adults) field study in apple trees	BAS 516 07 F [1)]	Population development	no unacceptable effects up to 5 x 0.75 kg/ha
T. pyri (nymphs, adults) field study in cherry orchard	BAS 516 07 F [1)]	Population development	no unacceptable effects up to 5 x 0.75 kg/ha

[1)] conducted with the minor change formulation BAS 516 00 F

Earthworms

Acute toxicity:	LC_{50}: 567 mg as/kg Product BAS 500 00 F: LC_{50}: 282 mg form./kg (35.2 mg as/kg)	New representative formulations: BAS 500 06 F: LC_{50} = 385.6 mg/kg (73.9 mg as/kg) $LC_{50\ corr}$ = 192.8 mg/kg [1)] (37.0 mg as/kg) [1)] BAS 516 07 F: LC_{50} > 1000 mg/kg $LC_{50\ corr}$ > 500 mg/kg [1)]
	Metabolite BF 500-6: LC_{50} > 1000 mg/kg	$LC_{50\ corr}$ > 500 mg/kg [1)]
	Metabolite BF 500-7: LC_{50} > 1000 mg/kg	$LC_{50\ corr}$ > 500 mg/kg [1)]
Reproductive toxicity:	NOEC: 1 L BAS 500 00 F/ha (0.443 mg as/kg)	Study with as ongoing New representative formulations: BAS 500 06 F: NOEC = 30.0 mg/kg (5.8 mg as/kg) BAS 516 07 F: NOEC = 40.0 mg/kg
		Metabolite BF 500-6: NOEC $\geq$ 320 mg/kg $NOEC_{corr} \geq$ 160 mg/kg [1)]
		Metabolite BF 500-7: NOEC $\geq$ 320 mg/kg $NOEC_{corr} \geq$ 160 mg/kg [1)]
Field studies:		New representative formulations: BAS 500 06 F: No adverse effects on naturally occurring earthworm populations at 6.25 L/ha on grass land (1.666 mg as/kg) BAS 516 07 F: No adverse effects on naturally occurring earthworm populations at 4.5 kg/ha

[1)] endpoint corrected to account for $logP_{ow}$ > 2 and high peat content (10%) in test soil

Soil macro-organisms other than earthworms

No endpoints are given in the EU Review Report. Available information on reproductive toxicity is shown in the table below.

Metabolite BF 500-6 (*Folsomia candida*):	NOEC ≥ 1000 mg/kg dry soil
Metabolite BF 500-7 (*Folsomia candida*):	NOEC ≥ 800 mg/kg dry soil
BAS 500 06 F (*Folsomia candida*):	NOEC = 125 mg/kg dry soil (23.9 mg as/kg dry soil)
BAS 516 07 F (*Folsomia candida*):	NOEC = 250 mg/kg dry soil
BAS 500 06 F (*Hypoaspis aculeifer*):	NOEC ≥ 90 mg/kg dry soil (17.3 mg as/kg dry soil)
BAS 516 07 F (*Hypoaspis aculeifer*):	Study in preparation

Furthermore, an organic matter decomposition study was conducted with BAS 500 06 F resulting in a NOEC of 1.25 L/ha.

Soil micro-organisms

Nitrogen mineralization:	No effects up to 10 L product/ha (2.5 kg as/ha) BF 500-6: No effect up to 750 g/ha BF 500-7: No effect up to 375 g/ha	New representative formulations: BAS 500 06 F: No unacceptable effects up to 12.5 L/ha (3.33 mg as/kg dry soil). BAS 516 07 F: No unacceptable effects up to 24 kg/ha
Carbon mineralization:	No effects up to 10 L product/ha (2.5 kg as/ha) BF 500-6: No effect up to 750 g/ha BF 500-7: No effect up to 375 g/ha	New representative formulations: BAS 500 06 F: No requirement anymore, thus no additional study conducted BAS 516 07 F: No unacceptable effects up to 24 kg/ha

Non-target plants

No endpoints are given in the EU Review Report. The following information is available for the new representative formulations:

BAS 500 06 F: ER_{50} (seedling emergence) > 1.25 L/ha, ER_{50} (vegetative vigor) > 1.25 L/ha

BAS 516 07 F: ER_{50} (vegetative vigor) > 5.4 kg/ha

Further studies will be conducted with BAS 516 07 F.

2 THE ACTIVE SUBSTANCE AND THE PLANT PROTECTION PRODUCT

Active substance

The actice substance pyraclostrobin was approved by the EU with the reference specification referred to in EU Directive 2004/30/EC. The minimum purity of the active substance specified in the Inclusion Directive is 975 g/kg. The manufacturing impurity dimethylsulfate (DMS) was considered to be of toxicological concern and must not exceed a concentration of 0.0001 % (1 ppm w/w) in the technical product.

The equivalency of the current technical pyraclostrobin to the reference specification and the impurity profile of all active substance batches used for (old and new) toxicological and ecotoxicological studies will be shown in the supplemental dossier. Due to continuous optimisation of the commercial production process the current active substance content is higher and the current concentration of technical impurities is lower compared to the reference specification. Further important information on the identity of the active substance is given in chapter 3.1.

Plant protection product

The representative formulations chosen for renewal of approval are BAS 500 06 F and BAS 516 07 F.

BAS 500 06 F (200 g/L pyraclostrobin, EC) is the result of further optimising the previous representative formulation BAS 500 00 F (250 g/L pyraclostrobin, EC).

BAS 516 07 F (26.7 % w/w boscalid, 6.7 % w/w pyraclostrobin, WG) was not involved in the Annex I evaluation process of either pyraclostrobin or boscalid.

For both formulations a complete set of studies and other documents will be provided in the supplemental dossier.

3 SPECIFIC CONCLUSIONS BASED ON PREVIOUS EVALUATION

3.1 Identity, physical/chemical/technical properties and methods of analysis

Identity
During the Annex I inclusion process two five batch analysis reports were submitted and evaluated. The first one was based on lab / pilot plant samples (tox-batches), while for the second one representative samples of the commercial production plant were used. The current reference specification is based on the analysed production plant samples. However, in the EU Review Report (SANCO/1420/2001-Final) a remark was made that the reference specification was based on pilot plant data.
According to current requirements a new five batch analysis will be submitted in the supplemental dossier based on the commercially produced technical material.

Phys.-chem. properties
Purified pyraclostrobin is a white to light beige crystalline solid, while the commercially produced technical material is an amber-coloured glass-like solid.
Pyraclostrobin is neither flammable, nor explosive, nor corrosive, nor oxidizing. The pure active ingredient (PAI) has a vapour pressure of 2.6×10^{-8} Pa at 20°C. It is slightly soluble in pure water (1.9 mg/L at 20°C and pH 5.8). Its n-octanol/water partition coefficient (log P_{ow}) is 3.99. Due to the lack of acidic protons pyraclostrobin does not dissociate. The active substance absorbs light in the UV area (absorption bands around at 205 and 275 nm), showing sensitivity to photolysis (measured with PAI).

Analytical methods

Product chemistry
Analytical methods to determine significant impurities and the relevant impurity dimethyl sulfate in the technical material were evaluated during the Annex I inclusion process. Updated analytical methods will be submitted as appropriate. In addition, the new CIPAC method for the determination of the active substance will be contained in the supplemental dossier.
Validated analytical methods for the determination of the active substance and of dimethyl sulfate in the new representative formulations will be submitted in the AIR 3 dossier.

Consumer safety
New methods for data generation and monitoring purposes were developed for plant matrices and animal tissues (including blood as body fluid). The methods cover the new guideline requirements (OECD, SANCO 825) and the metabolites identified during metabolism studies. The methods are fully validated. For proving the validity of the cow feeding study, the common moiety method used as data generation method will be validated for the main metabolites (especially 500M04 in milk). In order to support the poultry feeding study, the common residue analytical method used for sample analysis will be provided as supplemental information. Independent lab validation studies will be submitted for plants (additional commodities) and for livestock.

Environmental fate

New methods for monitoring purposes and generation of residue data were developed for soil, surface and ground water and air. The methods cover the new guideline requirements and the metabolites identified during metabolism studies. The methods were also fully validated. An independent lab validation will be provided for surface and ground water.

3.2 Mammalian toxicology

3.2.1 Absorption, distribution, metabolism and excretion in mammals

With the exception of the below described study all relevant data were already reviewed in the Annex I inclusion process.

The comparison of in-vitro-metabolism in humans with species used in toxicological pivotal studies is a new data requirement according to Regulation 1107/2009. It will be addressed by a study with hepatocytes from humans, rat and rabbit (pools of females and males), which is currently ongoing and will be submitted with the supplemental dossier. Two different concentrations of pyraclostrobin will be incubated with hepatocytes from the different species and metabolite patterns will be compared via retention times on radio-HPLC and mass.

3.2.2 Acute toxicity

Pyraclostrobin has low acute toxicity via the oral and dermal route.

As the physical form of technical pyraclostrobin is a solidified melt it can not be inhaled. Dissolved in acetone it was toxic to rats when inhaled as an aerosol for 4 hours. A study performed 1997 revealed an acute toxicity value of 0.31 mg/L < LC_{50} < 1.07 mg/L with no and 100% mortality at 0.31 and 1.07 mg/L, respectively. The derived LC_{50} in the List of Endpoints was 0.69 mg/L. A new study performed in 2002 at concentrations of 0.52, 0.65 and 0.85 mg/L, using also acetone as solvent, revealed an LC_{50} of 0.58 mg/L. A substantially different result was obtained in a second new acute inhalation study performed in 2001, using Solvesso 200 as a solvent, revealing an acute inhalation toxicity value of 4.07 < LC_{50} < 7.3. Thus, the solvent used affects – together with the particle size distribution of the generated aerosol – the acute inhalation toxicity of pyraclostrobin.

With regard to local effects pyraclostrobin is irritant to the skin and non-irritant to the eye. In a maximization assay no sensitizing effects were observed.

No phototoxic potential was seen with pyraclostrobin in a 3T3 NRU cell phototoxicity test according to OECD 432. Thus no impact on human health risk is expected by exposure to UV light.

3.2.3 Short-term toxicity

With the exception of a new 28-day inhalation toxicity study all relevant data were already reviewed in the Annex I inclusion process.

Subchronic oral toxicity studies were performed in rats, mice and dogs. Mice proved to be the most sensitive species. According to the former EU review the lowest dose tested (50 ppm) was still an effect level based on effects on body weight gain and serum urea levels in males. A NOAEL was set at 30 ppm (~ 4 mg/kg) based on body weight data after 91 days of treatment in the mouse carcinogenicity study (0, 10, 30, 120 ppm). The 90-day respectively 1-year NOAELs in rats and dogs were 500 and 200 ppm, respectively.
Common effects in all three species were effects on body weight, hypertrophy of the duodenal mucosa and effects on red and white blood cell parameters. Mainly in female rats an increased incidence of extramedullary hematopoiesis was observed. Apart from the correction of clerical errors the newly submitted report amendments to the rat and mouse 90-day studies indicate no treatment-related effects on hemosiderin deposition in the spleen.
Whereas in female rats and male dogs (12-months study) increased white blood cell counts were recorded, a (partially substantial) decrease of white blood cell counts was observed in mice. This was mainly due to an absolute decrease of lymphocyte counts down to 25% of the control value. The effects on lymphocyte counts in mice were accompanied by effects on lymphoid organs.
Effects on the liver were mainly observed in rats with males being the more susceptible sex. The effects on the liver consisted of hepatocellular hypertrophy. Secondary to the markedly reduced body weights, absolute liver weights were decreased in males whereas relative liver weights were unchanged. In females effects on body weight were much less pronounced, resulting in a statistical significant increase of absolute and or relative liver weight at dose levels > 500 ppm. The decreased absolute and increased relative liver weights in mice were secondary to the reduced terminal body weight. Histopathologically a reduced incidence or severity of diffuse fatty infiltration was noted, which likewise was considered secondary to the observed body weight effects.

The 28-day dermal toxicity study in rats did not reveal signs of systemic toxicity up to the highest dose tested (250 mg/kg). In contrast, local dermal toxicity was observed based on the irritant properties of pyraclostrobin.

Upon request of US-EPA a 28-day inhalation toxicity study was performed in 2005 at concentrations of 1, 30, and 300 mg/m³ (0.001, 0.03 and 0.3 mg/L) using acetone as solvent to generate an inhalable aerosol. The high concentration was about half of the acute inhalation LC_{50} of 0.58 mg/L (see chapter “Acute toxicity” above). The 4 and 3 cases of mortality in male and female high concentration rats between study days 7 and 24 were the consequence of this high dose. Adverse effects on body weight development were noted at 300 mg/m³, whereas no treatment-related clinical biochemistry differences were noted between vehicle (acetone) and treated groups. The only exception was a slight increase of white blood cell counts, which at the differential blood count correlated to an increase of polymorphonuclear neutrophils. This was indicative of a mild inflammatory process probably in the respiratory tract. Histopathology identified the nasal cavity, the larynx, the lung and the duodenum as target organs. The NOAEC in this study was 1 mg/m³. An additional 28-day inhalation study with intermediate dose levels (3 and 10 mg/m³) is currently conducted in order to support the risk assessment.

3.2.4 Genotoxicity

All relevant data were already reviewed in the Annex I inclusion process. The mutagenic potential of pyraclostrobin was studied in a number of in-vitro and in-vivo test systems. None of the test systems revealed any evidence of a mutagenic potential of pyraclostrobin.

3.2.5 Long-term toxicity

The main and most relevant data were reviewed in the Annex I inclusion process. New data to be submitted pertain additional histopathological and statistical evaluations of the already submitted long-term studies in rats and mice, data on additional dose groups performed in parallel to the rat long-term studies as well as data on an additional carcinogenicity study in female mice at a higher dose than that used in the already submitted study. All these data were generated for and/or submitted to US-EPA. The additional animal studies were all terminated latest after 13 months of treatment as the MTD was either reached in the main studies or exceeded in the additional studies. No gross or histopathological examination of the animals was performed.

The 24-months chronic toxicity study in Wistar rats performed at dose levels of 0, 25, 75 and 200 ppm revealed slight body weight effects and marginal effects on a few clinical chemistry parameters. No treatment-related findings were noted in ophthalmology, hematology and urinalysis. Likewise, no adverse histopathological effects including neoplastic lesions were noted.
The newly submitted supplementary chronic toxicity studies were performed at dose levels of 400 ppm in males and females and 600 ppm in females. A consistent and often statistically significant decrease of food consumption and body weight/body weight gain was observed in treated groups. Dose dependent effects on clinical pathology parameters consistent to those observed in the regular 90-day subchronic and 24-months chronic toxicity study were noted in both sexes.

The 24-months carcinogenicity study in Wistar rats was likewise performed at dose levels of 0, 25, 75 and 200 ppm. No treatment-related mortality or clinical signs were noted. Impaired body weight development was observed in both sexes at 200 ppm. Additionally, a decrease of food consumption was noted in high dose males during the first 3 months of treatment. No treatment-related neoplastic findings were noted. The only treatment-related histopathological finding was an increased incidence of liver cell necrosis in males at the high dose level. The NOAEL for systemic toxicity was 75 ppm, whereas the NOAEL for carcinogenicity was at least 200 ppm.
The newly submitted supplementary carcinogenicity studies in rats were performed at dose levels of 400 ppm in males and females and 600 ppm in females. No treatment-related clinical signs or mortality was recorded. Food consumption and body weight development was impaired at all dose levels.

Based on a request from EPA an extended histopathological evaluation on the low and mid dose males of the rat long-term studies was performed with specific attention to the incidence of histiocytic sarcoma. These data were reported in report amendments to the respective studies. Neither in the chronic nor in the carcinogenicity study the additional neoplastic, pre-neoplastic and non-neoplastic findings did alter the conclusions in the original reports.

Pyraclostrobin was administered to groups of 50 male and 50 female B6C3F1 mice at dietary concentrations of 0, 10, 30 and 120 ppm and additionally 180 ppm (females only) for 18 months. No treatment related mortality, clinical signs, effects on food consumption, hematology (blood smears) or histopathology were observed. The only treatment-related findings consisted of impaired body weight development in high dose males (120 ppm) and high dose females (180 ppm). Based on the observed body weight effects the NOAEL for systemic toxicity was 30 ppm (4.1 mg/kg) in males and 120 ppm (20.5 mg/kg) in females.

Upon request of US-EPA a supplemental mouse carcinogenicity study was performed in female mice at a dietary dose of 360 ppm. As the absolute body weight and the body weight gain was markedly decreased, the study was terminated after 7 months in agreement with US-EPA. Besides slightly increased food consumption, decreased absolute and increased relative organ weights, no other findings were noted. No histopathological examinations were performed.

The supplementary studies described above as well as an additionally submitted statistical evaluation of body weight and food consumption data of the rat and mouse long-term studies indicate that these studies were performed at adequate dose levels.

3.2.6 Reproductive studies

All relevant data were reviewed in the Annex I inclusion process.

A 2-generation reproduction study performed at dose levels of 0, 25, 75 and 300 ppm revealed parental toxicity at the high dose as indicated by reduced food consumption and impaired body weight development. No adverse effects on reproductive performance including sperm and ovarian parameters as well as estrus cycle were observed. Pup survival and birth weights were not affected by treatment, while pup body weight development was impaired in both generations at the high dose level. Secondary to the impaired body weight development of pups a slight delay in vaginal opening was observed. The mean value for the day of vaginal opening was still within the historical control range. Lower thymus, spleen and brain pup weights at weaning as well as increased relative liver weights were likewise secondary to the impaired body weight development of pups. Based on the effects described above the parental and developmental NOAEL was determined at 75 ppm (~ 8.2 mg/kg bw/day), whereas the reproductive NOAEL was at least 300 ppm (~ 32.6 mg/kg bw/day).

Oral administration of pyraclostrobin to pregnant Wistar rats at dose levels of 0, 10, 25 and 50 mg/kg bw/day elicited overt maternal toxicity at 50 mg/kg bw/d and was still toxic to the dams at ≥ 25 mg/kg bw/d as indicated by impaired body weight development and/or reduced food consumption and corrected body weight gain. There was no evidence of an increase in the incidence of malformations up to the highest dose tested. A statistically significant increase of skeletal variations was observed at the high dose. However, all incidences were within the historical control range. Nonetheless, in the EU review these variations were considered as potentially treatment-related. Consequently, the NOAEL for developmental toxicity was set at 25 mg/kg bw/day whereas the NOAEL for maternal toxicity was 10 mg/kg bw/day.

The developmental toxicity study in Himalayan rabbits performed at dose levels of 0, 5, 10 and 20 mg/kg bw/day revealed maternal toxicity down to the low dose level as indicated by dose dependent reduction of mean food consumption (up to 90%) and mean body weight loss during the initial phase of treatment. The high maternal toxicity at the high dose resulted in embryotoxicity (increased early post-implantation loss) and reduced litter sizes. The incidence of external or soft tissue malformations or variations of any type was not affected by treatment, but there was an apparent increase in the occurrence of skeletal malformations. However, the incidences were within the historical control range and were thus considered incidental (see Addendum 4 to the DAR). Based on the increased embryotoxicity at ≥ 10 mg/kg bw/day the developmental NOAEL was set at 5 mg/kg bw/day, whereas the maternal NOAEL was considered < 5 mg/kg bw/day. A maternal NOAEL of 3 mg/kg bw/day was determined in a supplemental study employing dose levels of 0, 3 and 5 mg/kg bw/day.

3.2.7 Neurotoxicity studies

All relevant data were reviewed in the Annex I inclusion process.

Pyraclostrobin was tested in an acute and in a short-term (90-day) neurotoxicity study in rats. These studies included extensive functional observation batteries as well as neurohistopathological investigations. No indications of a specific neurotoxic potential of pyraclostrobin were observed confirming the lack of toxic properties of this kind as suggested by the other toxicological studies.

3.2.8 Other toxicological studies

3.2.8.1 Toxicity studies on metabolites

A number of environmental and plant/lifestock metabolites were investigated mainly in genotoxicity studies. One exception is Reg.No. 298327 which is – apart from its occurrence in the environment - a major plant and lifestock metabolite, for which a more extended testing program was performed. In the following the metabolites are sorted according to their BASF internal Reg. No. (registration number). If other denominators are available these are indicated as well. With the exception of 3 Ames tests performed with water metabolites all studies were not yet submitted/reviewed in the EU.

Reg.No. 78810 (500M51) – Plant metabolite
A set of 3 in-vitro studies (Ames (OECD 471), mouse lymphoma L5178Y$^{TK+/-}$ (OECD 476) and in-vitro micronucleus assay (OECD 487)) was performed. All studies were negative, i.e. did not indicate a genotoxic potential.

Reg.No. 298327 (500M04, BF 500-5) – Plant, lifestock and environmental metabolite
Reg.No. 298327 is of low acute toxicity (LD_{50} > 2000 mg/kg), is not irritant to the skin and eye and is not a skin sensitizer (Maximization assay).
In a 90-day subchronic toxicity study in rats the metabolite proved to be considerably less toxic than pyraclostrobin. No effects on food consumption and body weight development were observed up to the limit dose of 1000 mg/kg bw/day. Hematology effects were restricted to the high dose and consisted of altered red blood cell parameters. No hematological effects were observed in females. Likewise, clinical chemistry effects were noted in high dose males only. Urinalysis revealed in both sexes at the high dose increased urine volumes accompanied by a lower specific gravity of the urine. In males at ≥ 300 mg/kg bw/day an increased incidence of crystals of unknown origin was noted in the sediment. Consistent to the hematological effects in high dose males an increased severity of extramedullary hematopoiesis was noted in the spleen. The findings in the urine were accompanied by a number of histopathological findings in the kidneys and the ureter in males at ≥ 300 mg/kg bw/day and females at 1000 mg/kg bw/day. Thus the NOAEL in this study was 100 mg/kg bw/day, i.e. roughly 30 fold higher than that in the corresponding pyraclostrobin study. Furthermore, the toxicological profile was considerably different to the parent as indicated by the absence of effects on the liver and duodenum, while no effects on the kidney were observed with pyraclostrobin.
In-vitro gene mutation assays in bacteria (Ames) and mammalian cells (HPRT) with Reg.No. 298327 were negative, whereas the in-vitro chromosome aberration test in V79 cells revealed a clastogenic activity in presence of metabolic activation. However, an in-vivo micronucleus assay in mice did not confirm the in-vitro results. In a parallel study with radioactive Reg.No. 298327 it was demonstrated that the bone marrow of mice was exposed to the metabolite. Thus overall the studies showed the absence of a genotoxic potential of this metabolite.

Reg.No. 411847 (500M60, BF 500-11) - Environmental metabolite
A negative Ames test was submitted with the initial EU dossier. Based on a Dutch request additional genotoxicity studies were conducted. While the gene mutation assay in mammalian cells (HPRT) was negative, the in-vitro analysis for chromosomal damage was positive without metabolic activation. However, the higher tier in-vivo study did not reveal any clastogenic activity in mouse erythrocytes. In this study the presence of the metabolite in blood plasma was confirmed, indicating the exposure of the target organ. Thus overall the studies showed the absence of a genotoxic potential of this metabolite.

Reg.No. 413038 (500M76, BF 500-14) – Environmental metabolite
A negative Ames test was submitted with the initial EU dossier. Based on a Dutch request additional genotoxicity studies were conducted. While the gene mutation assay in mammalian cells (HPRT) was negative, the in-vitro analysis for chromosomal damage was positive in presence and absence of a metabolizing system. However, the higher tier in-vivo study did not reveal any clastogenic activity in mouse erythrocytes. In this study the presence of the metabolite in blood plasma was confirmed, indicating the exposure of the target organ. Thus overall the studies showed the absence of a genotoxic potential of this metabolite.

Reg.No. 4916420 (500M49) – Lifestock metabolite
A set of 3 in-vitro studies (Ames, mouse lymphoma $L5178Y^{TK+/-}$ and in-vitro micronucleus assay) was performed. All studies were negative, i.e. did not indicate a genotoxic potential.

Reg.No. 4916421 (500M24) – Plant metabolite
The gene mutation assays in bacteria (Ames) and mammalian cells (mouse lymphoma assay) were negative, whereas the in-vitro micronucleus assay indicated a clastogenic potential. However, the higher tier in-vivo mouse micronucleus assay did not reveal an increased incidence of micronucleated polychromatic erythrocytes. An analysis of blood plasma levels of Reg.No. 4916421 is currently ongoing to demonstrate the exposure of the target organ. Thus overall the studies showed the absence of a genotoxic potential of this metabolite.

3.2.8.2 Supplementary studies on the active substance

In a 14-day dietary study, groups of 10 male and 10 female Wistar rats were administered pyraclostrobin at dietary dose levels of 0, 50, 500 and 1500 ppm. Beside the expected effects on food consumption and body weight development at ≥ 500 ppm, a dose and time-dependent decrease of serum iron levels was noted in both sexes at ≥ 500 ppm, with males being more severely affected than females. This study was submitted to US-EPA and will be thus also submitted in the AIR 3 dossier.

Upon a request of US-EPA immunotoxicity studies according to OPPTS 870.7800 were performed. Based on the observation that the effects on immune organs and parameters were more pronounced in mice, the studies were performed in female B6C3F1 mice, i.e. the same strain as used for the subchronic and long-term studies in mice. As the study was performed in the US with mice from a different breeder, an abbreviated range-finding study was performed at dietary dose levels of 0, 500 and 1500 ppm. In contrast to the 90-day study conducted in 1997, the dietary dose of 1500 ppm was not tolerated and the animals had to be killed for animal welfare reasons after 3 days of treatment. At 500 ppm marginal signs of toxicity were noted. These consisted mainly of slightly reduced food consumption and body weight gain in males, lower red blood cell parameters as well as lower thymus weights in female and hyperplasia of the duodenal mucosa.
Two different types of immunotoxicity studies were performed, i.e. a study determining the activity of natural killer (NK) cells and a study to investigate the humoral immunity (T-cell dependent antibody response – TDAR) in sheep red blood cell immunized mice. The latter study was repeated, because the immune response of control animals in the first trial was way out of the historical control range. All three studies were performed in female B6C3F1 mice at dose levels of 0, 50, 200 and 750 ppm with 50 mg/kg bw/day of Cyclophosphamide as positive control for the TDAR studies or anti asialo GM1 for the NK cell study. In all studies impaired body weight development was noted at 750 ppm. At the same dose, spleen and thymus weights were significantly decreased. This was also true for the (absolute) number of spleen cells for high dose females.
The second TDAR study revealed no difference in total spleen TDAR response, however, due to the lower number of splenocytes, the relative TDAR response was at 750 ppm. No differences between control and the 50 and 200 ppm dose were noted. In contrast, no TDAR response was observed with the positive cyclophosphamide.
The activity of NK cells (effector cell) was determined by their cytotoxic action on cultured YAC-1 mouse lymphoma cells (target cell). Cytotoxicity was monitored by the release of ^{51}Cr from preloaded YAC-1 cells. Various effector to target cell ratios were tested (200:1 to 6.25:1).

While NK cells from the positive control animals displayed a significant decrease of lytic activity at effector to target cell ratios of 200:1 to 50:1, no difference between pyraclostrobin and control animal NK cell activity was noted.
Based on the immunotox studies performed, pyraclostrobin treated animals did not show an impaired immune function on humoral and cellular level.

3.2.8.3 Endocrine disrupting properties

There is no indication for an endocrine disruption potential of pyraclostrobin. The organ weight changes of male (testes, epididymis (dogs only)), and female sexual organs (ovaries) and endocrine glands (adrenals, thyroid (dogs only)) observed in subchronic studies at higher dose levels were secondary to the observed decrease of terminal body weights and typically not accompanied by histopathological findings. The decrease of absolute and relative adrenal weights in female mice was accompanied by a decrease of lipid deposition in the x-zone, which is specific to mice and of unknown function. The decreased lipid content is considered secondary to the impaired body weight development. No histopathological effects on the thyroid were observed in rodents and dogs. Likewise, long-term administration of pyraclostrobin did not indicate treatment-related effects on neoplastic or non-neoplastic findings in sexual organs or endocrine tissues.
In the 2-generation study administration of pyraclostrobin had no effect on the estrous cycle, the number, morphology and motility of sperm as well as on male or female fertility. The statistically significant increase of the precoital time in F_1 high dose females (mean of 2.8 days) was considered incidental, as the precoital time was within the historical control range and was identical to the precoital time of F_0 control females. Likewise, the slightly prolonged vaginal opening in high dose F_1 females was within the historical control range and most probably due to a general retardation of development, since the body weights/body weight gains were clearly impaired at the high dose group, especially in females.

3.2.9 Medical data

Literature searches in the past did not reveal relevant information on pyraclostrobin health effects on humans. The BASF internal medical monitoring programme is designed as a general health check-up and includes a general physical examination including neurological status, red and white blood cell counts and liver enzymes. Adverse health effects suspected to be related to pyraclostrobin exposure have not been observed.

Literature and internal data surveys will be updated for the AIR 3 dossier submission.

3.2.10 Acute data for the representative formulation

New representative formulations (BAS 500 06 F and BAS 516 07 F) have been chosen for the renewal of approval. The representative formulation of the initial submission (BAS 500 00 F) will be not a part of the dossier. A set of new acute toxicity studies was/is being conducted for the two representative formulations.

BAS 500 06 F is of moderate acute toxicity by the oral and inhalation route of exposure and of very low toxicity by the dermal route. It is irritant to the skin and – according to CLP criteria – irritant to the eye. Furthermore, the formulation is a sensitizer by the dermal route.

The acute toxicity potential of BAS 516 07 F is determined on the basis of studies performed with BAS 516 07 F and with the very similar formulation BAS 516 00 F, which is considered to be valid for BAS 516 07 F, too. BAS 516 07 F is of low acute toxicity by the oral, dermal and inhalation route of exposure and is not irritant to the skin and eye. The existing Buehler assay does not indicate a sensitizing potential. A mouse local lymph node assay is currently conducted to determine the sensitizing properties of the formulation according to the current requirements.

Moreover, product specific dermal absorption data will be determined for both products. New risk assessments for operators, bystanders, residents and re-entry workers will be provided for both representative formulations.

3.3 Residues

All relevant information on the first Annex I review and the endpoints used in consumer dietary assessments can be found in the monograph of pyraclostrobin and in the SANCO/1420/2001-Final document (EU Review Report). Before Annex I inclusion, EFSA and several Member States have assessed the safety of established and new MRLs covering both, European uses and import tolerances.

For the current renewal of approval under Regulation 1107/2009, a data gap analysis according to new guidelines, new guidance documents and new policies on exposure assessments was performed. New studies / evaluations were initiated where considered necessary.

Furthermore, a literature search is being performed, and scientific publications will be included into the supplemental dossier when considered relevant and being of sufficient high quality. Hence, adequate summaries will be provided in the appropriate dossier chapters.

3.3.1 Metabolism in primary crops

Although there are existing metabolism studies in grapes, potato and wheat (all foliar application), two new crop metabolism studies have been conducted for the new AIR 3 dossier for covering a broader use spectrum. New crop metabolism studies were performed in wheat (seed treatment application) and in paddy rice. Besides these new studies, a cabbage metabolism study will be provided. This study was performed for achieving the Japanese registration. Furthermore, a supplementary document to the grape metabolism study was prepared upon request of Japan. Purpose of the document was to clarify the way of metabolite identification which is based on extracts from grape leaves. The investigations are confirming the results from the previous studies and support the existing residue definition (parent only for MRL setting and risk assessment purposes).

BAS 500 F is intensively metabolised by mainly three key transformation steps:
- N-Desmethoxylation and O-desmethylation of the side chain
- Hydroxylation of the aromatic ring systems
- Cleavage between the ring systems

The contribution of these reactions followed by subsequent conjugation leads to a large number of metabolites. The metabolic pathways in all crops were qualitatively similar. Pyraclostrobin (BAS 500 F) and its desmethoxy metabolite 500M07 (synonym: BF 500-3) formed the major part of the residue followed by the cleavage products 500M04 (synonym: BF 500-5, plus conjugates) and 500M24. In order to address the relevance assessment for metabolites (based on the Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, EFSA Journal 2012), BASF proposed (presubmission meeting with BfR in November 2012) a stepwise approach for limited genotoxicity testing of metabolites by read across groupings and the TTC Cramer Class III. This proposal provides a rationale and a starting point for the tox testing of metabolites considered as representative plant metabolites.

3.3.2 Metabolism in livestock

For Annex I inclusion (Directive 91/414/EEC) metabolism studies were carried out in two livestock species (lactating goats, laying hens) and in the rat. In ruminants and poultry, pyraclostrobin (BAS 500 F) is rapidly absorbed, distributed and excreted. In goats dosed with 12 mg/kg feed, the residues in fat and muscle mainly consisted of unchanged parent and its N-desmethoxy metabolite (synonym: 500M07, BF 500-3). Metabolites are formed in liver and kidney by hydroxylation of the chlorophenyl and the tolyl ring and by cleavage of the molecule. Low extractability could be observed in liver, but the parent compound was present in all matrices. In hens dosed with 12 mg/kg feed, the residues were below 0.010 mg/kg; consequently no further investigations were carried out. In fat and eggs, pyraclostrobin and the desmethoxy metabolite 500M07 formed the major part of the residue whereas in liver hydroxylation reactions were predominant. As in plant matrices, cleavage products (as 500M04 plus conjugate 500M05 and 500M49 / 500M51) have been identified in tissues, milk and eggs.

The studies are still meeting today's requirements. Upon request of the Australian authority, an in-vitro comparison study was performed for goat and cow cell cultures. The study which is provided in context of the AIR 3 dossier confirmed the comparable pathway in both test species.

In order to address the relevance assessment for metabolites (based on the Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, EFSA Journal 2012), BASF proposed (presubmission meeting with BfR in November 2012) a stepwise approach for limited genotoxicity testing of metabolites by read across groupings and the TTC Cramer Class III. This proposal provides a rationale for the tox testing of metabolites considered as representative livestock metabolites.

3.3.3 Metabolism in fish

A bioaccumulation study has been performed in 1999. It was provided in context of the Annex I inclusion according to Directive 91/414/EEC. The study includes the identification of metabolites in edible and inedible portions of bluegill sunfish.

Pyraclostrobin is registered in the majority of crops being intended as fish feed item. In most of these crops residue levels above LOQ occur. In addition, the conduct of a fish metabolism is a new data requirement according to Regulation 283/2013; a working document on the study conduct was published in II/2013. The study is currently ongoing in rainbow trouts; it will be completed in II/2014. In order to allow a full comparison, in-vitro investigations on fish cell cultures will be provided as supplemental information.

3.3.4 Metabolism in rotational crops

A confined rotational crop study was performed in context of the Annex I inclusion (Directive 91/414/EEC). The residue levels and the nature of residues were investigated in three different succeeding crops (radish, lettuce, wheat) at an application rate of 900 g as/ha. In the study the ^{14}C-chlorophenyl and ^{14}C-tolyl labelled pyraclostrobin was applied to bare soil. The total residues in the edible parts of the succeeding crops were very low after all plant back intervals. There is no accumulation of pyraclostrobin or its degradation products in the parts of plants used for human food or animal feed consumption. The study is still considered scientifically valid; it is meeting the requirements included in OECD guideline 502.

In order to prove that no metabolite consisting solely of the pyrazole moiety is formed in soil (see 3.4.1) and taken up by plants, preliminary investigations with the ^{14}C-pyrazole labelled pyraclostrobin were performed. ^{14}C-pyrazole labelled pyraclostrobin was applied at the maximum seasonal rate of 500 g as/ha to bare soil; after an aging period of 30 DAT crops were planted / sown. The investigations of total radioactivity in representative plant matrices are indicating very limited uptake. The study will be completed in II/2014 and included in the AIR 3 dossier.

3.3.5 Residue trials in plants

The previous residue data package prepared for Annex I inclusion according to Directive 91/414/EEC has been reviewed to identify which new trials are necessary to have a complete data package for the intended GAPs. During the first annex I inclusion, grapes, banana (import tolerance) and small grain cereals were submitted as representative uses.
The representative uses in this application are potatoes, small grain cereals and maize. In case of potatoes, two residue studies will be provided in context of the supplemental dossier. They have been evaluated before during national approval processes, but were not yet subject of any peer review. In order to support their intended use pattern, several new residue studies for cereals and maize have been conducted using the representative formulation BAS 500 06 F.

3.3.6 Livestock feeding studies

In order to set MRLs for food of animal origin, livestock feeding studies have been performed in cows and poultry. The cow feeding study was already provided in context of the Annex I inclusion according to Directive 91/414/EEC and is still considered as valid. For the USA registration, a poultry feeding study was conducted. After discussions with the RMS Germany, the poultry study is provided as supplemental information. In the study, animals were dosed at three dose levels. Even at the highest dose level, no detectable residues were found.

3.3.7 Effects of processing

The high temperature hydrolysis study was submitted during the Annex I submission according to Directive 91/414/EEC and is therefore considered as peer reviewed. Pyraclostrobin was hydrolytically stable under all test conditions employed in this study. New wheat and oat processed fraction studies will be included in the AIR 3 dossier. A processing study performed in maize was previously submitted within draft Registration Reports to several authorities. Since it has not been peer reviewed until this point in time it will be additionally provided in the AIR 3 dossier.

3.3.8 MRLs

Pyraclostrobin was included in Annex I to Directive 91/414/EEC on 01 June 2004. A summary of current MRLs can be found at the Commission website (http://ec.europa.eu/sanco_pesticides/public/index.cfm). Under Regulation (EC) No 396/2005, EFSA provided a reasoned opinion on the review of the existing MRLs; the final version was published in 2011. The points raised by EFSA for crops which are not discussed in this dossier will be covered by a separate submission. Refernces according to article 12 like Overall, the renewal of approval is not expected to substantively impact the current MRLs.

3.3.9 Consumer Exposure

In accordance with current practice, dietary exposure assessments will be conducted using the EFSA PRIMo Model. No intake exceedences are anticipated based on the ADI / ARfD values of 0.03 mg/kg bw/d, respectively. Since Annex I inclusion in 2004, the consumer exposure has been assessed several times by EFSA and national authorities in context of the MRL re-evaluation (Art. 12) and the setting of MRLs for new uses (Art. 10).

3.4 Environmental fate and behaviour

All relevant information on the first Annex I review and the endpoints used in environmental risk assessments can be found in the monograph of pyraclostrobin and in the SANCO/1420/2001-Final document (EU Review Report). Furthermore, information on environmental endpoints is given in chapter 1 of this document.

For the current renewal of approval under Regulation 1107/2009, a data gap analysis according to new guidelines, new guidance documents and new procedures in kinetic evaluations and exposure assessments was performed, and new studies / evaluations were initiated where considered necessary.

Furthermore, a literature search is being performed, and scientific publications will be included into the dossier when considered endpoint relevant and being of sufficient high quality. Hence, adequate summaries will be provided in the appropriate dossier chapters.

3.4.1 Soil

Concerning route of degradation in soil, metabolism studies with ^{14}C-chlorophenyl and ^{14}C-tolyl labelled pyraclostrobin showed high amounts of bound residues and also some mineralization. In order to prove that no metabolite consisting solely of the pyrazole moiety is missed, an aerobic soil metabolism and a soil photolysis study with ^{14}C-pyrazole labelled pyraclostrobin were performed. The results clearly confirm the understanding of pyraclostrobin degradation in soil. No new metabolites were found.

Concerning the rate of degradation in aerobic soil, a new laboratory study was performed with parent in four soils since the old study did not provide a full material balance. The degradation rates for parent are in the same range as obtained in the old studies. In addition, the degradation rates for the minor metabolite BF 500-3 were estimated. For the major soil metabolites BF 500-6 and BF 500-7 extra metabolite studies were done since no reliable degradation rate could be calculated from the parent study. For the soil metabolites BF 500-4 and BF 500-5 which appear in soil in measurable amounts only under strong anaerobic conditions, aerobic degradation rate studies are initiated to allow for groundwater risk assessment under the new regulation 1107/2009. Due to their structure, the expected DT_{50} is very short.

A new field dissipation study (4 sites: DK, DE, IT, FR) addresses the degradation behaviour of pyraclostrobin in soil according to the new requirements of 1107/2009, i.e. dissipation processes like photolysis are excluded.

Where necessary, kinetic re-evaluations of the already peer-reviewed studies according to FOCUS are performed and will be provided in the supplemental dossier.

Due to deficiencies of the old studies (missing material balance), new (confirmatory) adsorption values will be generated for metabolites BF 500-6 and BF 500-7. Both metabolites have extremely low water solubilities and adsorb very quickly to soil. Therefore, adsorption tests can be done only with one concentration and no Freundlich isotherm can be provided. In order to allow a groundwater leaching assessment also for the anaerobic metabolites BF 500-4 and BF 500-5, adsorption studies are initiated.

3.4.2 Water

An aerobic mineralization study according to guideline OECD 309, Aerobic Mineralization in Surface Water, was performed to fulfill the new data requirement according to Regulation 1107/2009.

Since irradiation is an important degradation/dissipation route for pyraclostrobin in water and the old irradiated water/sediment study did not provide a full material balance, a new irradiated water/sediment study including full material balance was performed. The route of degradation in surface waters was confirmed.

Furthermore, kinetic re-evaluations of the already peer-reviewed studies are performed according to FOCUS and will be provided in the supplemental dossier.

3.4.3 Air

Based on its physical-chemical properties, pyraclostrobin has no potential for volatilisation (vapour pressure 2.6 x 10^{-8} Pa at 20°C). No new data on volatilization will be generated. The half-life in air will be calculated according to the newest guideline (Atkinson).

3.4.4 Predicted environmental concentrations (PEC)

Predicted environmental concentrations will be calculated and reported in the supplementary dossier for the compartments soil, groundwater, surface water and sediment. For the calculations current modelling tools and guidance for the parameter selection as recommended by FOCUS will be applied.

The parameter selection will be based on the available studies including open literature if the assessment concludes the appropriateness of the study and the reported values.

3.5 Ecotoxicology

Relevant information on the first Annex I review and the endpoints used in environmental risk assessments can be found in the monograph of pyraclostrobin and in the SANCO/1420/2001-Final document (EU Review Report). Furthermore, information on ecotoxicological endpoints is given in chapter 1 of this document.

A number of additional studies has been conducted in order to cover new requirements as well as to address the risk of new representative formulations, which constitute a significant part of ecotoxicologial studies.

Furthermore, a literature search is being performed, and scientific publications will be included into the dossier when considered endpoint relevant and being of sufficient high quality. Hence, adequate summaries will be provided in the appropriate dossier chapters.

3.5.1 Terrestrial vertebrates

Birds

For pyraclostrobin one acute toxicity study in bobwhite quail is available. This study was already evaluated during the previous Annex I inclusion process and is still of relevance for deriving the acute toxicity endpoint. One acute toxicity study with the canary is being conducted on request of US-EPA and will be submitted in the AIR 3 dossier.

The two short-term toxicity studies in bobwhite quail and mallard duck evaluated during the previous Annex I inclusion process are no longer part of the required data as outlined in the new EFSA GD and the new EU Regulation 283/2013.

Two reproductive toxicity studies are available for pyraclostrobin, one in bobwhite quail and one in mallard duck. Both studies were already evaluated during the previous Annex I inclusion process and are still of relevance for deriving the reproductive toxicity endpoint.

An updated risk assessment will be conducted in line with EFSA's Bird and Mammal Guidance Document (2009), considering all available toxicity data.

To assess the risk of the new representative formulations, acute toxicity studies in bobwhite quail were conducted with BAS 500 06 F and BAS 516 07 F.

Mammals

For pyraclostrobin one acute toxicity study in rat is available. This study was already evaluated during the previous Annex I inclusion process and is still of relevance for deriving the acute toxicity endpoint.

The reproductive toxicity endpoint previously submitted for Annex I inclusion requires revision due to new guidance by EFSA for birds and mammals risk assessment. In accordance to the latest EFSA GD, the relevant studies for deriving the reproductive toxicity endpoint for pyraclostrobin is the two-generation reproduction toxicity study in rat, as well as the developmental toxicity studies in rat and rabbit. All three studies were already evaluated during the previous Annex I inclusion process.

Updated risk assessments will be submitted that follow the latest guidance of EFSA (2009). Envisaged refinement parameters for higher tier risk assessments include new studies on residue decline in young plants under Northern and Southern European conditions. In addition, new studies are available on ecological parameters like choice of focal species, PT and PD and on the ecologically relevant chronic toxicity endpoint for the wild mammal reproductive risk assessment. Further, a field-effect study was conducted in Central Europe and will be used as weight of evidence for the acute and long-term risk assessment to mammals.

To assess the risk of the new representative formulations at higher tier, acute dietary studies were conducted on rat and wood mouse to provide more realistic information about the potential risk to small mammals. Further, a synopsis document will be compiled to summarize the available data on acute oral and dietary testing with formulations. A body-burden modeling will be submitted as supporting line of evidence.

Amphibians

New literature data indicate potential risk of pyraclostrobin to amphibians. To address this topic a study was performed under worst-case laboratory conditions. In addition a semi-field study was conducted to evaluate the risk to amphibians under more relevant exposure conditions. These results will be presented and discussed in detail together with further literature information within the supplemental dossier.

3.5.2 Aquatic organisms

The standard acute and chronic studies on fish, daphnids, algae and sediment dwellers are available and have already been evaluated during the previous Annex I inclusion process. These studies are still of relevance for deriving the acute and chronic toxicity endpoints to be used in the aquatic risk assessment. Further data were generated to cover standard requirements in other countries (particularly in the US) and were not evaluated during the Annex I inclusion process. These data include studies on marine organisms conducted for marine/estuarine risk assessment. The ecotoxicological endpoints generated on these species are partly lower (e.g. for mysids) or are higher or in line (e.g. fish data) with previous endpoints. However, the extensive information available for fresh water organisms including a mesocosm study with a wide range of taxa and relevant, more realistic exposure conditions provide the pertinent information for risk assessment of edge of field freshwater ecosystems, and the overall aquatic risk assessment will not be changed significantly due to the additional data.

To more specifically address the risk from sediment exposure, a spiked sediment study on *C. riparius* has been conducted with the active substance. The endpoints indicate low risk from sediment exposure. The endpoints obtained in the alga study on *Pseudokirchneriella subcapitata* have been recalculated resulting in a new endpoint for one of the assessment parameters. However, the changes are minor and of no impact on the overall risk assessment.

An outdoor mesocosm study is available, which has already been previously evaluated by the RMS, but which was not included in the List of Endpoints in the EU Review Report. This study has been re-evaluated according to current mesocosm evaluation requirements and provides essential information for the risk assessment of aquatic organisms, including aquatic macro-invertebrates, molluscs, phyto- and zooplankton. Fish, however, are not sufficiently addressed in the mesocosm study. Instead a number of additional species as well as different exposure scenarios were tested allowing to considerably reduce any uncertainties regarding the risk to fish. This information has been largely available already during the Annex I inclusion process, however, additional information from new studies is available which further supports the previous assessment.

Several additional metabolites were considered for ecotoxicological testing mainly based on new e-fate studies. All results obtained so far indicate low ecotoxicological potential of these metabolites. Toxicity values are either orders of magnitude above respective values for the active substance or above the limit of solubility.

The required studies (plus studies for other countries) were conducted for the new representative formulations. The results are largely in line with those previously evaluated and confirm the dominant impact of the active substance pyraclostrobin on the aquatic toxicity of the formulations.

3.5.3 Terrestrial organisms

Honey bees

The acute oral and contact toxicity study conducted with the active substance (which has been evaluated during the Annex I inclusion process) was repeated since the old study showed some weakness in the response of the toxic reference. The new results confirm the low acute toxicity to honey bees, which is further confirmed by the results of the studies with both representative formulations.

In order to address new data requirements for chronic toxicity and effects on larvae/bee brood two new studies will be submitted: A semi-field study, showing that no unacceptable lethal or sublethal effects on honeybee colonies and bee brood development occur at rates of 1.25 L BAS 500 06 F/ha, and a laboratory chronic toxicity study, which is still ongoing.
Further, two studies to determine residues in honey bee food items will be submitted for refinement of the default exposure values stated in the new EFSA Guidance Document on Honey Bee Risk Assessment.

Non-target arthropods

Complete data packages of NTA studies will be submitted for both new representative formulations. The data for the new representative formulation BAS 500 06 F are in line with endpoints generated for the previous representative formulation (BAS 500 00 F). Data for the second representative formulation BAS 516 07 F indicate lower toxicity as compared to the previous formulation.

Soil organisms

New studies will be submitted for earthworm reproduction with the active substance (currently ongoing), the representative formulations (BAS 500 06 F and BAS 516 07 F) and two metabolites (BF 500-6 and BF 500-7). The metabolites showed no and the formulations medium toxicity.
Furthermore, field studies on natural occurring earthworm populations were conducted with both formulations indicating low risk to earthworms under realistic field conditions (including elevated application rates).

The toxicity of both new representative formulations and of the two soil metabolites was tested on Collembola and that of BAS 500 06 F also on soil mites. For consistency of the data package a soil mite study with BAS 516 07 F is in preparation. As for earthworms, the metabolites showed no chronic toxicity to Collembola. Both, Collembola and soil mites, showed less sensitivity to the formulations than earthworms.

New studies on nitrogen transformation were conducted for the two new representative formulations. For BAS 516 07 F in addition a soil respiration study is available. No unacceptable effects were observed at concentrations above expected environmental concentrations.

Non-target plants

The data from the available studies with the two new representative formulations indicate low risk to plants. Further studies will be conducted with BAS 516 07 F.

3.6 Definition of the residues

3.6.1 Plant & animal

In the previous Annex I inclusion process the following residue definitions were defined.

End-Point	**Active Substance: Pyraclostrobin**
	EU agreed endpoints (SANCO/1420/2001; Monograph 12945/ECCO/BBA/01, Vol. 1, list of endpoints) and endpoints intended for risk assessment
Residue definition in plant matrices for risk assessment	Pyraclostrobin (parent)
Residue definition in plant matrices for monitoring	Pyraclostrobin (parent)
Residue definition in animal matrices for risk assessment	For all matrices (except liver and milk fat): Pyraclostrobin (parent) For liver (except poultry liver) and milk fat: Pyraclostrobin and its metabolites analysed as the hydroxypyrazoles BF 500-5 and BF 500-8, sum expressed as pyraclostrobin
Residue definition in animal matrices for monitoring	Pyraclostrobin (parent)

Plant
In order to support the current residue definition for risk assessment in plant matrices, dietary exposure assessments will be provided for the metabolites 500M04, 500M24 and 500M76. The metabolites have been found in relevant plant matrices in levels below 10% TRR, but did not show structural similarity to the parent molecule. They have not been detected in significant amounts in the rat metabolism study.
The current residue definition for MRL setting and monitoring purposes, which is also in accordance with the one established by CXL, is considered as most suitable for supporting international trade.

Animal
In order to support the common moiety residue definition for risk assessment in animal matrices, dietary exposure assessments will be provided for the metabolites 500M04, 500M49 and 500M51. The metabolites have been found in relevant animal matrices, but did not show structural similarity to the parent molecule. They have not been detected in significant amounts in the rat metabolism study.
The current residue definition for MRL setting and monitoring purposes, which is also in accordance with the one established by CXL, is considered as most suitable for supporting international trade.

3.6.2 Soil, water and air

Following the previous Annex I evaluation, the active substance pyraclostrobin is given in the residue definition for soil, water and air. The future residue definition for the environmental compartments depends on the final results of the risk assessments for the metabolites (for details see below). Currently, the available new data do not indicate a change of the old residue definition (parent only) for monitoring purposes.

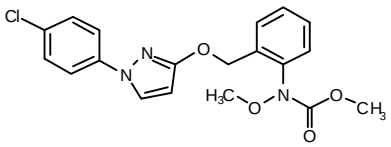
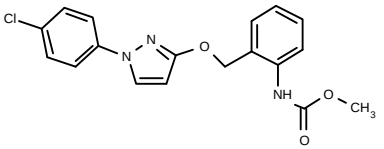
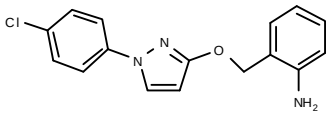
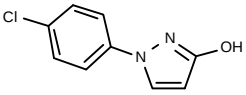
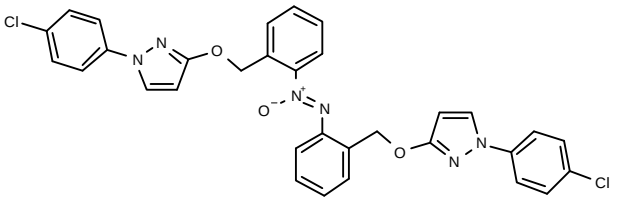
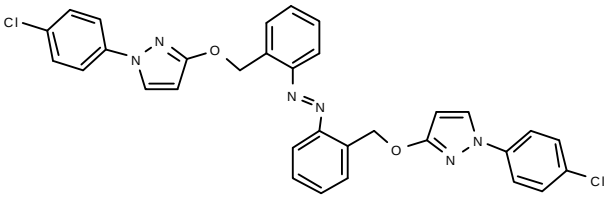
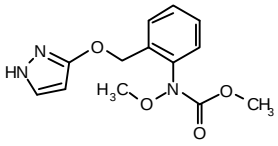
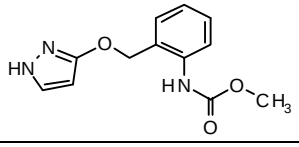
Soil
Risk assessments for soil organisms will be provided for parent and soil metabolites BF 500-6 and BF 500-7.
Metabolites BF 500-3, BF 500-4 and BF 500-5 do occur in amounts > 5% AR (applied radioactivity) only under strong anaerobic conditions. Due to their transient nature and immediate degradation under aerobic conditions, a terrestrial risk assessment is not considered necessary. Moreover, ecotox tests under anaerobic conditions, which would ensure sufficient exposure, do not provide living conditions for test organisms.
However, due to its occurrence in anaerobic sediment, a risk assessment for BF 500-3 will be provided for sediment organisms where exposure is considered possible over a longer time-period.

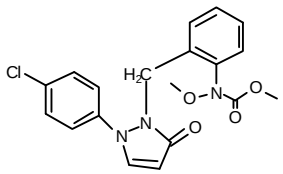
Groundwater
A groundwater risk assessment will be provided for pyraclostrobin and its metabolites BF 500-3, BF 500-4, BF 500-5, BF 500-6 and BF 500-7. Overall, the leaching risk is considered very low for all compounds either due to their high adsorption and/or their fast degradation in soil.

Surface water
Aquatic risk assessments will be provided for parent and the aqueous photoproducts BF 500-11, BF 500-13 and BF 500-14 occuring in the irradiated water/sediment studies. Risk assessments for sediment will be done for parent and metabolite BF 500-3.

Air
None of the metabolites showed any volatility so that the residue definition for air monitoring is parent only.

3.7 Overview of compounds currently identified for the environmental compartments

Compound designation	Reference code (Reg.No.)	Molecular weight	Structure
Pyraclostrobin (BAS 500 F)	304428	387	
BF 500-3 500M07 "des-methoxy"	340266	357	
BF 500-4 500M73 "aniline"	358672	300	
BF 500-5 500M04 "OH-pyrazole"	298327	194	
BF 500-6 500M01 "azoxy"	364380	611	 cis-trans isomerization possible
BF 500-7 500M02 "azo"	369315	596	 cis-trans isomerization possible
BF 500-11 500M60	411847	277	
BF 500-13 500M62	412785	247	

Compound designation	Reference code (Reg.No.)	Molecular weight	Structure
BF 500-14 500M76	413038	387	

3.8 Classification and Labelling

The classification and labelling according to Table 3.1 of Regulation (EC) No. 1272/2008 as amended by Commission Regulation (EC) No. 790/2009 is given as follows:

Classification:

Hazard class and Category:	Acute Tox. 3
Skin Irrit. 2	
Aquatic Acute 1	
Aquatic Chronic 1	
H-statements:	H331, H315, H400, H410

Labelling:

Pictogram:	GHS06, GHS09
Signal word:	Danger
H-statements:	H331, H315, H410

4 LIST OF STUDIES TO BE GENERATED, STILL ONGOING BUT NOT EVALUATED AND/OR NOT PEER REVIEWED

Reference is made to the notification of an active substance according to article 2 in the framework of the application for renewal of the active substance pyraclostrobin.

5 IDENTIFIED AREAS FOR WHICH DETAILED RE-EVALUATION IS NEEDED IN DOSSIER FROM APPLICANT AND IN EVALUATION BY RMS/Co-RMS.

Based on new information and new guidance available the following areas need to be re-evaluated in detail:

Identity, physical/chemical/technical properties and methods of analysis

Identity
- New five batch analysis representing current commercial production

Physical and chemical properties
- New information on the physical and chemical properties of the technical active substance according to modified registration requirements
- A number of new studies to characterize the physical and chemical properties of the two new representative formulations

Analytical methods
- Updated analytical methods for the active substance
- Validated analytical methods for the determination of the active substance and of dimethyl sulfate in the new representative formulations
- New residue analytical methods for risk assessment and MRL setting purposes according to current guidance requirements
- New analytical methods for soil/sediment, water and air according to current guidance requirements

Mammalian toxicology

- New subchronic inhalation studies relevant for setting an AEOLinhalation
- Immunotoxicity studies performed upon request of US-EPA according to OPPTS guidelines
- Genotoxicity studies for a number of metabolites; in addition acute and subchronic toxicity studies for one of the metabolites
- A number of acute toxicity studies and dermal penetration studies for the two new representative formulations

Residues

- New metabolism studies in plant and lifestock because of extension of uses and 1107/2009 data requirements
- Definition of the relevant residue for dietary risk assessment in light of the revised policy (as published in the EFSA Scientific Opinion of July 2012)
- New residue data (plant, processing)

Environmental fate and behaviour

- New kinetic evaluations of previously submitted data considering new scientific and technical developments
- New metabolism and degradation studies (laboratory and field) because of new developments and 1107/2009 requirements
- New adsorption/desorption studies for metabolites

Ecotoxicology

Birds and mammals
- New risk assessments according to the latest Guidance Document of EFSA (2009)

Amphibians
- Literature information and new studies addressing the risk to amphibians according to the new requirement in Regulation 1107/2009

Aquatic organisms
- New studies with active substance, new representative formulations and additional metabolites
- Consideration of proposed new Guidance Document and new PEC-calculations

Honey bees
- Acute and chronic risk to honey bees and bee brood according to new data requirements in Regulation 1107/2009

Non-target arthropods
- New studies with new representative formulations

Soil organisms
- New chronic studies with active substance, new representative formulations and metabolites

Non-target plants
- New studies with new representative formulations

6 Appendix

Overview of the GAPs of the representative uses intended to be used as the basis for the risk assessments provided in the supplemental dossier.

GAP for the representative formulation BAS 500 06 F

Crop	Member State or Country	Product name	F G or I	Pests or Group of pests controlled	Formulation		Application				Application rate per treatment				PHI (days)	Remarks:
					Type	Conc. of as g/L	Method Kind	Growth stage & season	Number max	interval between applications (min)	kg as/hL min max	water L/ha min max	kg as/ha max	L Product/ ha max		
Cereals	EU	Retengo	F	*Puccinia* spp., *R. secalis, P. teres*, (MEHITE)	EC	200	spraying	25 - 69	2	21 days	0.0625 - 0.25	100 - 400	0.25	1.25	35	Including physio-logical effects**
Maize	EU	Retengo	F	*Helmintho-sporium turcicum, Puccinia sorghi*	EC	200	spraying	30 - 65	1	-	0.05 - 0.2	100 - 400	0.2	1.0	*	Including physio-logical effects**

* Defined by growth stage at latest application
** In addition to disease control further effects (beyond fungicidal effects) will be addressed in the dossier

GAP for the representative formulation BAS 516 07 F

Crop	Member State or Country	Product name	F G or I	Pests or Group of pests controlled	Formulation		Application				Application rate per treatment				PHI (days)	Remarks:
					Type	Conc. of as g/kg	Method Kind	Growth stage & season	Number max	Interval between applications (min)	kg as/hL min max	water L/ha min max	kg as/ha max	kg Product/ ha max		
Potato	DE	Signum	F	*Alternaria* spp.	WG	267[1] + 67[2]	spraying	51 - 89	4	10 days	17 - 33[1] + 4 - 8[2]	200 - 400	67[1] + 17[2]	0.25	3	

[1] Boscalid
[2] Pyraclostrobin

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT L-CA, Section 1

IDENTITY OF THE ACTIVE SUBSTANCE

Reference List

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

There are no references submitted with this section.

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT L-CA, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

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Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 2.3/1	Kroehl T.	2013 a	Appearance of Pyraclostrobin technical material TC 2013/1399332 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 2.3/2	Kroehl T.	2010 a	Physical properties of Pyraclostrobin (BAS 500 F, Reg.No. 304 428 [redacted] technical material (TC/TGAI), produced at [redacted] 2010/1126095 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 2.4/1	Kroehl T., Behnken H.N.	2014 a	Mass, NMR, IR and UV/Vis spectra of Dimethylsulfate - minor component in Technical-Grade Pyraclostrobin 2014/1001441 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 2.8/1	Daum A.	2000 a	Determination of the dissociation constant of Reg.No. 304428 (BAS 500 F) 2000/1012252 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 2.9/1	Achhammer G.	2013 a	BAS 500 F - Pyraclostrobin - Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2013/1002641 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 2.9/2	Loehr S.	2011 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2011/1000601 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 2.9/3	Loehr S.	2011 b	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) 2011/1048145 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 2.11/1	Achhammer G.	2013 a	BAS 500 F - Pyraclostrobin - Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2013/1002641 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 2.11/2	Loehr S.	2011 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2011/1000601 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 2.11/3	Loehr S.	2011 b	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) 2011/1048145 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 2.13/1	Achhammer G.	2013 a	BAS 500 F - Pyraclostrobin - Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2013/1002641 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 2.13/2	Loehr S.	2011 a	Evaluation of physical and chemical properties according to Directive 94/37/EC (Regulation (EC) No 440/2008) 2011/1000601 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 2.13/3	Loehr S.	2011 b	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) 2011/1048145 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT L-CA, Section 3

FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162236 (version 1)
27-Feb-2017	Addition of a reference in 3.8, 3.9 and 3.10	BASF DocID 2017/1032488 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 3.8/1	Anonymous	2016 a	Safety data sheet - Pyraclostrobin techn. 2016/1223395 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 3.9/1	Anonymous	2016 a	Safety data sheet - Pyraclostrobin techn. 2016/1223395 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 3.10/1	Anonymous	2016 a	Safety data sheet - Pyraclostrobin techn. 2016/1223395 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

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Pyraclostrobin

DOCUMENT L-CA, Section 4

ANALYTICAL METHODS

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162237 (version 1)
27-Feb-2017	KCA 4.1.2/3: exchange of reference KCA 4.1.2/7-12: new references KCA 4.1.2/13-15: revised numbering KCA 4.1.2/7-16: new reference KCA 4.1.2/17-21: revised numbering KCA 4.2/5: exchange of reference changes are marked in yellow	BASF DocID 2017/1032489 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.1/1	Anonymous	2007 a	Determination of Pyraclostrobin in Pyraclostrobin technical, Pyraclostrobin technical concentrate, Pyraclostrobin emulsifiable concentrates and Pyraclostrobin water dispersible granules 2007/1017547 CIPAC - Collaborative International Pesticides Analytical Council, Harpenden Hertfordshire AL5 2HG, United Kingdom no Published	No	No	Not applicable	CIPAC
KCA 4.1.2/1	Tilting N., Sopena-Vazquesz F.	2014 a	Validation of analytical method L0166/01: Determination of BAS 500 F (Pyraclostrobin) and its metabolites Reg.No. 364380 (500M01), Reg.No. 369315 (500M02) and Reg.No. 340266 (500M07) in soil using LC/MS/MS 2013/1184817 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.1.2/2	Zangmeister W.	2010 a	Validation of analytical method L0161/01: Determination of BAS 500 F in soil at LOQ 0.001 mg/kg 2010/1075848 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
~~KCA 4.1.2/3~~	~~Obermann M.~~	~~2009 a~~	~~Validation of analytical method APL0500/03: Determination of pesticides in water by HPLC/MS~~ ~~2008/1042150~~ ~~BASF SE, Limburgerhof, Germany Fed.Rep.~~ ~~yes~~ ~~Unpublished~~	~~No~~	~~Yes~~	~~New data for AIR3 renewal~~	~~BASF~~
KCA 4.1.2/3	Obermann M.	2005 a	Validation of analytical method APL0500/01: Determination of pesticides in water by HPLC/MS 2005/1026675 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.1.2/4	Tilting N.	2012 a	Validation of method L0182/01: Determination of BAS 500 F and its metabolites Reg.No. 412053 (500M59), Reg.No. 411847 (500M60), Reg.No. 412785 (500M62), Reg.No. 413038, and Reg.No. 377613 in ground-surface- and tapwater using LC-MS/MS 2012/1009641 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2/5	Obermann M.	2014 a	Validation of analytical method L0182/02 for the determination of BF 500-5 (Reg.No. 298327), metabolite of BAS 500 F, in ground- and surface water by LC-MS/MS 2014/1004891 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.1.2/6	Penning H.	2012 a	Validation of analytical method L0197/01: Method for the determination of BAS 500 F (Pyraclostrobin) in air by LC-MS/MS 2012/1220256 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.1.2/7	Catchpole G. Hidding B.	2016 a	Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin) - Validation of an analytical method for the analysis of Reg.No. 5916421 in plasma using HPLC-MS (control procedure: 13/0173_04) 2016/1327657 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2/8	Catchpole G. Hidding B.	2016 d	Validation of an analytical method for the analysis of Reg.No. 411847 (metabolite of BAS 500 F, Pyraclostrobin) in plasma using HPLC-MS (control procedure: 99/0251_01) 2016/1321627 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.1.2/9	Catchpole G. Hidding B.	2016 b	Validation of an analytical method for the analysis of Reg.No. 413038 (metabolite of BAS 500 F, Pyraclostrobin) in plasma using HPLC (control procedure: 99/0249_01) 2016/1311551 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.1.2/10	Catchpole G. Hidding B.	2017 a	Validation of an analytical method for the analysis of Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) and Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) in plasma using HPLC-MS (control procedure: 14/0701_04) 2016/1333356 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2/11	Catchpole G. Hidding B.	2016 c	Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Validation of an analytical method for the analysis of Reg.No. 399379 (metabolite of BAS 500 F) in 1% Carboxymethyl cellulose in drinking water (w/V) using HPLC-UV 2016/1230128 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.1.2/12	Tanaka T.	2016 a	Validation for determination method of Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) in 0.5 w/v% carboxymethyl cellulose solution 2016/1230112 BSRC - Biosafety Research Center, Iwata Shizuoka 582-2, Japan yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.1.2/13	Leite R.	2005 a	Validation study of the SOP-PA.0243 for determination of Pyraclostrobin and its metabolite (BF 500-3) residues in coffee (grain), soybean (grain) and wheat (grain) 2005/1037978 BASF SA, Resende, Brazil yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2/14	Courtois J.	2014 a	Validation of analytical method L0220/01 for the detrmination of metabolite 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS 2014/1001721 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.1.2/15	Eilers B. et al.	2014 a	Method for the determination of 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS 2014/1001641 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 4.1.2/16	Jose W.F.P. de	2015 a	Validation of BASF Method Number L0076/09 for the determination of BAS 500 F and its metabolite 500M07 in citrus (whole fruit), dry beans (seeds), tomato (whole fruit), soybeans (grain) and wheat (grain) using HPLC-MS/MS and UPLC-MS/MS 2015/3004795 BASF SA, Guaratingueta, Brazil yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2/17	Eilers B., Taraschewski I.	2014 b	Validation of analytical method 446/2 (L0058/03) for the determination of BAS 500 F (Reg.No. 304428) and its metabolites 500M04 (Reg.No. 298327) and 500M85 (Reg.No. 399530) in animal matrices by LC-MS/MS 2013/1400972 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.1.2/18	Tilting N. et al.	2014 a	Method for determination of BAS 500 F (Reg.No. 304428) and its metabolites 500M04 (Reg.No. 298327) and 500M85 (Reg.No. 399530) in animal matrices by LC-MS/MS 2014/1138680 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 4.1.2/19	Hopf B.	2010 a	Validation of the analytical method L0151/01: Method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices 2010/1018944 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.1.2/20	Hopf B.	2011 a	Technical procedure: Method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices - BASF Method Number L0151/01 2011/1018046 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 4.1.2/21	Malinsky D.S., Riley M.E.	2000 a	Method validation of BASF analytical method D9902: Method for determination of residues of BAS 500F and its metabolite BF 500-16 in hen tissues using LC/MS/MS 2000/5004 BASF Corp. Agricultural Products Center, Research Triangle Park NC, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.2/1	Scherthan D.	2011 a	Independent laboratory validation of the BASF analytical method 421/0: Method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using LC/MS/MS 2011/1268146 RLP AgroScience GmbH, Neustadt/Weinstrasse, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 4.2/2	Dias C.M. et al.	2013 a	Multi-residue method for the analysis of pesticides in arabica coffee using liquid chromatography/tandem mass spectrometry 2014/1145909 <none>, <none>, <none> no Published	No	No	Not applicable	public
KCA 4.2/3	Schacherl A.	2010 a	Independent laboratory validation (ILV) of an analytical method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices 2010/1123694 Eurofins Agroscience Services GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 4.2/4	Lagunas-Allue L. et al.	2012 a	Comparison of four extraction methods for the determination of fungicide residues in grapes through gas chromatography-mass spectrometry 2012/1366723 <none>, <none>, <none> no Published	No	No	Not applicable	public

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
~~KCA 4.2/5~~	~~Rutt D., Jones G.~~	~~2014 b~~	~~Independent Laboratory Validation of BASF method L0182/02: Pyraclostrobin and its metabolites BF 500-5, BF 500-12, BF 500-11, BF 500-13, BF 500-14 and BF 500-15 in ground- and surface- water by LC/MS/MS~~ ~~2014/7000022~~ ~~JRF America, Audubon PA, United States of America~~ ~~yes~~ ~~Unpublished~~	~~No~~	~~Yes~~	~~New data for AIR3 renewal~~	~~BASF~~
KCA 4.2/5	Bianca C.M.	2015 a	Independent laboratory validation of BASF method L0182/02: BAS 500 F (Pyraclostrobin) and its metabolites BF 500-5 (Reg.No. 298327), BF 500-12, BF 500-11, BF 500-13, BF 500-14, BF 500-15 in ground- and surface- water by LC/MS/MS 2015/7001873 JRF America, Audubon PA, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

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DOCUMENT L-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-July-2014		BASF DocID 2014/1162238 (version 1)
09-June-2015	The document was amended in order to correct an error regarding the data protection claim of one study (KCA 5.2.3/1). New or changed text is marked in yellow.	BASF DocID 2015/1109582 (version 2)
05-August-2015	The document was amended in order to correct an error (KCA 5.1.1/1 was not characterized as vertebrate study). New or changed text is marked in blue.	BASF DocID 2015/1183829 (version 3)
27-Feb-2017	The following new references have been added: KCA 5.1.1/2-4 KCA 5.1.2/2-3 KCA 5.2.4/1 and KCA 5.2.5/1 KCA 5.4.2/1-2 KCA 5.4.3/2-4 KCA 5.6.1/1 KCA 5.8.1/27 KCA 5.8.1/39-49 The new references and the necessary re-numbering of already contained studies are marked in green.	BASF DocID 2017/1032490 (version 4)
30-May-2017	The document was amended in order to correct errors (KCA 5.1.1/4, KCA 5.2.4/1 and KCA 5.2.5/1 were not characterised as vertebrate studies). Furthermore, a new reference has been added (KCA 5.8.1/53). New or changed text is marked in grey.	BASF DocID 2017/1175847 (version 5)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1.1/1	[redacted] et al.	2014 a	Interim report: Further investigations of metabolites in rat plasma samples after dosing with 14C-Pyraclostrobin 2014/1136557 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.1.1/2	[redacted] et al.	2014 b	Further investigations of metabolites in rat plasma samples after dosing with 14C-Pyraclostrobin 2014/1315930 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.1.1/3	[redacted]	2016 a	14C-Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Study on kinetics in Wistar rats after oral administration 2015/1241735 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.1.1/4	[redacted]	2016 a	Metabolism investigation of 14C-Reg.No. 399379 (metabolites of BAS 500 F, Pyraclostrobin) in faeces and urine of male Wistar rats 2015/1198492 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1.2/1	Funk D. et al.	2014 a	Comparative in-vitro metabolism with 14C-BAS 500 F 2014/1001562 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.1.2/2	Funk D., Bellwon P.	2016 a	Influence of WWL229 on the in-vitro-metabolism of 14C-BAS 500 F with human hepatocytes and human liver cytosol 2016/1225031 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.1.2/3	Funk D., Bellwon P.	2016 b	Metabolisation of 14C-BAS 500 F in rat serum 2016/1225032 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.2.3/1	[redacted] et al.	2001 a	BAS 500 F 40% in Solvesso (technical active ingredient) - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2001/1010625 [redacted] yes Unpublished	Yes	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.2.3/2	[redacted]	2002 a	BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2002/1012053 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.2.3/3	[redacted]	2003 a	Amendment No. 1: BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2003/1009200 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.2.3/4	[redacted]	2003 b	Amendment No. 2: BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2003/1009427 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.2.4/1	[redacted]	2000 a	Amendment No. 1: BAS 500 .. F - Acute dermal irritation/corrosion in rabbits 2000/1018990 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.2.5/1	[redacted]	2000 b	Amendment No. 1: BAS 500 .. F - Acute eye irritation in rabbits 2000/1018989 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.2.7/1	Cetto V., Landsiedel R.	2012 a	BAS 500 F (Pyraclostrobin) - In vitro 3T3 NRU phototoxicity test 2012/1189936 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.2.7/2	Cetto V., Landsiedel R.	2014 a	Amenment No. 1 - BAS 500 F (Pyraclostrobin) - In vitro 3T3 NRU phototoxicity test 2014/1092412 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.3.2/1	[redacted] et al.	1999 a	BAS 500 F - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months 1999/10195 [redacted] yes Unpublished	Yes	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.3.2/2	[redacted] et al.	1999 b	Amendment No. 1: BAS 500 F - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months 1999/11899 [redacted] yes Unpublished	Yes	No	Not applicable	BASF
KCA 5.3.2/3	[redacted]	2000 a	Amendment No. 2: BAS 500 F - Subchronic oral toxicity study in Wistar rats administration in the diet for 3 months 2000/1012360 [redacted] yes Unpublished	Yes	No	Not applicable	BASF
KCA 5.3.2/4	[redacted]	2003 a	Amendment No. 3: BAS 500 F - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months 2003/1013399 [redacted] yes Unpublished	Yes	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.3.2/5	[redacted]	2004 a	Amendment to the report for Japanese registration: BAS 500 F - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months 2004/1027673 [redacted] yes Unpublished	Yes	No	Not applicable	BASF
KCA 5.3.2/6	[redacted] et al.	1998 a	BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl BR mice - Administration in the diet for 3 months 1998/11345 [redacted] yes Unpublished	Yes	No	Not applicable	BASF
KCA 5.3.2/7	[redacted] et al.	1999 c	Amendment No. 1: BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl mice - Administration in the diet for 3 months 1999/11900 [redacted] yes Unpublished	Yes	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.3.2/8	[redacted]	2003 b	Amendment No. 2: BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl mice - Administration in the diet for 3 months 2003/1013400 [redacted] yes Unpublished	Yes	No	Not applicable	BASF
KCA 5.3.3/1	[redacted] et al.	2005 a	BAS 500 F - Subacute inhalation study in Wistar rats - 20 aerosol exposures during 4 weeks 2005/1013950 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.3.3/2	[redacted] et al.	2014 a	Bas 500 F (Pyraclostrobin) - Repeated dose 28-day inhalation toxicity study with recovery period in Wistar rats, aerosol exposure 2014/1003946 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.4.2/1	[redacted]	2016 a	Amendment No 1: Cytogenetic study in vivo with BAS 500 F in the mouse micronucleus test. Single oral administration 2016/1309356 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.4.2/2	[redacted]	2016 c	14C-BAS 500 F- Study on the kinetics in mice 2016/1225931 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.4.3/1	Cayir A. et al.	2012 b	Micronuclei, nucleoplasmic bridges, and nuclear buds induced in human lymphocytes by the fungicide Signum and its active ingredients (Boscalid and Pyraclostrobin) 2012/1366624 <none>, <none>, <none> no Published	No	No	Not applicable	public
KCA 5.4.3/2	Schulz M., Landsiedel R.	2010 a	BAS 536 02 F - Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and preincubation test) 2010/1059892 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.4.3/3	[redacted]	2010 a	BAS 536 02 F - Micronucleus test in bone marrow cells of the mouse 2010/1141998 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.4.3/4	[redacted]	2014 a	Amendment No. 1 to the report - BAS 536 02 F - Micronucleus test in bone marrow cells of the mouse 2013/1403103 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.5/1	[redacted] et al.	1999 d	BAS 500 F - Chronic toxicity study in Wistar rats - Administration in the diet for 24 months 1999/11672 [redacted] yes Unpublished	Yes	No	Not applicable	BASF
KCA 5.5/2	[redacted]	2002 a	Amendment No. 1: BAS 500 F - Chronic toxicity study in Wistar rats. Administration in the diet for 24 months 2002/1005113 [redacted] yes Unpublished	Yes	No	Not applicable	BASF
KCA 5.5/3	[redacted] et al.	1999 e	BAS 500 F - Carcinogenicity study in Wistar rats - Administration in the diet for 24 months 1999/11868 [redacted] yes Unpublished	Yes	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.5/4	[redacted]	2002 b	Amendment No. 1: BAS 500 F - Carcinogenicity study in Wistar rats. Administration in the diet for 24 months 2002/1005114 [redacted] yes Unpublished	Yes	No	Not applicable	BASF
KCA 5.5/5	[redacted]	2002 c	Summary of results - BAS 500 F - First supplementary chronic toxicity study in Wistar rats - Administration in the diet for 24 months 2002/1004125 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.5/6	[redacted]	2002 d	Summary of results - BAS 500 F - Second supplementary chronic toxicity study in female Wistar rats - Administration in the diet for 24 months 2002/1004126 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.5/7	[redacted]	2002 e	Summary of results - BAS 500 F - First supplementary carcinogenicity study in Wistar rats - Administration in the diet for 24 months 2002/1004123 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.5/8	[redacted]	2002 f	BAS 500 F - Second supplementary carcinogenicity study in female Wistar rats - Administration in the diet for 24 months 2002/1004124 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.5/9	[redacted]. et al.	2005 a	BAS 500 F: Terminated carcinogenicity study in female B6C3F1 mice - Administration in the diet for 7 months 2005/1026477 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.5/10	[redacted] et al.	2002 a	Chronic and oncogenicity studies with BAS 500 F: Further evaluations of body weight, food consumption and food efficiency 2002/5002875 [redacted] no Unpublished	Yes	No	Not applicable	BASF
KCA 5.6.1/1	[redacted] et al.	2002 a	BAS 500 F - One generation range-finding reproduction toxicity study in Wistar rats; continuous dietary administration 2002/1004187 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.6.2/1	Schneider S. et al.	2014 a	Influence of maternal stress on gestational parameters and prenatal development in Himalayan rabbits - 53rd Annual Meeting and ToxExpo - March 23-27. 2014 - Phoenix Arizona 2014/1177661 <none>, <none>, <none> no Published	Yes	No	Not applicable	public

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.6.2/2	Greenaway J.B. et al.	2001 a	Anatomy of the lumbosacral spinal cord in rabbits 2001/1031903 <none>, <none>, <none> no Published	Yes	No	New data for AIR3 renewal	public
KCA 5.6.2/3	Viertel B., Trieb G.	2002 a	The Himalayan rabbit (Oryctolagus cuniculus L.): Spontaneous incidences of endpoints from prenatal developmental toxicity studies 2003/1034159 <none>, <none>, <none> no Published	Yes	No	Not applicable	public
KCA 5.8.1/1	Anonymous	2014 d	Export files of (Q)SAR-profiling modules of the OECD toolbox for Pyraclostrobin metabolites 2014/1172955 <none>, <none>, <none> no Unpublished	No	No	Not applicable	BASF
KCA 5.8.1/2	Anonymous	2014 a	In vitro AMES mutagenicity model with metabolic activation S9 - OASIS/TIMES QSAR analysis of Pyraclostrobin metabolites - AMES prediction 2014/1172952 <none>, <none>, <none> no Unpublished	No	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/3	Anonymous	2014 b	In vitro chromosomal aberration with metabolic activation S9 - OASIS/TIMES QSAR analysis of Pyraclostrobin metabolites prediction for chromosomal aberration 2014/1172953 <none>, <none>, <none> no Unpublished	No	No	Not applicable	BASF
KCA 5.8.1/4	Serafimova R. et al.	2013 a	Q(SAR) model reporting format (QMRF) ames mutagenicity with S9 2013/1414242 Assen Zlatarov University, Bourgas, Bulgaria no Unpublished	No	No	Not applicable	BASF
KCA 5.8.1/5	Mekenyan O. et al.	2013 a	Q(SAR) Model Reporting Format (QMRF) In vitro chromosomal aberration with S9 2013/1414460 <none>, <none>, <none> no Published	No	No	Not applicable	public
KCA 5.8.1/6	Anonymous	2014 c	Prediction and applicability domain analysis for models: Mutagenicity model (CAESAR) (version 2.1.9) - Mutagenicity SarPy model (version 1.0.4-BETA) - Pyraclostrobin 2014/1172954 <none>, <none>, <none> no Unpublished	No	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/7	Esdaile D.J.	2012 a	Plant and animal metabolites of BAS 500 F: Structure-activity hazard identification screen using DEREK 2011/1022602 CiToxLAB Hungary Ltd., Veszprem, Hungary no Unpublished	No	No	Not applicable	BASF
KCA 5.8.1/8	Cramer G.M., Ford R.A.	1977 a	Estimation of toxic hazard - A decision tree approach 1978/1001324 <none>, <none>, <none> no Published	No	No	Not applicable	public
KCA 5.8.1/9	Kroes R. et al.	2003 a	Structure-based thresholds of toxicological concern (TTC): Guidance for application to substances present at low levels in the diet 2004/1036074 <none>, <none>, <none> no Published	No	No	Not applicable	public
KCA 5.8.1/10	Munro I.C. et al.	1996 a	Correlation of structural class with no-observed-effect levels: A proposal for establishing a threshold of concern 1996/1005180 <none>, <none>, <none> no Published	No	No	Not applicable	public

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/11	[redacted]	1997 a	Study on the acute oral toxicity of Pyrazolon in rats 1997/10963 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/12	[redacted]	1997 b	Study on the acute dermal irritation/corrosion of Pyrazolon in the rabbit 1997/10964 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/13	[redacted]	1997 c	Study on the acute eye irritation of Pyrazolon in the rabbit 1997/10965 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/14	[redacted]	1997 a	Pyrazolon - Maximization test in guinea pigs 1997/10968 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/15	[redacted] et al.	2013 a	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Repeated dose 90-day oral toxicity study in Wistar rats - Administration via the diet 2013/1042164 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/16	Woitkowiak C.	2012 a	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2012/1220416 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/17	Schulz M., Landsiedel R.	2012 b	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in CHO cells (HPRT locus assay) 2012/1272482 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/18	Schulz M., Landsiedel R.	2012 a	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro chromosome aberration assay in V79 cells 2012/1185707 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/19	[redacted]	2013 a	Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2013/1026779 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/20	[redacted]	2013 a	14C-Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Study on the kinetics in mice 2012/1278425 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/21	Woitkowiak C.	2014 a	Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium/Escherichia coli, reverse mutation test 2013/1323364 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/22	Schulz M., Landsiedel R.	2014 f	Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells (TK+/- locus assay, microwell version) 2013/1298449 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/23	Schulz M., Landsiedel R.	2014 e	Reg.No. 5916421 (metabollite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells (cytokinesis block method) 2013/1363549 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/24	[redacted]	2014 a	Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin): Micronucleus assay in bone marrow cells of the mouse 2013/1389659 [redacted]. yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/25	Grauert E., Kamp H.	2014 a	Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin) - Concentration control analyses in 30% Dimethylsulfoxide / 70% Polyethyleneglycol 400 (v/v) 2014/1105774 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/26	Becker M., Kamp H.	2014 a	Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin) - Plasma analysis for external studies 2014/1145915 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/27	[redacted] et al.	2016 a	Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin) - Investigation of the bioavailability in blood of the mouse 2016/1236540 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/28	Woitkowiak C.	2013 b	Reg.No. 5916420 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella Typhimurium / Escherichia Coli reverse mutation assay 2013/1281928 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/29	Schulz M., Landsiedel R.	2014 d	Reg.No. 5916420 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells (TK+/- locus assay, microwell version) 2013/1298447 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/30	Schulz M., Landsiedel R.	2014 c	Reg.No. 5916420 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells (cytokinesis block method) 2013/1361921 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/31	Woitkowiak C.	2013 a	Reg.No. 78810 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2013/1255749 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/32	Schulz M., Landsiedel R.	2014 b	Reg.No. 78810 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells (TK+ locus assay, microwell version) 2013/1298448 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/33	Schulz M., Landsiedel R.	2014 a	Reg.No. 78810 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells (cytokinesis block method) 2013/1361922 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/34	Engelhardt G., Leibold E.	2003 b	In vitro gene mutation test with Reg.No. 413 038 in CHO cells (HPRT locus assay) 2003/1004384 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/35	Schulz M., Landsiedel R.	2013 b	Reg.No. 413038 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro chromosome aberration assay in V79 cells 2012/1044766 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/36	Schulz M., Landsiedel R.	2014 g	Amendment No. 1 - Reg.No. 413038 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro chromosome aberration assay in V79 cells 2014/1145892 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/37	[redacted]	2012 e	Reg.No. 413038 (metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2012/1220183 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/38	Engelhardt G., Hoffmann H.D.	1999 a	Salmonella typhimurium/Escherichia coli reverse mutation assay (AMES standard plate test and prival preincubation test) with Reg.No. 369315 1999/10736 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/39	Woitkowiak C.	2016 a	Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium/Escherichia coli - Reverse mutation assay (prival modification) 2016/1200488 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/40	Schulz M., Landsiedel R.	2016 a	Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells (TK Locus assay, microwell version) 2016/1225056 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/41	Chang S.	2016 a	Reg.No. 369315 (Metabolite of BAS 500 F, Pyraclostrobin): Micronucleus Test In Human Lymphocytes In Vitro 2016/1135652 Envigo CRS GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/42	[redacted] et al.	2017 a	Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Repeated-dose 28-day oral toxicity study in Wistar rats - Administration by gavage 2016/1288407 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/43	Woitkowiak C., Landsiedel R.	2016 a	Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium / Escherichia coli - Reverse mutation assay 2016/1119837 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/44	Schulz M., Landsiedel R.	2016 b	Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y lymphoma cells (TK+/- Locus assay, microwell version) 2016/1171390 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/45	Chang S.	2016 b	Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in human lymphocytes in vitro 2016/1109533 Envigo CRS GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/46	[redacted]	2016 a	Reg.No. 399379 (Metabolite of BAS 500 F, Pyraclostrobin), micronucleus assay in bone marrow cells of the mouse 2016/1295116 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/47	Grauert E., Hidding B.	2017 a	Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Homogeneity and concentration control analyses in 0.5% Carboxymethyl cellulose /steril water (w/v) 2017/1020704 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/48	[redacted]	2016 b	14C-Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Study on the kinetics on mice 2016/1225930 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/49	[redacted]	2017 a	Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin): Transgenic mice (MUTA mouse) gene mutation assay 2017/1002442 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/50	Schulz M., Landsiedel R.	2012 c	Reg.No. 411847 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in CHO cells (HPRT locus assay) 2012/1148607 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/51	Engelhardt G., Leibold E.	2003 a	In vitro chromosome aberration assay with Reg.No. 411847 in V79 cells 2003/1004383 BASF AG, Ludwigshafen/Rhein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.1/52	[redacted]	2012 d	Reg.No. 411847 (metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2012/1218557 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.1/53	Naumann S.	2017 a	Reg.No. 412785 (metabolite of Pyraclostrobin) - Micronucleus test in human lymphcytes in vitro 2017/1024661 Envigo CRS GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 5.8.2/1	[redacted] et al.	2003 a	BAS 500 F - Determination of iron in urine and serum of Wistar rats - Administration in the diet over 14 days 2003/1009534 [redacted] no Unpublished	Yes	No	Not applicable	BASF
KCA 5.8.2/2	[redacted]	2011 a	A 28 day oral (dietary) range finding study of BAS 500 F in B6C3F1 mice 2011/1194286 [redacted] no Unpublished	Yes	No	Not applicable	BASF
KCA 5.8.2/3	[redacted]	2012 f	A 28-day oral (dietary) natural killer cell immunotoxicity study of BAS 500 F in female B6C3F1 mice 2011/1035857 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.2/4	[redacted]	2012 e	A 28-day oral (dietary) antibody forming cell immunotoxicity study of BAS 500 F in female B6C3F1 mice 2012/1020986 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.8.2/5	[redacted]	2012 d	A 28-day oral (dietary) antibody forming cell immunotoxicity study of BAS 500 F in female B6C3F1 mice 2012/1084176 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 5.8.2/6	Houck K.A. et al.	2009 a	Profiling bioactivity of the ToxCast Chemical Library using Bio MAP primary human cell systems 2009/1130882 <none>, <none>, <none> no Published	No	No	Not applicable	public
KCA 5.8.2/7	Shah I. et al.	2011 a	Using nuclear receptor activity to stratify hepatocarcinogens 2011/1295091 <none>, <none>, <none> no Published	No	No	Not applicable	public

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8.2/8	Kleinstreuer N.C.	2011 a	Environmental impact on vascular development predicted by High-throughput screening 2011/1296591 <none>, <none>, <none> no Published	No	No	Not applicable	public
KCA 5.8.2/9	Sipes N.S. et al.	2013 a	Profiling 976 toxcast chemicals across 331 enzymatic and receptor signaling assays 2013/1371960 <none>, <none>, <none> no Published	No	No	Not applicable	public
KCA 5.8.3/1	Orton F. et al.	2011 a	Widely used pesticides with previously unknown endocrine activity revealed as in vitro antiandrogens 2011/1291251 <none>, <none>, <none> no Published	No	No	Not applicable	public
KCA 5.8.3/2	Reif D.M. et al.	2010 a	Endocrine profiling and prioritization of environmental chemicals using ToxCast data 2010/1231552 <none>, <none>, <none> no Published	No	No	Not applicable	public

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.9.3/1	Gergely R.M. et al.	2008 a	Acute pesticide poisoning associated with Pyraclostrobin fungicide - Iowa, 2007 2008/1102036 <none>, <none>, <none> no Published	Yes	No	Not applicable	public

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DOCUMENT L-CA, Section 6

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

Reference List

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Version history[1]

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18-Jul-2014		BASF DocID 2014/1162239 (version 1)
27-Feb-2017	KCA 6.3.1/3: new reference changes are marked in yellow	BASF DocID 2017/1032919 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.1/1	Abdel-Baky S.	2001 a	Freezer storage stability of BAS 500 F and BF 500-3 in plant matrices including processed commodities 2001/5000232 BASF Agro Research RTP, Research Triangle Park NC, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.2.1/1	Hamm R.T.	1998 a	Metabolism of BAS 500 F in grapes 1998/10988 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	No	Not applicable	BASF
KCA 6.2.1/2	Hamm R.T.	2000 a	Amendment No. 1: Metabolism of BAS 500 F in grapes 2000/1000201 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.2.1/3	Bross M.	2004 a	Pyraclostrobin (BAS 500 F) - Grape metabolism: Additional information on the investigations of grape leaves 2004/1000758 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 6.2.1/4	Sato K.	2000 a	Metabolic fate of BAS 500 F in Chinese cabbage 2000/1018512 The Institute of Environmental Toxicology, Mitsukaido-shi Ibaraki 303-0043, Japan yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.2.1/5	Rabe U., Kloeppner U.	2014 a	Metabolism of 14C-Pyraclostrobin in rice 2013/1134958 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.2.1/6	Birk B., Kloeppner U.	2013 a	Metabolism of 14 C-Pyraclostrobin (14C-BAS 500 F) in wheat after seed treatment 2012/1158148 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.2.3/1	Bross M., Lutz T.	2009 a	In vitro investigations of the metabolism of BAS 500 F in goat and cow 2009/1067176 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.2.5/1	[redacted]	1999 a	Bioaccumulation and metabolism of (14C)-BAS 500 F in bluegill sunfish 1999/11348 [redacted] yes Unpublished	Yes	No	Not applicable	BASF
KCA 6.2.5/2	[redacted]	2014 a	The metabolism of 14C-BAS 500 F in rainbow trout (Oncorhynchus mykiss) 2014/1001601 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.1/1	Schulz H.	2004 a	Study on the residue behaviour of BAS 510 F and BAS 500 F in potatoes after application of BAS 516 00 F under field conditions in The Netherlands, Germany, United Kingdom, Denmark, France North and South, 2003 2004/1015948 Institut Fresenius Chemische und Biologische Laboratorien AG, Taunusstein, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.3.1/2	Schulz H.	2006 a	Study on the residue behaviour of Dimethomorph and Pyraclostrobin in potatoes after treatment with BAS 536 01 F under field conditions in France (N & S), Denmark, Germany, Belgium, Italy, Spain and Greece, 2005 2006/1000581 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.3.1/3	Galvez O. Moreno S.	2016 a	Study on the residue behaviour of Boscalid (BAS 510 F) and Pyraclostrobin (BAS 500 F) on potato after treatment with BAS 516 07 F under field conditions in South Europe, season 2015 2016/1000547 Agricultura y Ensayo SL, Sevilla, Spain yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.2/1	Plier S.	2013 a	Determination of residues of BAS 500 F in barley after two applications of BAS 500 06 F in Germany, Netherlands, France (North), France (South), Greece, Italy and Spain, 2011 2012/1067587 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.3.2/2	Plier S.	2011 a	Determination of residues of BAS 500 F in barley after two applications of BAS 500 06 F in Germany, United Kingdom, Denmark, France (North), France (South), Greece, Italy and Spain, 2010 2011/1135916 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.2/3	Erdmann H.-P.	2010 a	Study on the residue behaviour of BAS 700 F, Epoxiconazole and Pyraclostrobin in barley after application of BAS 702 01 F, BAS 700 00 F, BAS 500 06 F and BAS 480 38 F under field condition in France, Spain, Italy and Germany, 2009 2010/1006342 Agro-Check Dr. Teresiak & Erdmann GbR, Lentzke, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.3.2/4	Tandy R.	2012 a	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in winter barley after treatment with either BAS 556 03 F, BAS 500 06 F or BAS 555 00 F in Northern and Southern Europe during 2011 2012/1194990 Eurofins Agroscience Services, Melbourne Derbyshire DE73 8AG, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.2/5	Meyer M.	2013 a	Study on the residue behaviour of Fluxapyroxad (BAS 700 F) and Pyraclostrobin (BAS 500 F) in barley after treatment with either BAS 703 04 F or BAS 700 00 F or BAS 500 06 F under field conditions in Germany, N-France, Spain and Greece, 2012 2013/1282605 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.3.2/6	Plier S.	2011 b	Determination of residues of BAS 500 F in wheat after two applications of BAS 500 06 F in Germany, United Kingdom, France (North), Denmark, France (South), Greece, Italy and Spain, 2010 2011/1135915 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.2/7	Plier S.	2013 b	Determination of residues of BAS 500 F in wheat after two applications of BAS 500 06 F in Germany, United Kingdom, Netherlands, France (South), Greece, Italy and Spain, 2011 2012/1067588 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.3.2/8	Tandy R.	2012 b	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in winter wheat after treatment with either BAS 556 03 F, BAS 500 06 F or BAS 555 00 F in Northern andSouthern Europe during 2011 2012/1194991 Eurofins Agroscience Services, Melbourne Derbyshire DE73 8AG, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.2/9	Tandy R.	2014 a	Amendment No. 1: Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in winter wheat after treatment with either BAS 556 03 F, BAS 500 06 F or BAS 555 00 F in Northern and Southern Europe during 2011 2014/1090810 Eurofins Agroscience Services, Melbourne Derbyshire DE73 8AG, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.3.2/10	Meyer M.	2013 b	Study on residue behaviour of Fluxapyroxad (BAS 700 F), Pyraclostrobin (BAS 500 f) in wheat after treatment with either BAS 703 04 F, BAS 700 00 F or BAS 500 06 F under field conditions, Germany, United Kingdom, Spain, Southern France, 2012 2013/1336790 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.3.3/1	Aitken A.	2013 a	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) in maize following one application of BAS 500 06 F to 8 trials in 2012 - SEU and NEU 2013/1308888 Charles River, Tranent EH33 2NE, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.3/2	Aitken A.	2014 b	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) in maize following one application of BAS 500 06 F to 1 trial in 2013 – NEU 2014/1001741 Charles River Laboratories, Tranent East Lothian EH33 2NE, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.3.3/3	Schulz H., Ziske J.	2010 a	Study on the residue behaviour of Pyraclostrobin and Epoxiconazole in maize after treatment with BAS 512 04 F under field conditions in Germany, Northern France, United Kingdom, the Netherlands, Italy, Greece, Southern France and Spain 2008 2010/1025690 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.3.3/4	Schulz H.	2010 a	1st addendum to the report - Study on the residue behaviour of Pyraclostrobin and Epoxiconazole in maize after treatment with BAS 512 04 F under field conditions in DE, N-FR, UK, NL, IT, GR, S-FR and ES, 2008 2010/1080941 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.3/5	Fleischer G.	2013 a	Study on the residue behaviour of BAS 480 F, BAS 500 F and BF 500-3 (500M07) in corn after treatment with BAS 512 04 F under field conditions in Germany, 2011 2013/1065883 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 6.3.3/6	Schulz H.	2010 b	Residue behaviour of Epoxiconazole and Pyraclostrobin in maize after treatment with BAS 512 04 F and BAS 500 06 F under field conditions in Germany Northern France United Kingdom the Netherlands Italy Greece Southern France and Spain 2009 2010/1039144 SGS Institut Fresenius GmbH, Taunusstein, Germany Fed. Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.3.4/1	Morgenthal K.	2014 b	500M79: Residue analysis in plant matrices by LC-MS/MS 2014/1001661 IES - Innovative Environmental Services Ltd., Witterswil, Switzerland yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.4.1/1	[redacted]	2000 a	A meat and egg magnitude of the residue study with BAS 500 F in laying hens 2000/5005 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 6.5.1/1	Hueben M.	2014 a	High temperature hydrolysis - Simulated processing of 14C-Pyraclostrobin (14C-BAS 500 F) 2014/1136542 Fraunhofer-Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.5.3/1	Plier S.	2013 c	Determination of residues of BAS 500 F (Pyraclostrobin) in wheat and its processed products after two applications of BAS 500 06 F in Germany 2012/1067586 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.5.3/2	Plier S.	2013 d	Determination of residues of BAS 700 F (Fluxapyroxad) and BAS 500 F (Pyraclostrobin) in oat and its processed products after two applications of BAS 703 04 F in Germany, 2012 2013/1037950 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.5.3/3	Braun D.	2011 a	Determination of residues of BAS 500 F (Pyraclostrobin) and BAS 480 F (Epoxiconazole) in maize and its processed products after one application of BAS 512 04 F in Germany 2010/1144336 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 6.6.1/1	Rabe U., Kalyon B.	2014 b	Confined indicator rotational crop study with 14C-Pyraclostrobin 2014/1001761 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.9/1	Bross M., Mackenroth C.	2014 a	Pyraclostrobin (BAS 500 F): Refinement of the dietary exposure assessment of Pyraclostrobin metabolites in plant and animal commodities 2014/1001541 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 6.9/2	Xiangwei Y. et al.	2012 a	Dissipation of Pyraclostrobin and its metabolite BF-500-3 in maize under field conditions 2012/1366722 <none>, <none>, <none> no Published	No	No	Not applicable	public

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DOCUMENT L-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 7.1.1.1/1	Kuhnke G., Hassink J.	2014 a	Metabolism of (pyrazole-3-C14) BAS 500 F in soil under aerobic conditions 2013/1337273 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.1.1.3/1	Hassink J., Hermann M.	2014 a	Soil photolysis of (pyrazole-3-C14) BAS 500 F 2013/1341955 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.1.2.1.1/1	Kuhnke G., Hassink J.	2014 a	Metabolism of (pyrazole-3-C14) BAS 500 F in soil under aerobic conditions 2013/1337273 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.1.2.1.1/2	Staudenmaier H., Kuhnke G.	2013 a	Rate of degradation of 14C-Pyraclostrobin (BAS 500 F) in aerobic soil 2011/1102370 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 7.1.2.1.1/3	Eickler B.	2014 a	Kinetic evaluation of aerobic soil degradation of BAS 500 F - Pyraclostrobin: Determination of trigger and modeling endpoints according to Focus Degradation Kinetics 2014/1093424 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 7.1.2.1.2/1	Tornisielo A., Sacchi R.R.	2011 a	Rate of degradation of BF 500-6 on European soils under aerobic conditions 2011/1142307 BASF SA, Guaratingueta, Brazil yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.1.2.1.2/2	Tornisielo A., Sacchi R.R.	2011 b	Rate of degradation of BF 500-7 on European soils under aerobic conditions 2011/1142308 BASF SA, Guaratingueta, Brazil yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.1.2.1.2/3	Ebert D., Dalkmann P.	2014 a	Rate of degradation of BF 500-4 (Reg.No. 358672) in soil under aerobic conditions 2013/1294779 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 7.1.2.1.2/4	Schoof S., Possienke M.	2013 a	Rate degradation of BF 500-5 (Reg.No. 298327) in soil under aerobic conditions 2013/1294780 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.1.2.1.3/1	Pape L.	2014 a	Kinetic evaluation of anaerobic soil degradation studies for BAS 500 F - Pyraclostrobin according to Focus 2014/1000701 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 7.1.2.1.4/1	Ebert D., Dalkmann P.	2014 a	Rate of degradation of BF 500-4 (Reg.No. 358672) in soil under aerobic conditions 2013/1294779 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 7.1.2.2.1/1	Eickler B.	2014 b	Kinetic evaluation of two field dissipation studies with BAS 500 F - Pyraclostrobin conducted in Germany, Sweden and Spain: Determination of trigger and modeling endpoints according to Focus Degradation Kinetics and EFSA 2014/1093423 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 7.1.2.2.1/2	Bayer H., Marwitz A.	2014 a	Field soil dissipation study of BAS 500 F (Pyraclostrobin) in the formulation BAS 500 14 F on bare soil at four different sites in Europe, 2011-2012 2013/1348661 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.1.2.2.1/3	Pape L.	2014 a	Kinetic evaluation of a field dissipation study for BAS 500 F - Pyraclostrobin conducted in 2011 and 2012: Determination of best-fit endpoints according to Focus 2014/1105763 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 7.1.2.2.1/4	Pape L.	2014 b	Kinetic evaluation of a field dissipation study for BAS 500 F - Pyraclostrobin conducted in 2011 and 2012: Determination of modeling endpoints according to Focus 2014/1105764 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 7.1.2.2.1/5	Tilting N. et al.	2014 a	Stability of residues of BAS 500 F (Pyraclostrobin, Reg.No 304428) and its metabolites 500M01 (Reg.No. 364380), 500M02 (Reg.No. 369315), and 500M07 (Reg.No. 340266) in various soils under frozen conditions 2014/1000723 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.1.3.1.2/1	Ebert D. et al.	2014 a	Study of the adsorption behaviour of BF 500-6 (Reg.No. 364380) on different soils 2014/1000624 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 7.1.3.1.2/2	Ebert D. et al.	2014 a	Study of the adsorption behaviour of BF 500-7 (Reg.No. 369315) on different soils 2014/1000625 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.1.3.1.2/3	Tuffnail W.	2014 b	Adsorption/desorption of BF500-4 (Reg.No. 358672) on soil 2014/1000721 Quotient Bioresearch Ltd., Rushden Northamptonshire NN10 6ER, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.1.3.1.2/4	Tuffnail W.	2014 a	Adsorption/desorption of BF500-5 (Reg.No. 298327) on soil 2014/1000722 Quotient Bioresearch Ltd., Rushden Northamptonshire NN10 6ER, United Kingdom yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.2.1.1/1	Scharf J.	1999 b	Hydrolysis of BAS 500 F 1999/10060 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 7.2.1.1/2	Ebert D.	2011 a	Amendment No. 1: Hydrolysis of BAS 500 F 2011/1201705 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	No	Not applicable	BASF
KCA 7.2.2.2/1	Ebert D., Possienke M.	2013 a	14C-BAS 500 F (Pyraclostrobin): Aerobic mineralisation in surface water 2013/1002741 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.2.2.3/1	Wiedemann G.	2013 a	Kinetic evaluation of BAS 500 F - Pyraclostrobin in water/sediment systems under aerobic conditions 2012/1165029 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 7.2.2.4/1	Miles B.	2012 b	Kinetic evaluation of BAS 500 F in water/sediment systems under aerobic conditions 2012/1021122 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 7.2.2.4/2	Ebert D.	2012 a	Degradation of BAS 500 F in water/sediment under irradiated conditions 2011/1101715 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 7.3.1/1	Hassink J.	2013 a	Photochemical oxidative degradation of BAS 500 F (QSAR estimates) 2013/1350648 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

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DOCUMENT L-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-July-2014		BASF DocID 2014/1162241 (version 1)
09-June-2015	The document was amended in order to correct an error regarding the data protection claim of one study (KCA 8.2.1/6). New or changed text is marked in yellow.	BASF DocID 2015/1162001 (version 2)
27-Feb-2017	KCA 8.1.2.2/2: new reference KCA 8.1.2.2/3-6: revised numbering KCA 8.2.8/3: new reference KCA 8.2.8/4-8: revised numbering KCA 8.3.1.1.1/2: new reference KCA 8.3.1.1.2/2: new reference Changes are marked in blue.	BASF DocID 2017/1032920 (version 3)

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Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.1.1.1/1	[redacted]	2014 a	BAS 500 F (Pyraclostrobin) - Acute Toxicity in the canary (Serinus canaria) after single oral administration (LD50) 2013/1400375 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 8.1.2.1/1	[redacted]	2009 a	BAS 500 06 F: Acute toxicity testing study - Repeated dose oral toxicity study in Wistar rats 2009/1108893 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 8.1.2.1/2	[redacted]	2011 a	BAS 500 06 F - Repeated dose oral toxicity study in Wistar rats - Administration via the diet over 5 days 2011/1146588 [redacted] No Unpublished	Yes	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.1.2.1/3	[redacted]	2014 a	BAS 500 06 F: Study to assess avoidance and effects in wood mice (Apodemus sylvaticus) upon dietary exposure 2012/1129348 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 8.1.2.1/4	Mastitsky S. et al.	2014 a	A body burden model to assess the acute dietary risk posed by formulated Pyraclostrobin (BAS 500 06 F) to the common vole, Microtus arvalis 2014/1001603 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 8.1.2.2/1	[redacted]	2014 a	Field study on the acute and long-term effects of a Pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray in spring to cereals on populations of small mammals (wood mice and common voles) in Central Europe (Germany) 2014/1000041 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.1.2.2/2	Ziesemann B.	2016 a	Field study on the acute and long-term effects of a Pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray on meadows to populations of common voles in Central Europe (Germany) 2015/1126803 RIFCon GmbH, Hirschberg, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.1.2.2/3	Moreno S.	2013 a	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) on wheat (young plants) after treatment with BAS 500 06 F under field conditions in North and South Europe, season 2012 2013/1045207 Agricultura y Ensayo SL, Alcala de Guadaira, Spain yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.1.2.2/4	Martin T.	2013 a	Study on the residue behavior of Pyraclostrobin (BAS 500 F) on pea (young plants) after the application of BAS 500 06 F under field conditions in France (North), Germany, United Kingdom, Italy and Spain, 2012 2013/1044539 Agrologia SL, Utrera, Spain yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.1.2.2/5	Erzgraeber B.	2013 a	Dissipation of BAS 500 F - Pyraclostrobin on young plants (wheat and peas) - Trials conducted in the Northern Zone of Europe - Calculation of DT50 / DT90 dissipation times 2013/1078114 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 8.1.2.2/6	Erzgraeber B.	2013 b	Dissipation of BAS 500 F - Pyraclostrobin on young plants (wheat and peas) - Trials conducted in the Southern Zone of Europe - Calculation of DT50 / DT90 dissipation times 2013/1291161 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 8.1.4/1	Belden J. et al.	2010 a	Acute toxicity of fungicide formulations to amphibians at environmentally relevant concentrations 2014/1143801 <none>, <none>, <none> no Published	Yes	No	Not applicable	public

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.1.4/2	Bruehl C. et al.	2013 a	Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline? 2014/1143855 <none>, <none>, <none> no Published	Yes	No	Not applicable	public
KCA 8.2.1/1	[redacted]	2000 a	Flow-through acute toxicity of BAS 500 F to the rainbow trout, Oncorhynchus mykiss 2000/5034 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 8.2.1/2	[redacted]	2000b	Flow-through acute toxicity of BAS 500 F to the bluegill sunfish, Lepomis macrochirus 2000/5033 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 8.2.1/3	[redacted]	2000 c	Flow-through acute toxicity of BAS 500 F to the sheepshead minnow, Cyprinodon variegatus 2000/5032 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.1/4	[redacted]	2007 a	Reg.No. 340266 (metabolite BF 500-3 of BAS 500 F): Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in a static system over 96 hours 2007/1010836 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 8.2.1/5	[redacted]	2014 a	Acute toxicity of Reg.No. 298327 (BF 500-5, metabolite of Pyraclostrobin) to rainbow trout (Oncorhynchus mykiss) in a 96-hour static test 2013/1349200 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 8.2.1/6	[redacted]	2000 a	BAS 500 F - Acute toxicity study on the rainbow trout (Oncorhychus mykiss) after short time exposure over 0.5, 2 and 8 hours in a flow-through system followed up by a post exposure period 2000/1014919 [redacted] yes Unpublished	Yes	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.2.1/1	[redacted]	2000 i	Early life stage toxicity of BAS 500 F to the fathead minnow, Pimephales promelas 2000/5053 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 8.2.2.1/2	[redacted]	2001 j	Early life stage toxicity of BAS 500 F to the sheepshead minnow, Cyprinodon variegatus 2000/5247 [redacted] yes Unpublished	Yes	Yes	New data for AIR3 renewal	BASF
KCA 8.2.4.1/1	Bergtold M. Janson G.	2006 a	Acute toxicity of Reg.No. 340266 (metabolite of BAS 500 F) to Daphnia magna STRAUS in a 48 hour static test 2006/1038907 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.4.1/2	Kuhl R., Frank C.	2014 b	Acute toxicity of Reg.No. 298327 (BF 500-5, metabolite of Pyraclostrobin) to Daphnia magna in a static 48-hour immobilization test 2013/1349201 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.4.2/1	Boeri R.L. et al.	2000 d	Flow-through acute toxicity of BAS 500 F to the mysid, Americanysis bahia 2000/5031 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.4.2/2	Boeri R.L. et al.	2000 e	Flow-through mollusc shell deposition test with BAS 500 F 2000/5042 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.5/1	Wyskiel D.C. et al.	2004 a	BAS 500 F: A flow-through life-cycle toxicity test with the saltwater mysid Americamysis bahia 2004/5000004 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.5/2	Dinehart S.	2013 a	BAS 500 F: Life-cycle toxicity test of the saltwater mysid, Americamysis bahia, conducted under flow-through test conditions 2013/7002075 ABC Laboratories Inc., Columbia MO, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.5.3/1	Kuhl R., Wydra V.	2013 a	Effects of BAS 500 F (Pyraclostrobin) on the development of sediment dwelling larvae of Chironomus riparius in a sediment-water system - Exposed via spiked sediment 2012/1185699 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.5.3/2	Kuhl R., Wydra V.	2013 b	Effects of Reg.No. 340266 (metabolite of BAS 500 F (Pyraclostrobin), synonymous: 500M07, BF 500-3) on the development of sediment dwelling larvae of Chironomus riparius in a sediment-water system - exposed via spiked sediment 2013/1237446 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.5.3/3	Backfisch K.	2014 b	Chronic toxicity of Reg. No. 364380 (BF 500-6; metabolite of Pyraclostrobin) to the non-biting midge Chironomus riparius - A spiked sediment study 2014/1001481 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.5.3/4	Backfisch K.	2014 a	Chronic toxicity of Reg.No. 369315 (BF 500-7; Metabolite of Pyraclostrobin) to the non-biting midge Chironomus riparius - A spiked sediment study 2014/1001482 BASF SE, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.5.4/1	Gaertner K.	2013 a	BAS 500 F: Whole sediment acute toxicity to a marine amphipod (Leptocheirus plumulosus) 2013/7000055 ABC Laboratories Inc., Columbia MO, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.6.1/1	Dohmen G.P.	1999 a	Effect of BAS 500 F on the growth of the green alga Pseudokirchneriella subcapitata 1999/11020 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	No	Not applicable	BASF
KCA 8.2.6.1/2	Hoffmann F.	2009 a	Effect of BAS 500 F on the growth of the green alga Pseudokirchneriella subcapitata - Additional calculation of the inhibition values for growth rate and yield data after a test period of 72 h 2009/1037148 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.6.1/3	Hoffmann F.	2006 a	Effect of BF 500-3 (Reg.No. 340266, metabolite of BAS 500 F) on the growth of the green alga Pseudokirchneriella subcapitata 2006/1038445 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.6.1/4	Kuhl R., Frank C.	2014 c	Toxicity of Reg.No. 298327 (BF 500-5, metabolite of Pyraclostrobin) to Pseudokirchneriella subcapitata in an algal growth inhibition test 2013/1349202 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.6.2/1	Boeri R.L. et al.	2000 f	Growth and reproduction toxicity test with BAS 500 F and the freshwater alga, Navicula pelliculosa 2000/5046 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.6.2/2	Boeri R.L. et al.	2000 g	Growth and reproduction toxicity test with BAS 500 F and the freshwater alga, Anabaena flos-aquae 2000/5036 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.6.2/3	Boeri R.L. et al.	2000 k	Growth and reproduction toxicity test with BAS 500 F and the marine alga, Skeletonema costatum 2000/5035 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.7/1	Boeri R.L. et al.	2000 h	Growth and reproduction toxicity test with BAS 500F and the duckweed, Lemna gibba G3 2000/5037 T.R. Wilbury Laboratories Inc., Marblehead MA, United States of America yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.8/1	Dohmen G.-P.	2000 a	The effect of BAS 500 00 F on aquatic ecosystems - An outdoor mesocosm investigation 2000/1000011 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	No	No	Not applicable	BASF
KCA 8.2.8/2	Dohmen G.P.	2013 a	Mesocosm study evaluation (BAS 500 00 F) - The effect of BAS 500 00 F on aquatic ecosystems - An outdoor mesocosm investigation 2012/1357084 BASF SE, Limburgerhof, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF
KCA 8.2.8/3	Hommen U.	2016 a	MDD calculation for data sets of the BASF pond study on Pyraclostrobin (BASF DocID 2000/1000011) 2016/1345507 Fraunhofer-Institut fuer Molekularbiologie und Angewandte Oekologie, Schmallenberg, Germany Fed.Rep. no Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.2.8/4	Ding Y. et al.	2010 a	Toxicity of sediment associated pesticides to Chironomus dilutus and Hyalella azteca 2014/1143797 <none>, <none>, <none> no Published	No	No	Not applicable	public

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.2.8/5	Morrison S.A. et al.	2013 a	Acute toxicity of Pyraclostrobin and Trifloxystrobin to Hyalella azteca 2014/1143857 <none>, <none>, <none> no Published	No	No	Not applicable	public
KCA 8.2.8/6	Bringolf R.B. et al.	2007 a	Contaminant sensitivity of freshwater mussels - Acute and chronic toxicity of technial-grade pesticides to glochidia and juveniles of freshwater mussels (Unionidae) 2014/1143854 <none>, <none>, <none> no Published	No	No	Not applicable	public
KCA 8.2.8/7	Hooser E.A. et al.	2012 a	Acute toxicity of three Strobilurin fungicide formulations and their active ingredients to tadpoles 2014/1143796 <none>, <none>, <none> no Published	Yes	No	Not applicable	public
KCA 8.2.8/8	Hartman E.A.H. et al.	2014 a	Chronic effects of Strobilurin fungicides on development, growth and mortality of larval great plains toads (Bufo cognatus) 2014/1143856 <none>, <none>, <none> no Published	Yes	No	Not applicable	public

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.3.1/1	Mack P.	2014 a	Determination of residues of BAS 556 03 F in nectar, pollen and flowers of sunflowers after one application in 2013 2014/1000204 Eurofins Agroscience Services EcoChem GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.3.1/2	Barth M.	2014 a	Determination of residues of BAS 556 03 F in oilseed rape inflorescences and their respective honeybee food items 2014/1000182 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.3.1.1.1/1	Sekine T.	2013 a	Effects of BAS 500 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory 2013/1003210 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.3.1.1.1/2	Amsel K.	2016 a	Acute toxicity of BAS 500 F to the bumblebee Bombus terrestris L. under laboratory conditions 2016/1000530 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.3.1.1.2/1	Sekine T.	2013 a	Effects of BAS 500 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory 2013/1003210 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.3.1.1.2/2	Amsel K.	2016 a	Acute toxicity of BAS 500 F to the bumblebee Bombus terrestris L. under laboratory conditions 2016/1000530 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.4.1/1	Friedrich S.	2014 a	Sublethal toxicity of BAS 500 F (Pyraclostrobin) to the earthworm Eisenia fetida in artificial soil 2014/1000461 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.4.1/2	Ganssmann M.	2013 a	Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms Eisenia fetida in artificial soil 2013/1003174 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.4.1/3	Ganssmann M.	2013 b	Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms Eisenia fetida in artificial soil with 10% peat 2013/1224029 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 8.4.2/1	Ganssmann M.	2013 c	Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction of the collembola Folsomia candida in artificial soil with 5% peat 2013/1068054 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF
KCA 8.4.2/2	Ganssmann M.	2013 d	Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction of the collembola Folsomia candida in artificial soil with 5% peat 2013/1224030 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. yes Unpublished	No	Yes	New data for AIR3 renewal	BASF

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT L-CA, Section 9

LITERATURE DATA

Reference List

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Datapoint	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 9/1	Zander-El-Metwally M., Esswein U.	2014 a	Literature search report - Pyraclostrobin - Final draft - BASF confidential 2014/1172994 BASF SE, Ludwigshafen/Rhein, Germany Fed.Rep. no Unpublished	No	No	Not applicable	BASF

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Pyraclostrobin

DOCUMENT M-CA, Section 1

IDENTITY OF THE ACTIVE SUBSTANCE

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 1 IDENTITY OF THE ACTIVE SUBSTANCE

CA 1.1 Applicant

BASF SE
Crop Protection Division
P.O. Box 120
67114 Limburgerhof
Germany

Contact person:

(a) Contact: [redacted]
BASF SE
Agricultural Center
P.O. Box 120
67114 Limburgerhof
Germany
Telephone: [redacted]
Telefax:
E-mail:

(b) Alternative: [redacted]
BASF SE
Agricultural Center
P.O. Box 120
67114 Limburgerhof
Germany
Telephone: [redacted]
Telefax:
E-mail:

CA 1.2 Producer

Manufacturer of pyraclostrobin (legal entity):

BASF SE
67056 Ludwigshafen
Germany
Crop Protection Division:
P.O. Box 120
67114 Limburgerhof
Germany

Contact person: Please refer to CA 1.1 Applicant.

CA 1.3 Common Name Proposed or ISO-accepted and synonyms

Pyraclostrobin

CA 1.4 Chemical Name (IUPAC and CA nomenclature)

IUPAC: Methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)-(N-methoxy)carbamate

CA: Carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester

CA 1.5 Producer's Development Code Numbers

BASF Number: BAS 500 F

BASF Registry Number: Reg.No. 304428

CA 1.6 CAS, EC and CIPAC Numbers

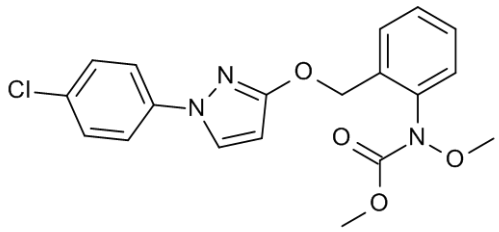
CAS: 175013-18-0

CIPAC: 657

EINECS not assigned

CA 1.7 Molecular and Structural Formula, Molar Mass

Structural formula:



Molecular formula: $C_{19}H_{18}ClN_3O_4$

Molecular mass: 387.8 g/mol

CA 1.8 Method of Manufacture (synthesis pathway) of the active substance

Confidential information - data provided in Document J.

CA 1.9 Specification of Purity of the Active Substance in g/kg

Minimum purity 975 g/kg, in accordance with 2004/30/EC (Annex I Inclusion Directive)

CA 1.10 Identity and Content of Additives (such as Stabilisers) and impurities

CA 1.10.1 Additives

Confidential information - data provided in Document J.

CA 1.10.2 Significant impurities

Confidential information - data provided in Document J.

CA 1.10.3 Relevant impurities

The manufacturing impurity dimethylsulfate (DMS) is considered to be of toxicological concern and must not exceed a concentration of 0.0001% in the technical product (Commission Directive 2004/30/EC of 10 March 2004).

CA 1.11 Analytical Profile of Batches

Confidential information - data provided in Document J.

BASF
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Pyraclostrobin

DOCUMENT M-CA, Section 2

PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

Compiled by:

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 2 PHYSICAL AND CHEMICAL PROPERTIES OF THE ACTIVE SUBSTANCE

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.1 Melting point and boiling point			Information was already reported and peer reviewed in the context of the EU Review Report SANCO/1420/2001-Final, 8. September 2004: Melting point: 63.7-65.2°C (99.8%) Boiling point: no boiling point up to decomposition at 200°C (99.8%)		EU Review Report SANCO/1420/2001-Final, 8. September 2004
CA 2.2 Vapour pressure, volatility			Information was already reported and peer reviewed in the context of the EU Review Report SANCO/1420/2001-Final, 8. September 2004: Vapour pressure: 2.6 x 10^{-8} Pa, 20°C Henry's law constant: 5.307 x 10^{-6} Pa m^3 mol^{-1}		EU Review Report SANCO/1420/2001-Final, 8. September 2004
CA 2.3 Appearance (Physical state, colour)	OPPTS 830.6303, FP0062/005, OPPTS 830.6302, FP0038/008, OPPTS 830.6303, FP0039/006	TGAI COD-001833 96.8%	Regarding the appearance of the purified active substance information was already reported and peer reviewed in the context of the EU Review Report SANCO/1420/2001-Final, 8. September 2004: Appearance PAI (99.8%): white to light beige crystalline solid Pyraclostrobin technical material (amorphous) was determined to be a solid, amber, glass like material.	Y	EU Review Report SANCO/1420/2001-Final, 8. September 2004 [see 2013/1399332 Kroehl T. 2013 a]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
		TGAI 10-510020 99.4%	Pyraclostrobin technical material (crystalline) was determined to be a solid, light yellow, odourless, fine crystalline powder.	Y	[see 2010/1126095 Kroehl T. 2010 a] Note: In the application erroneously the wrong study was mentioned.
CA 2.4 Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	OECD 101		Information was already reported and peer reviewed in the context of the EU Review Report SANCO/1420/2001-Final, 8.September 2004 UV-Vis absorption: 2.5 x 10^4 L mol^{-1} cm^{-1} at 205 nm 2.4 x 10^4 L mol^{-1} cm^{-1} at 275 nm (22°C, 99.8%)		EU Review Report SANCO/1420/2001-Final, 8. September 2004
		Dimethyl-sulfate SHBC4978V 99.8%	**Spectra of dimethyl sulfate (relevant impurity):** **Mass Spectrometry:** CI mass spectrum: monoisotopic molecular mass of protonated dimethyl sulfate at m/z = 127 u EI mass spectrum: confirms the accurate monoisotopic molecular mass of dimethyl sulfate as m/z = 125.9981 u. The spectrum shows characteristic fragment ions at 125 u, 96 u, 95 u, 79 u, 78 u, 66 u and 65 u, which also confirm the structure of dimethyl sulfate. **NMR Spectroscopy:** ^{1}H-NMR: The spectrum shows the methyl protons as the characteristic peak of dimethyl sulfate at a chemical shift of 3.93 ppm. ^{13}C-NMR: The spectrum shows the characteristic peak of the methyl carbon of dimethyl sulfate at a chemical shift of 58.5 ppm. The multiplet at 77.0 ppm is the solvent $CDCl_3$.	Y	[see 2014/1001441 Kroehl T., Behnken H.N. 2014 a]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
			IR Spectroscopy: 2968 cm^{-1}: C-H asymmetric stretching vibration of methyl groups 1459 cm^{-1}: C-H asymmetric bending vibration of methyl groups 1390 cm-1: S-O2 asymmetric stretching vibration of sulfates derivatives 1199 cm^{-1}: S-O_2 symmetric stretching vibration of sulfates derivatives 984 cm^{-1}: S-O symmetric stretching vibration of sulfates derivatives 827 cm^{-1}: S-O symmetric stretching vibration of sulfates derivatives The spectrum is in accordance with the proposed structure. **UV/Vis-Spectroscopy:** 199 nm: 0.109 $Lmol^{-1}cm^{-1}$ 279 nm: 0.019 $Lmol^{-1}cm^{-1}$ 290 nm: 0.017 $Lmol^{-1}cm^{-1}$		
CA 2.5 Solubility in water			Information was already reported and peer reviewed in the context of the EU Review Report SANCO/1420/2001-Final, 8. September 2004 1.9 ± 0.17 mg/L at 20°C in deionised water (pH of 5.8) There is no dissociation in water. Therefore, pH dependence on solubility is not applicable.		EU Review Report SANCO/1420/2001-Final, 8. September 2004

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.6 Solubility in organic solvents			Information was already reported and peer reviewed in the context of the EU Review Report SANCO/1420/2001-Final, 8. September 2004 <u>Solubility at 20°C:</u> n-heptane: 3.7 g/L 2-propanol: 30.0 g/L octanol: 24.2 g/L olive oil: 28.0 g/L methanol: 100.8 g/L acetone: >500 g/L ethyl acetate: >500 g/L acetonitrile: >500 g/L dichloro-methane: >500 g/L toluene: >500 g/L		EU Review Report SANCO/1420/2001-Final, 8. September 2004
CA 2.7 Partition co-efficient n-octanol/water			Information was already reported and peer reviewed in the context of the EU Review Report SANCO/1420/2001-Final, 8. September 2004 log Pow = 3.99 (20°C, 99.8%)		EU Review Report SANCO/1420/2001-Final, 8. September 2004
CA 2.8 Dissociation in water • dissociation constant(s) (pKa values) • identity of dissociated species • dissociation constant(s) (pKa values) of the active principle	OECD 112	PAI 01586-218 99.8%	The test substance does not dissociate, because no pH shift could be observed. Therefore, no pK_a could be determined.	Y	[see 2000/1012252 Daum A. 2000 a]

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.9 Flammability and self-heating	EEC A.10 EEC A.16	TGAI COD-001236 (solidified melt) 99.0% TGAI COD-001258 (crystalline) 99.9%	Neither the crystalline nor the amorphous material is considered highly flammable. No self-heating was detected.	Y Y Y	[see 2013/1002641 Achhammer G. 2013 a] [see 2011/1000601 Loehr S. 2011 a] [see 2011/1048145 Loehr S. 2011 b]
CA 2.10 Flash point	EEC A.9		Not applicable, as the test material has a melting point >40°C.		
CA 2.11 Explosive properties	EEC A.14	TGAI COD-001236 (solidified melt) 99.0% TGAI COD-001258 (crystalline) 99.9%	The test substance is not considered to exhibit a danger of explosion in the sense of the directive. The test for sensitivity to friction (friction test), impact (falling weight test) and thermal sensitivity (steel sleeve test) were all negative.	Y Y Y	[see 2013/1002641 Achhammer G. 2013 a] [see 2011/1000601 Loehr S. 2011 a] [see 2011/1048145 Loehr S. 2011 b]
CA 2.12 Surface Tension			Information was already reported and peer reviewed in the context of the EU Draft Assessment Report 2001 • 71.8 mN/m at 0.5% (w/w) (20°C) • 71.5 mN/m at 2.0% (w/w) (20°C)		EU Draft Assessment Report 2001 (Chapter B-2)

Test or Study & Data Point	Guideline and method	Test material purity and specification	Findings	GLP Y/N	Reference
CA 2.13 Oxidising properties	EEC A.17	TGAI COD-001236 (solidified melt) 99.0% TGAI COD-001258 (crystalline) 99.9%	The test material is not considered an oxidising substance in the sense of the directive, as the test mixture burned slower than the reference mixture.	Y Y Y	[see 2013/1002641 Achhammer G. 2013 a] [see 2011/1000601 Loehr S. 2011 a] [see 2011/1048145 Loehr S. 2011 b]
CA 2.14 Other studies			Not required		

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DOCUMENT M-CA, Section 3

FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162244 (version 1)
09-Jun-2015	3, 3.1, 3.2 and 3.6 were amended in order to better reflect the applicant's intention to achieve the renewal of approval for both, the use as fungicide and the use as plant growth regulator (new or changed text is marked in yellow).	BASF DocID 2015/1106130 (version 2)
27-Feb-2017	Chapters 3.8 - 3.10 were amended by the MSDS of pyraclostrobin TGAI and the text was updated where necessary (new or changed text is marked in blue).	BASF DocID 2017/1032923 (version 3)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 3 FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

Pyraclostrobin was included in Annex I of Directive 91/414/EEC on 1 June 2004 (entry into force) under Inclusion Directive 2004/30/EC for **the use as fungicide**. This use was amended on 22 April 2009 (entry into force) by Inclusion Directive 2009/25/EC to **the use as fungicide or plant growth regulator**. Both Annex I inclusion decisions were based on the same endpoints and provisions.
It is the intention of the applicant to achieve the renewal of approval for the active substance pyraclostrobin for the use as fungicide as well as the use as plant growth regulator. Therefore both uses are addressed in the chapters below if appropriate.

CA 3.1 Use of the Active Substance

Pyraclostrobin is a strobilurine **fungicide** which is used worldwide in many crops for the control of a broad range of important pathogens from the classes of ascomycetes, basidiomycetes, deuteromycetes and oomycetes. Pyraclostrobin is active against different fungal stages both on the plant surface and in the plant tissue. After application to the plant, the active ingredient is taken up via the leaf and then translocated at low rates via the transpiration flow. Due its relatively low mobility, it shows local systemic and translaminar activity. Because of its very high intrinsic activity, pyraclostrobin has been observed to have systemic effects in a number of authorized uses. By that, it can control fungal stages which have already become established in deeper tissue layers. Pyraclostrobin is thus suitable for preventative and curative treatments. Since the vapour pressure of pyraclostrobin is very low, a marked gas phase activity was not observed.
In addition to the **fungicidal effects, plant physiology is also affected** by the application of pyraclostrobin. Among these effects higher yield and better product quality in absence of diseases as well as improvement of the assimilation rate and delayed senescence have been reported. Some studies showed better stress tolerance to abiotic stresses (e.g. drought or frost). Furthermore, a reduction of physiological leaf spots in cereals has been observed after treatment with pyraclostrobin-containing products. Based on these effects, pyraclostrobin is approved as **plant growth regulator** in the EU in addition to its approval as fungicide.

CA 3.2 Function

Pyraclostrobin is used as a fungicide to control harmful diseases in a broad range of crops. In addition to the fungicidal effects, plant physiology is also affected by the application of pyraclostrobin (use as plant growth regulator). For details please see M-CA 3.1 and M-CA 3.6.

CA 3.3 Effects on Harmful Organisms

Pyraclostrobin is active against different fungal stages on and in the plant. When applied protectively, pyraclostrobin prevents not only the germination of fungal spores landing on the plant surface but also re-infection, since during these extremely energy-consuming phases fungi react very sensitively to disturbances of their mitochondrial respiratory chain. Due to its ability to penetrate into the leaf and its further translocation as well as its high intrinsic activity, it can also control fungal stages which have already become established in deeper tissue layers. Pyraclostrobin is thus suitable for preventative and curative treatments.

CA 3.4 Field of Use Envisaged

Agriculture

CA 3.5 Harmful Organisms Controlled and Crops or Products Protected or Treated

Pyraclostrobin is used to control a broad range of important fungal diseases such as

- *Alternaria* spp.
- *Bipolaris* spp.
- *Blumeriella* spp.
- *Botrytis* spp.
- *Cercospora* spp.
- *Colletotrychum* spp.
- *Drechslera* spp.
- *Exserohilum* spp.
- *Fusarium* spp.
- *Gnomonia* spp.
- *Guignardia* spp.
- *Hemileia* spp.
- *Kabatiella* spp.
- *Leptosphaeria* spp.
- *Monilinia* spp.
- *Neofabrea* spp.
- *Phomopsis* spp.
- *Phyllosticta* spp.
- *Plasmopara* spp.
- *Podosphaera* spp.
- *Puccinia* spp.
- *Pyrenophora* spp.
- *Ramularia* spp.
- *Rhynchosporium* spp.
- *Sclerotinia* spp.
- *Septoria* spp.
- *Sphaerotheca* spp.
- *Stemphylium* spp.
- *Uncinula* spp.
- *Uromyces* spp.
- *Venturia* spp.
- *Wilsonomyces* spp.

Pyraclostrobin is used in a wide range of crops including (representative uses in bold):

Cereals

Corn

Beets

Legume crops
- Dry pulses (dry harvest)
  - Beans: field beans
  - Peas: chickpeas, field peas, chickling vetch
- Legume vegetables (fresh harvest)
  - Beans (with and without pods)
  - Peas (with and without pods)

Vegetables
- Brassica vegetables
  - Head cabbage
  - Leafy cabbage
  - Flowering cabbage
  - Brussel sprouts
- Bulb vegetables
  - Onions (incl. spring onions)
  - Shallot
  - Garlic
- Root and tuber vegetables
  - Carrots
  - **Potato**
  - Radish
  - Parsnip
- Stem vegetables
  - Leek
  - Celery
  - Asparagus
  - Fennel
- Fruiting vegetables
  - Cucurbits with edible peel (cucumber, gherkins, courgette)
  - Cucurbits with inedible peel (melon, pumpkin, zucchini)
  - Tomato
  - Aubergine
  - Pepper
- Leafy vegetables and herbs
  - Lettuce and similar
  - Fresh herbs
  - Cichory, witloof

Perennial crops
- Berries and small fruits
  - Strawberries
  - Currants
  - Raspberries
- Grapes
- Hop
- Nuts
- Pomefruit
- Stonefruit

Others
- Ornamentals
- turf

CA 3.6 Mode of Action

Use as fungicide:
Pyraclostrobin belongs to the QoI group of fungicides. The mode of action is the inhibition of mitochondrial respiration resulting from a blockage of the electron transport from ubihydroquinone to cytochrome c by means of a binding to the ubihydroquinone oxidation centre (Qo) of the cytochrome bc1 complex (Complex III). This disrupts the mitochondrial electron transport chain, thus blocking phosphorylation further down in the respiratory chain. In consequence, this leads to a reduction of energy-rich ATP which is required to support a range of essential processes in the fungal cell such as maintenance of membrane potentials and concentration gradients up to DNA, RNA and protein biosynthesis. In the end, the various fungal development processes of spore germination, formation of infection structures, mycelium growth and sporulation are permanently disrupted.

Use as plant growth regulator:
In addition to the effects on yield through inhibition of fungal pathogens, pyraclostrobin also delivers a positive effect on yield through influence on the plant metabolism and physiology.
A decrease in ethylene levels has been demonstrated in several lab studies after treatment with pyraclostrobin. This can explain the observed delay in senescence. The reduction in ethylene levels can result in a variety of cellular changes being involved in yield gain (e.g. by an increased and longer photosynthetic performance during the vegetation period of a treated crop):
- enhancement of cytokinin levels resulting in increased chlorophyll concentrations in the leaves
- higher catalase and superoxide dismutase (SOD) activities reducing reactive O_2^--levels producing e.g. H_2O_2, resulting in reduced chlorophyll degradation

Furthermore, studies demonstrated that pyraclostrobin increases nitrate reductase, improving nitrogen assimilation and nitrogen use efficiency.

CA 3.7 Information on Occurrence or Possible Occurrence of the Development of Resistance and Appropriate Management Strategies

QoI resistant strains of some plant pathogenic fungal species were identified in some areas after intensive use and high selection pressure. Information which species are affected and to what extent can be followed in the annual minutes of the QoI Working Group of the Fungicide Resistance Action Committee (FRAC) on the FRAC web page (www.frac.info). Most important resistance mechanisms to QoIs are target site mutations, in particular the G143A in the cytochrome *b* (cyt *b*). G143A leads to strong resistance and reduced field efficacy. Two other mutations, F129L and G137R have been described, which lead to lower resistance factors. All QoI fungicides are in the same cross-resistance group and should be managed accordingly.

However, until now several fungal species did not develop field resistance to QoI fungicides. These include different rust species, *Pyrenophora teres* and others. *Puccinia triticina* experienced a comparable selection pressure as *Blumeria graminis* (a species which developed QoI resistance 1-2 years after market launch), but so far no sensitivity changes have been detected. Also, no adaptation has been found for the soybean rust pathogen *Phakopsora pachyrhizi*, despite intensive use of QoIs in large areas. The reason for this was elucidated by Grasso *et al*. (2006), who showed that the structure of the target gene is responsible: If an intron sequence follows directly after codon 143, the G143A does not occur, because the glycine codon 143 is part of the signal sequence which is essential for the intron recognition and plays an essential role in splicing during mRNA maturation. If this codon is altered, no functional complex III can be formed. Therefore it can be concluded, that for a species with an intron sequence directly after codon 143, the G143A is unlikely to occur. However, mutations F129L or G137R are possible. F129L occurs frequently in some European populations of *P. teres*, but due to low resistance factors caused by F129L, field efficacy of pyraclostrobin remains good (Semar *et al.* 2007). In other species like *P. triticina* or *P. pachyrhizi*, no mutations in the cyt *b* gene have been reported so far. The gene structure around codon 143 seems to be highly conserved within a species, even for isolates with a diverse geographic, host plant or historic (year of isolation) background. *C*yt *b* of thousands of *P. teres* isolates was analyzed in our laboratory in the last decade and all of them were identical. No G143A mutated isolates showed up in *P. triticina* or *P. pachyrhizi* after many years of QoI selection pressure which confirms the high intraspecific conservation of this gene sequence. Therefore, it is likely that the whole population of a species contains the intron at the same position when it has been detected for some isolates. However, *Botrytis cinerea* seems to be an exception. Two types of cyt *b* gene, (with and without intron, Jiang *et al.* 2009) exist in *B. cinerea* and the genotype without intron developed G143A in various crops and regions. So far published “intron pathogens” are listed in Table 3.7-1.

Table 3.7-1: Plant pathogenic fungi with intron after codon 143 in the cytochrome *b* gene

Pathogen	Disease	Host	Reference
Alternaria dauci	Leaf blight	Carrots	Stammler 2012
Alternaria grandis	Early blight	Tomatoes, potatoes	Stammler 2012
Alternaria solani	Early blight	Tomatoes, potatoes	Grasso *et al*. 2006
Alternaria tomatophila	Early blight	Tomatoes, potatoes	Stammler 2012
Bipolaris maydis	Southern leaf blight	Corn	Stammler 2012
Cercospora zea-maydis	Gray leaf spot	Corn	Stammler 2012
Cochliobolus carbonum	Northern leaf spot	Corn	Stammler 2012
Guignardia bidwellii	Black rot	Grapes	Miessner *et al.* 2011
Hemileia vastatrix	Rust	Coffee	Grasso *et al*. 2006
Monilinia fructicola	Blossom blight, brown rot	Stonefruits	Miessner & Stammler 2010
Monilinia laxa	Blossom blight, brown rot	Stonefruits	Miessner & Stammler 2010
Phakopsora pachyrhizi	Rust	Soy beans	Grasso *et al.* 2006
Phyllosticta citricarpa	Citrus black spot	Citrus species	Stammler *et al.* 2012
Puccinia spp.	Rust	Cereals, corn, others	Grasso *et al.* 2006
Pyrenophora teres	Net blotch	Barley	Grasso *et al.* 2006
Setosphaeria turcica	Northern leaf blight	Corn	Stammler 2012
Uromyces appendiculatus	Rust	Beans	Grasso *et al.* 2006

Resistance management strategies including modifiers such as limiting the number of QoI-applications, alternation and combination with other modes of action have been worked out by the FRAC QoI Working Group per crop and pathogen. These recommendations are annually reviewed and actual yearly sensitivity monitoring data are considered for adaptating the resistance management strategies in order to maintain the activity against various plant pathogens in different crops and regions. The current recommendations are available on the FRAC webpage (www.frac.info).

References

Grasso V., Palermo S., Sierotzki H., Garibaldi A. and Gisi U. (2006) Cytochrome *b* gene structure and consequences for resistance to Qo inhibitor fungicides in plant pathogens. *Pest Management Science* **62**, 465-472

Jiang J., Ding L., Michaelidis T.J., Hongye L. and Ma Z. (2009) Molecular characterization of field azoxystrobin-resistant isolates of *Botrytis cinerea*. *Pesticide Biochemistry and Physiology* **93,** 72-76

Miessner S. and Stammler G. (2010) *Monilinia laxa, M. fructigena* and *M. fructicola*: Risk estimation of resistance to QoI fungicides and identification of species with cytochrome b gene sequences. *Journal of Plant Diseases and Protection* **117**, 162-167

Miessner S., Mann W., and Stammler G. (2011) *Guignardia bidwellii*, the causal agent of black rot of grapevine has a low risk for QoI resistance. *Journal of Plant Diseases and Protection* **118**, 51-53

Semar M., Strobel D., Koch A., Klappach K. and Stammler G. (2007) Field efficacy of pyraclostrobin against populations of *Pyrenophora teres* containing the F129L mutation in the cytochrome *b* gene. *Journal of Plant Diseases and Protection* **114**, 117-119

Stammler G. (2012) Resistance risk of corn pathogens to QoI fungicides. *Outlooks on Pest Management* **23**, 211-214

CA 3.8 Methods and Precautions Concerning Handling, Storage, Transport or Fire

Report:	CA 3.8/1 Anonymous, 2016 a Safety data sheet - Pyraclostrobin techn. 2016/1223395
Guidelines:	EEC 1907/2006
GLP:	no

Personal protection: Control parameters

Components with workplace control parameters: No occupational exposure limits known.

Exposure controls

Personal protective equipment

Respiratory protection:
Suitable respiratory protection for lower concentrations or short-term effect: Particle filter with high efficiency for solid and liquid particles (e.g. EN 143 or 149, Type P3 or FFP3).

Hand protection:
Suitable chemical resistant safety gloves (EN 374) also with prolonged, direct contact (Recommended: Protective index 6, corresponding > 480 minutes of permeation time according to EN 374): E.g. nitrile rubber (0.4 mm), chloroprene rubber (0.5 mm), butyl rubber (0.7 mm) and other

Eye protection:
Safety glasses with side-shields (frame goggles) (e.g. EN 166)

Body protection:
Body protection must be chosen depending on activity and possible exposure, e.g. apron, protecting boots, chemical-protection suit (according to EN 14605 in case of splashes or EN ISO 13982 in case of dust).

General safety and hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wearing of closed work clothing is recommended. Store work clothing separately. Keep away from food, drink and animal feeding stuffs.

Precautions for safe handling

No special measures necessary if stored and handled correctly. Ensure thorough ventilation of stores and work areas. When using do not eat, drink or smoke. Hands and/or face should be washed before breaks and at the end of the shift. Remove contaminated clothing and protective equipment before entering eating areas.

Protection against fire and explosion:
Prevent electrostatic charge - sources of ignition should be kept well clear - fire extinguishers should be kept handy. Avoid influence of heat.
In addition, for crystalline pyraclostrobin:
Avoid dust formation. Dust can form an explosive mixture with air.

Conditions for safe storage, including any incompatibilities

Segregate from foods and animal feeds.
Further information on storage conditions: Keep away from heat. Protect against moisture. Protect from direct sunlight.

Storage stability:
Storage duration: 24 months

Protect from temperatures above: 40 °C
Changes in the properties of the product may occur if substance/product is stored above indicated temperature for extended periods of time.

Transport

Land transport

ADR

- Hazard class: 9
- Packing group: III
- ID number: UN 3077
- Hazard label: 9, EHSM
- Proper shipping name: ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains PYRACLOSTROBIN)

RID

- Hazard class: 9
- Packing group: III
- ID number: UN 3077
- Hazard label: 9, EHSM
- Proper shipping name: ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains PYRACLOSTROBIN)

Inland waterway transport

ADN

- Hazard class: 9
- Packing group: III
- ID number: UN 3077
- Hazard label: 9, EHSM
- Proper shipping name: ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains PYRACLOSTROBIN)

Sea transport

IMDG

- Hazard class: 9
- Packing group: III
- ID number: UN 3077
- Hazard label: 9, EHSM
- Marine pollutant: YES
- Proper shipping name: ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains PYRACLOSTROBIN)

Air transport

IATA/ICAO

- Hazard class: 9
- Packing group: III
- ID number: UN 3077
- Hazard label: 9, EHSM
- Proper shipping name: ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains PYRACLOSTROBIN)

Fire-fighting measures

Extinguishing media

Suitable extinguishing media: dry powder, foam, water spray
Unsuitable extinguishing media for safety reasons: carbon dioxide, water jet

Special hazards arising from the substance or mixture

Carbon monoxide, hydrogen chloride, carbon dioxide, nitrogen oxides, organochloric compounds might be released in case of fire.

Advice for fire-fighters

Special protective equipment:
Wear self-contained breathing apparatus and chemical-protective clothing.

Further information:
Keep containers cool by spraying with water if exposed to fire. Dispose of fire debris and contaminated extinguishing water in accordance with official regulations. Collect contaminated extinguishing water separately, do not allow to reach sewage or effluent systems. In case of fire and/or explosion do not breathe fumes.

CA 3.9 Procedures for Destruction or Decontamination

Report:	CA 3.9/1 Anonymous, 2016 a Safety data sheet - Pyraclostrobin techn. 2016/1223395
Guidelines:	EEC 1907/2006
GLP:	no

Waste treatment methods

Must be disposed of or incinerated in accordance with local regulations.

Contaminated packaging:
Contaminated packaging should be emptied as far as possible and disposed of in the same manner as the substance/product.

Methods and material for containment and cleaning up

For small amounts: Pick up with suitable appliance and dispose of.

For large amounts: Sweep/shovel up.
Dispose of absorbed material in accordance with regulations. Collect waste in suitable containers, which can be labeled and sealed. Clean contaminated floors and objects thoroughly with water and detergents, observing environmental regulations. Cleaning operations should be carried out only while wearing breathing apparatus.

CA 3.10 Emergency Measures in Case of an Accident

Report:	CA 3.10/1 Anonymous, 2016 a Safety data sheet - Pyraclostrobin techn. 2016/1223395
Guidelines:	EEC 1907/2006
GLP:	no

First-aid measures

Description of first aid measures

First aid personnel should pay attention to their own safety. If the patient is likely to become unconscious, place and transport in stable sideways position (recovery position). Immediately remove contaminated clothing.

If inhaled:
Keep patient calm, remove to fresh air, seek medical attention.

On skin contact:
Immediately wash thoroughly with soap and water, seek medical attention.

On contact with eyes:
Wash affected eyes for at least 15 minutes under running water with eyelids held open, consult an eye specialist.

On ingestion:
Immediately rinse mouth and then drink 200-300 ml of water, seek medical attention.

Most important symptoms and effects, both acute and delayed

The most important known symptoms are skin irritation caused by skin contact or irritation of the respiratory tract.

Indication of any immediate medical attention and special treatment needed

Treatment: Treat according to symptoms (decontamination, vital functions), no specific antidote known.

Personal precautions, protective equipment and emergency procedures

Use personal protective clothing. Avoid contact with the skin, eyes and clothing. Avoid dust formation.

Environmental precautions

Do not discharge into drains/surface waters/groundwater. Do not discharge into the subsoil/soil.

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DOCUMENT M-CA, Section 4

ANALYTICAL METHODS

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Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162245 (version 1)
27-Feb-2017	Addition of further metabolites in Table 4-1; CA 4.1.2/3: replacement of report and update of summary; CA 4.1.2/7 - CA 4.1.2/12: addition of new validation reports for new methods used to support additional toxicological studies (numbering of following documents and tables corrected accordingly); CA 4.1.2/16: addition of new validation report (numbers of following documents and tables corrected accordingly); CA 4.2/5: replacement by amended new report and update of summary; New or changed text is marked in yellow.	BASF DocID 2017/1032924 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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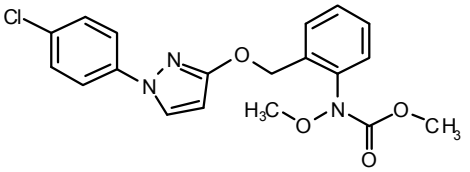
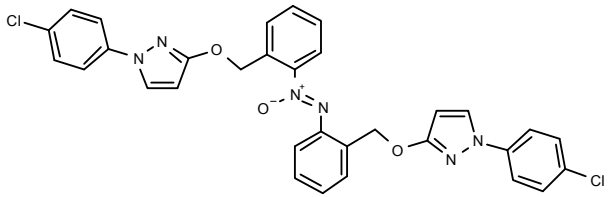
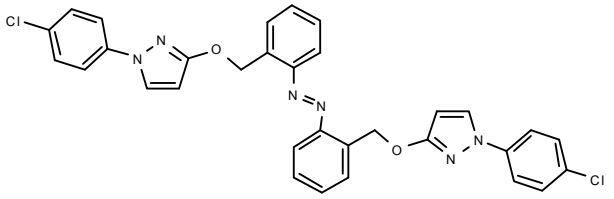
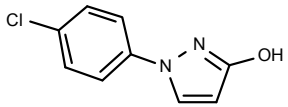
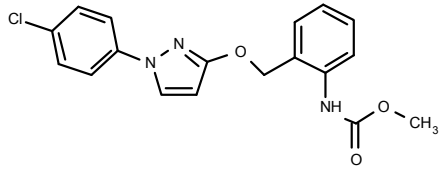
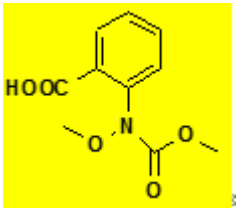
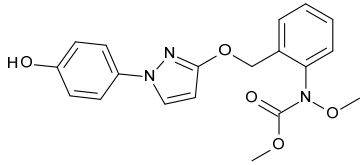
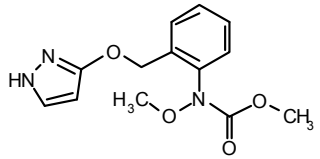
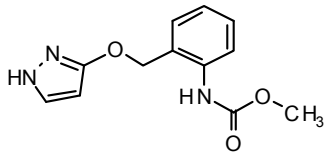
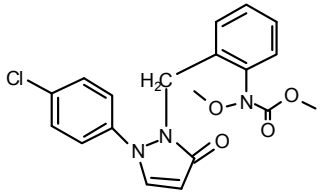
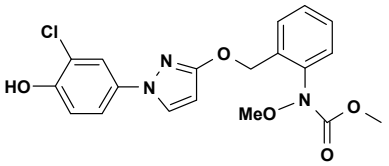
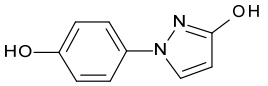
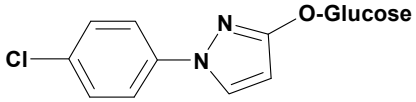
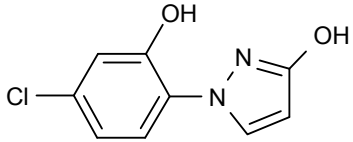
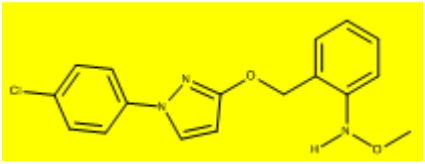
The table below provides an overview on the metabolites being of relevance for the analytical method section including the different code numbers that are available for each metabolite. Due to historic reasons (e.g. use of different metabolite codes in different study reports), it is unfortunately not possible to use always only one and the same metabolite code for a certain metabolite. In the following chapters and study summaries synonym metabolite codes are given in brackets where deemed to be helpful.

Table 4-1: Pyraclostrobin: Substances and metabolites, structures, codes, synonyms

Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**		
500M00	304428	BAS 500 F	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)-(N-methoxy)carbamate 175013-18-0		
500M01	364380	BF 500-6	N,N'-bis-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)diazene N-oxide not assigned	soil, sediment	
500M02	369315	BF 500-7	N,N'-bis-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)diazene not assigned	soil, sediment	

Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**		
500M04	298327	BF 500-5	1-(4-chlorophenyl)-1H-pyrazol-3-ol 76205-19-1	soil, water, crops, hen, goat	
500M07	340266	BF 500-3	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) carbamate 512165-96-7	soil, sediment, crops, hen, goat	
500M24	5916421		2-[methoxy1 methoxycarbonyl]amino benzoic acid not assigned	wheat, rat	
500M59	412053	BF 500-12	methyl N-(2-{[1-(4-hydroxyphenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-methoxy carbamate not assigned	water (photolysis)	
500M60	411847	BF 500-11	methyl N-methoxy N-{2-[(1H-pyrazol-3-yl)oxymethyl]phenyl}carbamate 175013-17-9	water (photolysis)	

Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**		
500M62	412785	BF 500-13	methyl N-[2-(1H-pyrazol-3-yloxymethyl) phenyl]carbamate not assigned	water (photolysis)	
500M76	413038	BF 500-14	methyl N-{2-[2-(4-chlorophenyl)-5-oxo-2,5-dihydro-pyrazol-1-ylmethyl]- phenyl} N-methoxy carbamate not assigned	water (photolysis)	
500M77	4001763	BF 500-16	methyl N-(2-{[1-(3-chloro-4 hydroxyphenyl)-1H-pyrazol-3-yl] oxymethyl}phenyl) N-methoxy carbamate not assigned	hen	
500M78	377613	BF 500-15	1-(4-hydroxyphenyl)-1H-pyrazol-3-ol not assigned	water (photolysis)	
500M79	5937091	not applicable	(2S,3R,4S,5S,6R)-2-[1-(4-chlorophenyl)pyrazol-3-yl]oxy-6-(hydroxymethyl)tetrahydropyran-3,4,5-triol not assigned	crops	

Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**		
500M85	399530	BF 500-8	1-(4-chloro-2-hydroxyphenyl)-1 H-pyrazol-3-ol not assigned	goat	
500M106	399379		N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) O-methylhydroxylamine not assigned	rat (*in-vivo*), human and rabbit (*in-vitro*)	

CA 4 ANALYTICAL METHODS

CA 4.1 Methods used for the generation of pre-approval data

CA 4.1.1 Methods for the analysis of the active substance as manufactured

(a) Determination of the pure active substance in the active substance as manufactured and specified in the dossier submitted in support of approval under Regulation (EC) No 1107/2009

Pyraclostrobin is determined according to method APL0250/01, which was submitted and evaluated within the previous Annex I inclusion process and which was included in the DAR. Please consider that APL0250/01 is a newly allocated method number replacing the previous method number CP266. The analytical method is described in BASF DocID 1996/11507 and the validation in BASF DocID 1997/10691.

After Annex I inclusion the following CIPAC method has been developed and will be submitted within this chapter. A summary can be found below.

Report:	CA 4.1.1/1 Anonymous, 2007a Determination of Pyraclostrobin in Pyraclostrobin technical, Pyraclostrobin technical concentrate, Pyraclostrobin emulsifiable concentrates and Pyraclostrobin water dispersible granules (CIPAC MT 657) 2007/1017547
Guidelines:	none
GLP:	no

Principle of the method
The content of pyraclostrobin is determined by reverse phase high performance liquid chromatography with UV detection and external calibration. The separation of the samples is achieved by using gradient elution, and quantification of the active ingredient by UV-detection at 275 nm and comparison with reference material.

Chromatographic conditions (typical)

Column:	stainless steel, 250 x 4.6 (i.d.) mm, packed with Zorbax Eclipse XDB-C 18 or equivalent material with the same selectivity
Mobile phase:	water - acetonitrile - acetic acid, 750 + 250 + 1 (v/v)
Column temperature:	ambient

Flow rate program:

time (min)	Flow rate (ml/min)
0	1.0
7.3	1.0
7.5	2.0
13.8	2.0
14.0	1.0
16.0	1.0

Detector wavelength:	275 nm
Injection volume:	5 µl
Run time:	approximately 16 min
Retention time:	approximately 6.3 min

Validation

The method was peer-reviewed in a CIPAC ring test with the following results:

Repeatability r	= 17 g/kg at 996 g/kg active substance content
Reproducibility R	= 20 to 29 g/kg at 996 g/kg active substance content

(b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured

Significant impurities and additives:

Confidential information - data provided in Document J.

Relevant impurities:

Dimethyl sulfate is determined according to method M97/0028/02, which was submitted and evaluated within the previous Annex I inclusion process and which was included in the confidential part of the DAR. The analytical method is described in BASF DocID 1999/11896 and the validation in BASF DocID 1999/11956.

CA 4.1.2 Methods for risk assessment

Note: The order of the study summaries is differing compared to the information given in the application submitted for renewal of approval. In case references are summarized that were not contained in the application or in case references listed in the application are not contained in this chapter, comments are made where appropriate.

(a) Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Since the analytical methods for determination of residues of pyraclostrobin and its metabolites in soil, water and air presented in the previous dossier are rather old, new analytical methods were developed for environmental matrices according to newest guidelines and considering the current state of art techniques used in analytical laboratories.

In addition to the studies listed in the application for renewal of approval, two further methods are submitted for completeness: A parent only method for soil (CA 4.1.2/2) and a multi-pesticide method for water (CA 4.1.2/3). These studies were used in the new field soil dissipation study (CA 7.1.2.2.1/2) for application verification analytics of petri dish soil samples directly after application and for spray broth analysis.

Soil

Pyraclostrobin (BAS 500 F) and its metabolites

Report:	CA 4.1.2/1 Tilting N., Sopena-Vazquesz F., 2014a Validation of analytical method L0166/01: Determination of BAS 500 F (Pyraclostrobin) and its metabolites Reg.No. 364380 (500M01), Reg.No. 369315 (500M02) and Reg.No. 340266 (500M07) in soil using LC/MS/MS 2013/1184817
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the methods

Soil samples (5 g) are placed into a PP tube and extracted with 25 mL acetonitrile by mechanical shaking for 30 min at 225 rpm. Subsequently, the sample is centrifuged and the liquid phase is decanted and evaporated to dryness. The extraction is repeated with 25 mL acetonitrile / water (80/20, v/v). After centrifugation, the liquid phase from the second extraction is separated and used to re-dissolve the dried residue from the first extraction. An additional dilution with acetonitrile / water (80/20, v/v) was performed when necessary (final volume = 50 mL) before LC-MS/MS measurement.

The method was validated at two fortification levels (0.001 and 0.01 mg kg^{-1}) for soils from different origins: two LUFA standard soils (LUFA 5M and LUFA 2.2 soils) and two soils freshly collected from the field (Italian and German field soil).

Recovery findings

The method L0166/01 was proved to be suitable to determine residues of pyraclostrobin and its metabolites BF 500-6 (500M01), BF 500-7 (500M02) and BF 500-3 (500M07) in different soils using LC-MS/MS with a limit of quantification (LOQ) of 0.001 mg kg^{-1} and a limit of detection (LOD) of 0.0002 mg kg^{-1}. Validation experiments were conducted in four different soils for pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3. The mean recovery values for pyraclostrobin and its metabolites were between 70 and 110%. The detailed results are given in Table 4.1.2-1 and Table 4.1.2-2.

Table 4.1.2-1: Validation results of a residue analytical method for pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3 in soil (LUFA 5M and LUFA 2.2)

Soil	Analyte	m/z	No. of replicates	Fortification level [mg kg^{-1}]	Mean Recovery [%]	RSD [%]
LUFA 5M	pyraclostrobin (BAS 500 F)	388 → 194	5	0.001	100.1	2.4
			5	0.01	96.5	2.8
		388 → 163	5	0.001	101.4	2.5
			5	0.01	95.7	1.6
	BF 500-6 (500M01)	611 → 417	5	0.001	101.9	8.9
			5	0.01	98.4	3.8
		611 → 223	5	0.001	102.0	4.5
			5	0.01	100.1	4.7
	BF 500-7 (500M02)	595 → 401	5	0.001	98.2	3.2
			5	0.01	96.3	3.6
		595 → 207	5	0.001	101.5	3.1
			5	0.01	97.7	5.0
	BF 500-3 (500M07)	358 → 164	5	0.001	103.4	4.4
			5	0.01	96.9	4.4
		358 → 132	5	0.001	98.7	5.3
			5	0.01	94.9	4.6
LUFA 2.2	pyraclostrobin (BAS 500 F)	388 → 194	5	0.001	99.3	2.2
			5	0.01	98.9	1.7
		388 → 163	5	0.001	102.8	2.1
			5	0.01	98.0	3.1
	BF 500-6 (500M01)	611 → 417	5	0.001	95.6	2.7
			5	0.01	96.3	7.5
		611 → 223	5	0.001	99.7	1.8
			5	0.01	98.8	5.1
	BF 500-7 (500M02)	595 → 401	5	0.001	89.2	1.5
			5	0.01	87.8	1.8
		595 → 207	5	0.001	96.5	3.2
			5	0.01	95.3	3.2
	BF 500-3 (500M07)	358 → 164	5	0.001	100.1	4.1
			5	0.01	97.2	3.0
		358 → 132	5	0.001	100.5	2.9
			5	0.01	98.8	2.5

Table 4.1.2-2: Validation results of a residue analytical method for pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3 in soil (Field Soil Italy and Field Soil Germany)

Soil	Analyte	m/z	No. of replicates	Fortification level [mg kg^{-1}]	Mean Recovery [%]	RSD [%]
Field Soil Italy	pyraclostrobin (BAS 500 F)	388 → 194	5	0.001	102.1	2.5
			5	0.01	103.6	2.2
		388 → 163	5	0.001	101.9	2.4
			5	0.01	104.2	4.7
	BF 500-6 (500M01)	611 → 417	5	0.001	97.0	5.8
			5	0.01	98.6	3.7
		611 → 223	5	0.001	96.6	4.2
			5	0.01	97.3	3.8
	BF 500-7 (500M02)	595 → 401	5	0.001	100.6	2.3
			5	0.01	95.6	4.0
		595 → 207	5	0.001	102.4	1.9
			5	0.01	96.8	1.7
	BF 500-3 (500M07)	358 → 164	5	0.001	97.6	2.4
			5	0.01	99.1	5.2
		358 → 132	5	0.001	100.7	3.4
			5	0.01	103.1	4.4
Field Soil Germany	pyraclostrobin (BAS 500 F)	388 → 194	5	0.001	100.2	3.5
			5	0.01	97.9	5.6
		388 → 163	5	0.001	98.3	1.5
			5	0.01	98.5	8.4
	BF 500-6 (500M01)	611 → 417	5	0.001	94.7	3.6
			5	0.01	88.8	7.6
		611 → 223	5	0.001	94.6	3.6
			5	0.01	88.8	8.9
	BF 500-7 (500M02)	595 → 401	5	0.001	97.4	3.4
			5	0.01	89.4	10.9
		595 → 207	5	0.001	101.0	3.5
			5	0.01	95.2	9.9
	BF 500-3 (500M07)	358 → 164	5	0.001	101.5	4.9
			5	0.01	100.3	8.0
		358 → 132	5	0.001	96.8	2.8
			5	0.01	95.8	6.0

Linearity	Good linearity (regression coefficients ≥ 0.977) was observed in the range of 0.01 ng mL^{-1} to 0.75 ng mL^{-1} for pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3.
Specificity	The method L0166/01 allows the specific determination of pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3 in soil. This was demonstrated on the basis of four different soils using the highly specific detection technique LC-MS/MS. Each compound could be determined at two different mass transitions. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte.
Limit of Quantification	The limit of quantification was 0.001 mg kg^{-1}.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-1 and Table 4.1.2-2.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	**The method L0166/01 used LC-MS/MS for the final determination of pyraclostrobin and its metabolites BF 500-6 (500M01), BF 500-7 (500M02) and BF 500-3 (500M07) in different soils with a limit of quantification (LOQ) of 0.001 mg kg^{-1} for pyraclostrobin and each metabolite.** **It could be demonstrated that method L0166/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3 in all tested soils.**

Report: CA 4.1.2/2
Zangmeister W., 2010a
Validation of analytical method L0161/01: Determination of BAS 500 F in soil at LOQ 0.001 mg/kg
2010/1075848
Guidelines: SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the methods

Soil samples (5 g) are placed into a PP tube and extracted with 25 mL methanol/water (80/20, v/v) by mechanical shaking for 30 min at 225 rpm. Subsequently, the sample is centrifuged for 5 minutes at 4000 rpm (20ºC). An additional dilution with methanol/water (80/20, v/v) was performed when necessary before LC-MS/MS measurement.

The method was validated at fortification levels of 0.001 and 0.01 mg kg^{-1} for LUFA standard soils (LUFA 2.2 and LUFA 5M).

Recovery findings

The method L0161/01 was proved to be suitable to determine residues of pyraclostrobin in soils using LC-MS/MS with a limit of quantification (LOQ) of 0.001 mg kg^{-1}. Validation experiments were conducted in two different soils for pyraclostrobin. The mean recovery values for pyraclostrobin were between 70 and 110%. The detailed results are given in Table 4.1.2-3.

Table 4.1.2-3: Validation results of a residue analytical method for pyraclostrobin in soil (LUFA 2.2 and LUFA 5M)

Soil	Analyte	m/z	No. of replicates	Fortification level [mg kg^{-1}]	Mean Recovery [%]	RSD [%]
LUFA 2.2	pyraclostrobin (BAS 500 F)	388 → 194	5	0.001	96.6	1.1
			5	0.01	93.7	4.4
		388 → 163	5	0.001	95.6	2.7
			5	0.01	94.0	3.5
LUFA 5M	pyraclostrobin (BAS 500 F)	388 → 194	5	0.001	93.0	3.0
			5	0.01	89.5	1.0
		388 → 163	5	0.001	93.1	1.0
			5	0.01	90.6	3.8

Linearity	Good linearity (regression coefficients ≥ 0.999) was observed in the range of 0.025 ng mL^{-1} to 1.00 ng mL^{-1} for pyraclostrobin.
Specificity	The method L0161/01 allows the specific determination of pyraclostrobin in soil. This was demonstrated on the basis of two different soils using the highly specific detection technique LC-MS/MS. Pyraclostrobin could be determined at two different mass transitions. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for pyraclostrobin.
Limit of Quantification	The limit of quantification was 0.001 mg kg^{-1}.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-3.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	**The method L0161/01 used LC-MS/MS for the final determination of pyraclostrobin in different soils with a limit of quantification (LOQ) of 0.001 mg kg^{-1}.** **It could be demonstrated that method L0161/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of pyraclostrobin in all tested soils.**

Water

Pyraclostrobin (BAS 500 F) and its metabolites

~~**Report:** CA 4.1.2/3~~
~~Obermann M., 2009a~~
~~Validation of analytical method APL0500/03: Determination of pesticides in water by HPLC/MS~~
~~2008/1042150~~
~~**Guidelines:** SANCO/825/00 rev. 7 (17 March 2004)~~
~~**GLP:** yes~~
~~(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)~~

Report: CA 4.1.2/3
Obermann M., 2005 a
Validation of analytical method APL0500/01: Determination of pesticides in water by HPLC/MS
2005/1026675
Guidelines: SANCO/825/00 rev. 7 (17 March 2004)
GLP: yes
(certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)

Note: BASF DocID 2008/1042150 was erroneously included in the renewal dossier submitted in July 2014 and had therefore to be exchanged by BASF DocID 2005/1026675.

Principle of the methods

The samples are diluted with a mixture of acetonitrile/water and acidified with formic acid prior to analysis. Final determination is performed by HPLC/MS using a YMC Pro C18 analytical column and a gradient mixture of water – acetonitrile with formic acid as modifier at a flow rate of 700 µL/min. Detection is accomplished using positive ion electrospray ionization mass spectrometry. The identity of the test item was proven by coincidence of the retention times with the retention times of the authentic reference item peaks and furthermore by MS-detection (m/z 388).

Recovery findings

The method APL0500/01 was proven to be suitable to determine various pesticide residues including pyraclostrobin in water using HPLC/MS with a limit of quantification (LOQ) of 0.001 mg L^{-1} and a limit of detection (LOD) of 0.0005 mg L^{-1}. Validation experiments were conducted in Ft-mix-water for various pesticides including pyraclostrobin. The mean recovery values for all compounds were between 70 and 110%. The detailed results are given for pyraclostrobin in Table 4.1.2-4.

Table 4.1.2-4: Validation results of a residue analytical method for pesticides in water by HPLC/MS

Analyte	No. of replicates	Fortification level [mg kg^{-1}]	Mean Recovery [%]	RSD [%]
Pyraclostrobin (BAS 500 F)	5	0.001	99.4	2.2
	5	0.01	104.9	3.0

Linearity

Linear regression was used for calibration using five calibration levels, which were prepared in water/acetonitrile/formic acid (80/20/0.1, v/v/v). Good linearity (regression coefficients > 0.995) was observed in the range of 0.5 ng/mL to 130 ng/mL.

Specificity

The identification and quantification of pyraclostrobin were based on the retention time and the use of reference items and by MS-detection. Under the described conditions, the method is specific for the determination of pyraclostrobin in water.
Under the given chromatographic conditions, no significant co-elution of one of the active ingredient peaks with an unknown component was observed.
As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is necessary.

Matrix Effects

No matrix effects were investigated within an independent experiment, therefore instrument recovery samples would be implemented within each analytical series, if no further information on matrix effects of the investigated matrix is available. Alternatively, matrix-matched standards would be used.

Limit of Quantification

The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested, resulting in an LOQ of 1 µg L^{-1} for pyraclostrobin.

Limit of Detection

The limit of detection (LOD) is defined by the lowest calibration level used, corresponding to a concentration of 0.5 µg L^{-1} for pyraclostrobin.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.1.2-4.

Standard Stability

An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.

Stability of Working Solutions	An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	**The method APL0500/01 used HPLC/MS for the final determination of different pesticides in water with a limit of quantification (LOQ) of 0.001 mg L^{-1} for each compound including pyraclostrobin.** **It could be demonstrated that method APL0500/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of pesticides including pyraclostrobin in water.**

Report:	CA 4.1.2/4 Tilting N., 2012a Validation of method L0182/01: Determination of BAS 500 F and its metabolites Reg.No. 412053 (500M59), Reg.No. 411847 (500M60), Reg.No. 412785 (500M62), Reg.No. 413038, and Reg.No. 377613 in ground- surface- and tapwater using LC-MS/MS 2012/1009641
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/825/00 rev. 8 (30 June 2010)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

The BASF method L0182/01 was developed for the determination of BAS 500 F (pyraclostrobin) and its metabolites BF 500-12 (500M59), BF 500-11 (500M60), BF 500-13 (500M62), BF 500-14 (500M76) and BF 500-15 (500M78) in water samples. A 50 mL sample aliquot is acidified by adding 500 µL of formic acid and extracted by SPE. After washing with 5 mL acidified water, the SPE column is dried. The column is eluted with 2 x 5 mL ethyl acetate and the combined extracts are evaporated at 40°C. Determination is conducted by HPLC-MS/MS. The limit of quantitation (LOQ) of the method for pyraclostrobin is 0.003 µg L^{-1} and for the metabolites the LOQ is 0.03 µg L^{-1} in water.
As matrix effects were observed during method development, matrix matched standards were used for calibration.

Recovery findings

The method proved to be suitable to determine pyraclostrobin and its metabolites BF 500-12, BF 500-11, BF 500-13, BF 500-14 and BF 500-15 in water. Validation experiments were conducted in ground-, surface- and tap water. Samples were spiked with the analytes at the limit of quantification (0.003 µg L^{-1} for pyraclostrobin, 0.03 µg L^{-1} for the metabolites) and 10 times higher (0.03 µg L^{-1} for pyraclostrobin, 0.3 µg L^{-1} for the metabolites). All average recovery values (mean of five replicates per fortification level and analyte) were between 70 and 110%. The detailed results are given in Table 4.1.2-5.

Table 4.1.2-5: Results of the method validation for the determination of pyraclostrobin and its metabolites in groundwater, surface water and tap water

Analyte	m/z	Matrix	Replicates	Fortification level [µg L^{-1}]	Mean recovery [%]	RSD [%]
Pyraclostrobin (BAS 500 F)	388->194	groundwater	5	0.003	87.9	7.4
			5	0.03	94.2	2.6
		surface water	5	0.003	89.3	4.1
			5	0.03	97.5	4.6
		tap water	5	0.003	89.6	4.9
			5	0.03	103.0	5.2
	388->163	groundwater	5	0.003	92.6	7.4
			5	0.03	99.0	5.3
		surface water	5	0.003	93.8	6.1
			5	0.03	100.4	2.6
		tap water	5	0.003	92.1	3.1
			5	0.03	104.4	9.4
BF 500-12 (500M59)	370->194	groundwater	5	0.03	95.9	1.7
			5	0.3	97.8	2.8
		surface water	5	0.03	97.1	1.8
			5	0.3	96.9	1.7
		tap water	5	0.03	98.9	1.3
			5	0.3	101.3	2.9
	370->278	groundwater	5	0.03	98.9	2.6
			5	0.3	95.8	3.9
		surface water	5	0.03	98.6	3.0
			5	0.3	97.8	2.3
		tap water	5	0.03	98.8	2.6
			5	0.3	101.8	3.2
BF 500-11 (500M60)	278->194	groundwater	5	0.03	95.7	3.8
			5	0.3	98.8	2.7
		surface water	5	0.03	86.7	1.8
			5	0.3	85.0	2.4
		tap water	5	0.03	97.3	2.1
			5	0.3	96.4	3.4
	278->149	groundwater	5	0.03	95.2	2.4
			5	0.3	95.6	3.4
		surface water	5	0.03	87.6	3.4
			5	0.3	88.4	1.9
		tap water	5	0.03	95.7	2.9
			5	0.3	95.9	3.1
BF 500-13 (500M62)	248->132	groundwater	5	0.03	97.0	1.4
			5	0.3	95.4	3.9
		surface water	5	0.03	91.2	1.9
			5	0.3	83.8	1.6
		tap water	5	0.03	99.3	1.6
			5	0.3	93.5	6.1
	248->216	groundwater	5	0.03	99.8	3.1
			5	0.3	97.7	4.0
		surface water	5	0.03	91.1	4.7
			5	0.3	82.0	2.4
		tap water	5	0.03	98.0	5.5
			5	0.3	94.8	7.6

Analyte	m/z	Matrix	Replicates	Fortification level [µg L^{-1}]	Mean recovery [%]	RSD [%]
BF 500-14 (500M76)	388->241	groundwater	5	0.03	97.4	5.7
			5	0.3	102.8	3.1
		surface water	5	0.03	97.6	3.7
			5	0.3	97.7	6.2
		tap water	5	0.03	100.6	9.7
			5	0.3	101.0	4.7
	388->300	groundwater	5	0.03	103.4	3.3
			5	0.3	99.3	5.3
		surface water	5	0.03	104.0	4.7
			5	0.3	102.1	3.4
		tap water	5	0.03	100.6	6.1
			5	0.3	105.0	5.6
BF 500-15 (500M78)	177->135	groundwater	5	0.03	92.2	1.0
			5	0.3	103.1	3.4
		surface water	5	0.03	95.0	3.7
			5	0.3	97.2	5.2
		tap water	5	0.03	96.7	2.3
			5	0.3	99.2	1.7
	177->132	groundwater	5	0.03	93.5	3.0
			5	0.3	99.8	4.2
		surface water	5	0.03	100.3	2.0
			5	0.3	103.3	4.8
		tap water	5	0.03	95.8	2.8
			5	0.3	99.4	3.1

Linearity	Good linearity ($r \geq 0.99$) was observed in the range of 0.005 ng mL^{-1} to 1.0 ng mL^{-1} for the two ion transitions of pyraclostrobin and its metabolites BF 500-11, BF 500-12, BF 500-13, BF 500-14 and BF 500-15 in three different water types (ground-, surface- and tap water).
Specificity	The method successfully determines pyraclostrobin and its metabolites BF 500-11, BF 500-12, BF 500-13, BF 500-14 and BF 500-15 in water. This was demonstrated on the basis of three different water types (groundwater, surface water and tap water) using HPLC-MS/MS, and monitoring two mass transitions for each analyte. The tested untreated water samples showed no significant interferences (< 30%) at the retention time of the analytes. Due to the high selectivity and specificity of HPLC-MS/MS an additional confirmatory technique was not necessary.
Limit of Quantification	The method has a limit of quantification of 0.003 µg L^{-1} for pyraclostrobin and 0.03 µg L^{-1} for each metabolite.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	**The method L0182/01 for the analysis of pyraclostrobin (BAS 500 F) and its metabolites BF 500-12 (500M59), BF 500-11 (500M60), BF 500-13 (500M62), BF 500-14 (500M76) and BF 500-15 (500M78) in ground-, surface- and tap water used HPLC-MS/MS for final determination, with limit of quantification of 0.003 µg L^{-1} for pyraclostrobin and 0.03 µg L^{-1} for each metabolite.** **It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries and is therefore applicable to correctly determine pyraclostrobin and its metabolites in ground- surface- and tap water samples.**

Report:	CA 4.1.2/5 Obermann M., 2014a Validation of analytical method L0182/02 for the determination of BF 500-5 (Reg.No. 298327), metabolite of BAS 500 F, in ground- and surface water by LC-MS/MS 2014/1004891
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

The objective of this validation study was to demonstrate the applicability and repeatability of method L0182/02 for the determination of BF 500-5 (500M04) in ground- and surface water by using HPLC-MS/MS. This method is complementary to method L0182/01, which is validated for pyraclostrobin and several of its other metabolites.

Principle of the method

A 50 mL water sample aliquot is acidified with formic acid and concentrated on a C18 SPE column. After eluting with ethyl acetate the eluate is evaporated to dryness and reconstituted with acetonitrile/water [20/80, v/v] before determination by HPLC-MS/MS.
The limit of quantitation (LOQ) of the method is 0.03 µg L^{-1} in water.

Recovery findings

Method L0182/02 was proved to be suitable to determine residues of BF 500-5 in ground- and surface water to a limit of quantification (LOQ) of 0.03 µg L^{-1}. Samples were spiked with the analyte at the limit of quantification (0.03 µg L^{-1}) and 10 times higher (0.3 µg L^{-1}). All average recovery values (mean of five replicates per fortification level) were between 70 and 110%. The detailed results are given in Table 4.1.2-6.

Table 4.1.2-6: Results of the method validation for the determination of BF 500-5 in ground- and surface water

Analyte	m/z	Matrix	Replicates	Fortification level [µg L^{-1}]	Mean recovery [%]	RSD [%]
BF 500-5 (500M04)	195->117	groundwater	5	0.03	98	5.9
			5	0.3	102	6.0
		surface water	5	0.03	110	5.6
			5	0.3	110	4.0
	195->153	groundwater	5	0.03	103	7.9
			5	0.3	101	6.7
		surface water	5	0.03	108	7.2
			5	0.3	105	1.9

Linearity	Good linearity ($r \geq 0.996$) was observed in the range of 0.05 ng mL^{-1} to 1.0 ng mL^{-1} for the two ion transitions of BF 500-5 in two different water types (ground- and surface water).
Specificity	The method successfully determines residues of BF 500-5 in ground- and surface water using HPLC-MS/MS, and monitoring two mass transitions. The tested untreated water samples showed no significant interferences (< 30%) at the retention time of the analytes. Due to the high selectivity and specificity of HPLC-MS/MS an additional confirmatory technique was not necessary.
Limit of Quantification	The method has a limit of quantification of 0.03 µg L^{-1}.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 10%, see Table 4.1.2-6.
Reproducibility	Reproducibility of the method was not determined within this validation study.
Conclusion	**The method L0182/02 for the analysis of BF 500-5 (500M04) in ground- and surface water used HPLC-MS/MS for final determination, with limit of quantification of 0.03 µg L^{-1}.** **It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BF 500-5 in ground- and surface water samples.**

Air

Pyraclostrobin (BAS 500 F)

Report: CA 4.1.2/6
Penning H., 2012a
Validation of analytical method L0197/01: Method for the determination of BAS 500 F (Pyraclostrobin) in air by LC-MS/MS
2012/1220256
Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

BASF method L0197/01 was developed for the determination of BAS 500 F - pyraclostrobin in air samples. The analyte is spiked to Tenax adsorption tubes. After sucking air through the glass tubes at 35°C and a relative humidity of $\geq$ 80% for 6 hours at approximately 90 L h^{-1}, the tube content is extracted by ultrasonication using acetone. Final determination of pyraclostrobin is achieved by LC-MS/MS. The limit of quantification is defined as the lowest fortification level used in the validation process corresponding to a concentration of 4.44 ng pyraclostrobin per L air.

Recovery findings

The results from the fortification experiments at two spiking levels (2.40 µg and 24.0 µg of the analyte) showed that the recoveries were between 70 and 110% (see Table 4.1.2-7).

Because no interferences in the control samples were observed, no blank correction was necessary. Therefore, in the following result table, only uncorrected recovery data are presented for two mass transitions of pyraclostrobin. All mean recovery values were in the range 103% – 108%. For each fortification level the %RSD values were <20% (range: 1.4 – 4.3%).

Table 4.1.2-7: Results of method validation of pyraclostrobin in air (uncorrected data)

Test system	Analyte	Replicates	Fortification level [ng L^{-1}]	1. Transition (388 -> 194)			2. Transition (388 -> 163)		
				Mean recovery [%]	SD [+/- %]	RSD [%]	Mean recovery [%]	SD [+/- %]	RSD [%]
Air	Pyraclostrobin	6	4.44	103	4.2	4.1	103	4.5	4.3
		6	44.4	108	1.6	1.5	108	1.6	1.4

Linearity

Good linearity ($r > 0.99$) was observed in the range of 0.24 ng mL^{-1} to 2.4 ng mL^{-1} for both mass transitions of pyraclostrobin.

Specificity

Under the described conditions method L0197/01 is specific for the determination of pyraclostrobin in air. Significant interferences (> 30% of LOQ) were not observed at the retention time and mass transitions of pyraclostrobin.

Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. Two mass transitions of pyraclostrobin were quantified.

Limit of Quantification

Limit of quantification should take into account relevant human and eco-toxicological-based limit values or exposure levels. Based on the equation described in SANCO/825/00 rev. 8.1, the limit of quantification should comply with the concentration C (mass of test item per volume air) calculated from the $AOEL_{inhalative}$. The $AOEL_{inhalative}$ value at time of method development was proposed to be 0.015 mg (kg body weight)$^{-1}$ day^{-1}.

Considering a body weight of 60 kg, a safety factor of 0.1 and an average respiratory volume of 20 m^3 per day, the limit of quantification (C) for the air method should be 0.0045 µg L^{-1}. Based on a collected air volume of 540 L, the amount spiked on the adsorber was 2.4 µg.

The lowest fortification level used for validation was 4.44 ng L^{-1} air and is therefore defined as limit of quantification.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%, see Table 4.1.2-7.

Reproducibility

Reproducibility of the method was not determined within this validation study.

Conclusion

Method L0197/01 was validated at concentrations of 4.44 ng L^{-1} air and 44.4 ng L^{-1} air. This method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of pyraclostrobin in air.

(b) Methods in soil, water and any additional matrices used in support of efficacy studies

Not relevant

(c) Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

As pyraclostrobin is classified as toxic, a residue analytical method in body fluids and tissues is required. Consequently, method 439/0, which was originally developed for animal matrices, had been adapted to blood. The adapted method was evaluated in the previous Annex I inclusion process and was considered to be suitable. It is based on LC-UV and uses LC-MS as confirmatory technique. In order to provide a method using up-to-date technology, method L0151/01 was developed and also validated for blood. The relevant data are provided below (see CA 4.1.2/19).

Methods for concentration control in feed or other matrices are reported, where necessary, along with the respective toxicological studies. Additionally, the following standalone validation studies are presented.

Report:	CA 4.1.2/7 Catchpole G., Hidding B., 2016 a Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin) - Validation of an analytical method for the analysis of Reg.No. 5916421 in plasma using HPLC-MS (control procedure: 13/0173_04)
	2016/1327657
Guidelines:	EC 1107/2009 of the European Parliament, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the methods

The aim of the study was the validation of an analytical method for the quantitative analysis of Reg.No. 5916421 (500M24, metabolite of pyraclostrobin) in the vehicle plasma by high performance liquid chromatography with MS detection (HPLC MS) according to control procedure number 13/0173 04. The study was performed by BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany.

30 µL of the plasma sample (mouse plasma) are extracted with acetonitrile. After protein precipitation, the sample is centrifuged. The clear supernatant is analyzed by LC-MS/MS using an Ascentis Express C18 column and an acetonitrile/water gradient with 0.01% formic acid as modifier. Detection is accomplished in ESI+ mode at a mass transition of 226 m/z → 150 m/z for quantification and 226 m/z → 145 m/z for confirmation. The results are calculated by direct comparison of the sample peak responses to those of external matrix-matched standards.

The limit of quantification (LOQ) of the method is 50 ng mL^{-1} for Reg.No. 5916421 in plasma.

Recovery findings

The method is suitable to determine residues of Reg.No. 5916421 in plasma. Samples are fortified with the analyte at the limit of quantification of 50 ng mL^{-1}, 20 times higher (1,000 ng mL^{-1}) and 200 times higher (10,000 ng mL^{-1}).

Mean recovery values (mean of five replicates per fortification level) are between 99 and 105% (see table below). Regarding the blank plasma sample and the solvent blank, no peak was observed in both chromatograms at the retention time of the test substance.

Table 4.1.2-8: Characteristics for the analytical method used for validation of Reg.No. 5916421 residues in plasma

Matrix	Analyte	Mass transition	Fortification Level [ng mL^{-1}]	Number of replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Plasma	Reg.No. 5916421	266→150	50	5	102	2.0	100	2.7
			1000	5	99	3.4		
			10000	5	100	2.1		
		266→145	50	5	105	8.5	103	5.6
			1000	5	105	2.6		
			10000	5	100	1.9		

Linearity Good linearity (r^2 > 0.99) is observed in both calibration curves covering the low (4 ng mL^{-1} to 75 ng mL^{-1}) and high concentration range (75 ng mL^{-1} to 1200 ng mL^{-1}) using matrix-matched standards.

Specificity Highly selective determination of the analyte using LC-MS/MS detection and monitoring two mass transitions was applied. There were no interferences at the corresponding retention time in any of the control specimens (blank value < 30 % LOQ).

Matrix Effects Matrix effects are negated by using matrix-matched standards, leading to acceptable recovery values.

Limit of Quantification The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested. LOQ is 50 ng mL^{-1} for Reg.No. 5916421 in plasma.

Limit of Detection The limit of detection (LOD) is calculated based on the signal to noise ratio S/N = 3 of the peak from the lowest fortification level, resulting in a value of 0.6 ng mL^{-1}.

Repeatability The relative standard deviations (RSD, %) for all fortification levels are below 20%.

Standard Stability	An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.
Stability of Working Solutions	An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.
Reproducibility	Reproducibility of the method was not determined within this validation study.

Conclusion

Control procedure number 13/0173_04 for the analysis of Reg.No. 5916421 (500M24, metabolite of pyraclostrobin) in plasma uses HPLC-MS/MS for final determination, with a limit of quantification of 50 ng mL^{-1}.
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly and accurately determine residues of Reg.No. 5916421 in plasma.

Report:	CA 4.1.2/8 Catchpole G., Hidding B., 2016 d Validation of an analytical method for the analysis of Reg.No. 411847 (metabolite of BAS 500 F, Pyraclostrobin) in plasma using HPLC-MS (control procedure: 99/0251_01) 2016/1321627
Guidelines:	EC 1107/2009 of the European Parliament, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the methods

The aim of the study was the validation of an analytical method for the quantitative analysis of Reg.No. 411847 (500M60, metabolite of pyraclostrobin) in the vehicle plasma by high performance liquid chromatography with MS detection (HPLC MS/MS) according to control procedure number 99/0251 01-03. The study was performed by BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany.
The plasma sample (mouse plasma) is extracted with acetonitrile (1 part plasma + 9 parts acetonitrile). After protein precipitation, the sample is centrifuged. The clear supernatant is analyzed by LC-MS/MS using a Luna C18 column and an acetonitrile/water gradient with 0.01% formic acid as modifier. Detection is accomplished in ESI+ mode at a mass transition of 278 m/z → 194 m/z for quantification and 278 m/z → 163 for confirmation. The results are calculated by direct comparison of the sample peak responses to those of external matrix-matched standards.
The limit of quantification (LOQ) of the method is 50 ng mL^{-1} for Reg.No. 411847 in plasma.

Recovery findings

The method is suitable to determine residues of Reg.No. 411847 in plasma. Samples are fortified with the analyte at the limit of quantification of 50 ng mL^{-1}, 10 times higher (500 ng mL^{-1}) and 100 times higher (5000 ng mL^{-1}).

Mean recovery values (mean of five replicates per fortification level) are between 92 and 102% (see table below). Regarding the blank plasma sample and the solvent blank, no peak was observed in both chromatograms at the retention time of the test substance.

Table 4.1.2-9: Characteristics for the analytical method used for validation of Reg.No. 411847 residues in plasma

Matrix	Analyte	Mass transition	Fortification Level [ng mL^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Plasma	Reg.No. 411847	278 → 194	50	5	102	1.5	97	6.7
			500	5	92	9.1		
			5000	5	98	4.3		
		278 → 163	50	5	102	1.1	97	6.6
			500	5	92	8.9		
			5000	5	98	4.3		

Linearity	Good linearity (r^2 > 0.99) is observed in both calibration curves covering the low (4 ng mL^{-1} to 73 ng mL^{-1}) and high concentration range (49 ng mL^{-1} to 586 ng mL^{-1}) using matrix-matched standards.
Specificity	Highly selective determination of the analyte using LC-MS/MS detection and monitoring two mass transitions was applied. There were no interferences at the corresponding retention time in any of the control specimens (blank value < 30 % LOQ).
Matrix Effects	Matrix effects are negated by using matrix-matched standards, leading to acceptable recovery values.
Limit of Quantification	The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested, resulting in an LOQ of 50 ng mL^{-1} for Reg.No. 411847 in plasma.
Limit of Detection	The limit of detection (LOD) is calculated based on the signal to noise ratio S/N = 3 of the peak from the lowest fortification level, resulting in a value of 0.01 ng mL^{-1}.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels are below 20%.
Standard Stability	An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.
Stability of Working Solutions	An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.
Reproducibility	Reproducibility of the method was not determined within this validation study.

Conclusion

Control procedure number 99/0251_01-03 for the analysis of Reg.No. 411847 (500M60, metabolite of pyraclostrobin) in plasma uses HPLC MS/MS for final determination, with a limit of quantification of 50 ng mL^{-1}.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly and accurately determine residues of Reg.No. 411847 in plasma.

Report:	CA 4.1.2/9 Catchpole G., Hidding B., 2016 b Validation of an analytical method for the analysis of Reg.No. 413038 (metabolite of BAS 500 F, Pyraclostrobin) in plasma using HPLC (control procedure: 99/0249_01) 2016/1311551
Guidelines:	EC 1107/2009 of the European Parliament, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the methods

The aim of the study was the validation of an analytical method for the quantitative analysis of Reg.No. 413038 (500M76, metabolite of pyraclostrobin) in the vehicle plasma by high performance liquid chromatography with MS detection (HPLC MS/MS) according to control procedure number 99/0249 01. The study was performed by BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany.

The plasma sample (mouse plasma) is extracted with acetonitrile (1 part plasma + 9 parts acetonitrile). After protein precipitation, the sample is centrifuged. The clear supernatant is analyzed by LC-MS/MS using a Luna C18 column and an acetonitrile/water gradient with 0.01% formic acid as modifier. Detection is accomplished in ESI+ mode at a mass transition of 388 m/z → 164 m/z for quantification and 388 m/z → 132 for confirmation. The results are calculated by direct comparison of the sample peak responses to those of external matrix-matched standards.

The limit of quantification (LOQ) of the method is 50 ng mL^{-1} for Reg.No. 413038 in plasma.

Recovery findings

The method is suitable to determine residues of Reg.No. 413038 in plasma. Samples are fortified with the analyte at the limit of quantification of 49 ng mL^{-1}, 20 times higher (980 ng mL^{-1}) and 100 times higher (9800 ng mL^{-1}).

Mean recovery values (mean of five replicates per fortification level) are between 92 and 98% (see table below). Regarding the blank plasma sample and the solvent blank, no peak was observed in both chromatograms at the retention time of the test substance.

Table 4.1.2-10: Recoveries for Reg.No. 413038 (metabolite of pyraclostrobin) in plasma

Matrix	Analyte	Mass transition	Fortification Level [ng mL^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Plasma	Reg.No. 413038	388 → 164	49	5	94	6.1	95	4.4
			980	5	92	2.3		
			9800	5	98	0.6		
		388 → 132	49	5	92	5.0	94	4.3
			980	5	92	2.3		
			9800	5	98	0.4		

Linearity	Good linearity ($r^2 \geq 0.999$) was observed in the range of 3.92 ng/mL to 1176.0 ng/mL using the two mass transitions described. 12 calibration levels, split into two distinct mass ranges (3.92 ng/mL – 73.5 ng/mL and 73.5 ng/mL – 1176.0 ng/mL), were used.
Specificity	Highly selective determination of the analyte using LC-MS/MS detection and monitoring two mass transitions was applied. There were no interferences at the corresponding retention time in any of the control specimens (blank value < 30 % LOQ).
Matrix Effects	Matrix effects are negated by using matrix-matched standards, leading to acceptable recovery values.
Limit of Quantification	The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested, resulting in an LOQ of 49 ng mL^{-1} for Reg.No. 413038 in plasma.
Limit of Detection	The limit of detection (LOD) is calculated based on the signal to noise ratio S/N = 3 of the peak from the lowest fortification level, resulting in a value of 0.02 ng mL^{-1}.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels are below 20%.
Standard Stability	An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.
Stability of Working Solutions	An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.
Reproducibility	Reproducibility of the method was not determined within this validation study.

Conclusion

Control procedure number 99/0249 01 for the analysis of Reg.No. 413038 (500M76, metabolite of pyraclostrobin) in plasma uses HPLC MS/MS for final determination, with a limit of quantification of 49 ng mL^{-1}.
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly and accurately determine residues of Reg.No. 413038 in plasma.

Report:	CA 4.1.2/10 Catchpole G., Hidding B., 2017 a Validation of an analytical method for the analysis of Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) and Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) in plasma using HPLC-MS (control procedure: 14/0701_04)
Guidelines:	2016/1333356 EC 1107/2009 of the European Parliament, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the methods

The aim of the study was the validation of an analytical method for the quantitative analysis for Reg.No. 399379 (500M106, metabolite of pyraclostrobin) and Reg.No. 298327 (500M04, metabolite of pyraclostrobin) in the vehicle plasma by high performance liquid chromatography with high-resolution MS detection (HPLC MS) according to control procedure number 4/0701 04-01. The study was performed by BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany.

The plasma sample (mouse plasma) is extracted with acetonitrile (1 part plasma + 9 parts acetonitrile). After protein precipitation, the sample is centrifuged. The clear supernatant is analyzed by LC-high-res-MS using a Accucore C18 column and an acetonitrile/water gradient with 0.01% formic acid as modifier. Detection is accomplished in positive full scan mode at an exact mass of m/z 330.10038 for Reg.No. 399379, and m/z 195.03197 for Reg.No. 298327. The results are calculated by direct comparison of the sample peak responses to those of external matrix-matched standards.

The limit of quantification (LOQ) of the method is 50 ng mL^{-1} for both analytes in plasma.

Recovery findings

The method is suitable to determine residues of Reg.No. 399379 and Reg.No. 298327 in plasma. Samples are fortified with the analyte at the limit of quantification of 50 ng mL^{-1}, 10 times higher (500 ng mL^{-1}) and 100 times higher (5000 ng mL^{-1}).

Mean recovery values (mean of five replicates per fortification level) are between 92 and 98% (see table below). Regarding the blank plasma sample and the solvent blank, no peak was observed in both chromatograms at the retention time of the test substance.

Table 4.1.2-11: Recoveries for Reg.No. 399379 and Reg.No. 298327 (metabolites of pyraclostrobin) in plasma

Matrix	Analyte	m/z (+/- 3 mDa)	Fortification Level [ng mL^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
Plasma	Reg.No. 399379	330.10038	50	4*	75	6.8	90	11.8
			500	5	97	5.3		
			5000	5	94	3.5		
	Reg.No. 298327	*195.03197*	50	4*	102	8.0	98	6.2
			500	5	93	3.2		
			5000	5	101	1.3		

* Outlier identified

Linearity Good linearity ($r^2 \geq 0.999$) was observed in the range of 4.104 ng/mL to 102.6 ng/mL and 76.95 ng/mL to 615.6 ng/mL (Reg.No. 399379) and 3.864 ng/mL to 72.45 ng/mL and 72.45 ng/mL to 579.6 ng/mL (Reg.No. 298327) using the accurate m/z described. At least six calibration levels distributed over each concentration range were used.

Specificity Highly selective determination of the analytes using LC with high resolution MS-detection was used, therefore no additional confirmatory technique is needed. There were no interferences at the corresponding retention time in any of the control specimens (blank value < 30 % LOQ).

Matrix Effects Matrix effects are negated by using matrix-matched standards, leading to acceptable recovery values.

Limit of Quantification The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested, resulting in an LOQ of 50 ng mL^{-1} for both analytes in plasma.

Limit of Detection The LODs of the entire analytical procedure following any sample dilution steps were assessed and found to be < 4.104 ng/mL (Reg.No. 399379) and 0.0546 ng/mL (Reg.No. 298327), which correspond to < 8.2 % and < 1 % of the respective LOQ.

Repeatability The relative standard deviations (RSD, %) for all fortification levels are below 20%.

Standard Stability An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.

Stability of Working Solutions	An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.
Reproducibility	Reproducibility of the method was not determined within this validation study.

Conclusion

Control procedure number 14/0701_04 for the analysis of Reg.No. 399379 and Reg.No. 298327 (metabolites of pyraclostrobin) in plasma uses HPLC with high resolution MS-detection for final determination, with a limit of quantification of 50 ng mL^{-1}.
It could be demonstrated that the current analytical method fulfills the requirements with regard to specificity, linearity, repeatability, limit of quantification and accuracy and is therefore applicable to correctly quantify Reg.No. 399379 (500M106, metabolite of pyraclostrobin) and Reg.No. 298327 (500M04, metabolite of pyraclostrobin) in plasma.

Report:	CA 4.1.2/11 Catchpole G., Hidding B., 2016 c Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Validation of an analytical method for the analysis of Reg.No. 399379 (metabolite of BAS 500 F) in 1% Carboxymethyl cellulose in drinking water (w/v) using HPLC-UV 2016/1230128
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the methods

The aim of the study was the validation of an analytical method for the quantitative analysis of Reg.No. 399379 (500M106, metabolite of pyraclostrobin) in the vehicle 1% carboxymethyl cellulose in drinking water (w/V) by high performance liquid chromatography with UV detection (HPLC-UV) according to control procedure number 14/0701 03-01. The study was performed by BASF SE, Experimental Toxicology and Ecology, Ludwigshafen, Germany.

The vehicle samples (1% carboxymethyl cellulose in drinking water) are transferred into appropriate volumetric flasks with 10 mL acetonitrile/water (1/1, v/v) and filled up to the mark with acetonitrile to obtain concentrations within the tested calibration range. After filtering the samples (cellulose filter, 0.2 µm) residues of Reg.No. 399379 are determined by HPLC-UV using a Chromolith Performance RP18e column and an acetonitrile/water gradient with 0.1% formic acid as modifier. Detection is accomplished at a wavelength of 278 nm (with 360 nm reference). The results are calculated by direct comparison of the sample peak responses to those of external matrix-matched standards.

The limit of quantification (LOQ) of the method is 0.1 mg mL^{-1} (10 mg per 100 mL) in aqueous 1% carboxymethyl cellulose for Reg.No. 399379.

Recovery findings

The method is suitable to determine residues of Reg.No. 399379 in 1% carboxymethyl cellulose in drinking water (w/v). Samples are fortified with the analyte at the limit of quantification (0.1 mg mL^{-1}), 50 times higher (5 mg mL^{-1}) and 2500 times higher (250 mg mL^{-1}).

Mean recovery values (mean of five replicates per fortification level) are between 99 and 104% (see table below). Regarding the blank vehicle sample, no peak was observed in the chromatogram at the retention time of the test substance.

Table 4.1.2-12: Recoveries for Reg.No. 399379 (metabolite of pyraclostrobin) in 1% carboxymethyl cellulose in drinking water (w/V)

Matrix	Analyte	Wave-length	Fortifi-cation Level [mg mL^{-1}]	Number of replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
1% CMC in drinking water (w/v)	Reg.No. 399379	278 nm	0.1	5	99	0.3	101	2.7
			5	5	104	0.5		
			250	5	99	0.8		

Linearity Good linearity ($r^2 > 0.99$) is observed in the calibration curve covering the range of 0.005 mg mL^{-1} to 0.051 mg mL^{-1} using matrix-matched standards.

Specificity There were no interferences at the corresponding retention time in any of the control specimens (blank value < 30 % LOQ).
Since UV-detection is used, no other signal suppression or depression can be expected.
As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is necessary.

Matrix Effects The effects of the sample matrix on HPLC-UV response is not tested within this validation study. Matrix-matched standards are used and lead to very good recovery values.

Limit of Quantification The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested, resulting in an LOQ of 0.1 mg mL^{-1} (10 mg per 100 mL) in 1% carboxymethyl cellulose in drinking water (w/V) for Reg.No. 399379.

Repeatability The relative standard deviations (RSD, %) for all fortification levels are below 20%.

Standard Stability An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.

Stability of Working Solutions An assessment of stability was not considered to be mandatory within this validation study. The duration of a complete analytical run is always below 24 hours and samples are always injected promptly after sample preparation.

Reproducibility Reproducibility of the method was not determined within this validation study.

Conclusion

Control procedure number 14/0701 03-01 for the analysis of Reg.No. 399379 (500M106, metabolite of pyraclostrobin) in 1% carboxymethyl cellulose in drinking water (w/V) uses HPLC UV for final determination, with a limit of quantification of 0.1 mg mL^{-1}.
It could be demonstrated that the method fulfills the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly and accurately determine residues of Reg.No. 399379 in 1% carboxymethyl cellulose in drinking water (w/v).

Report:	CA 4.1.2/12 Tanaka T., 2016 a Validation for determination method of Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) in 0.5 w/v% carboxymethyl cellulose solution 2016/1230112
Guidelines:	OECD Principles of Good Laboratory Practice
GLP:	yes (certified by Ministry of Health, Labour and Welfare, Tokyo, Japan)

Principle of the methods

The aim of the study was the validation of an analytical method for the quantitative analysis of Reg.No. 399379 (500M106, metabolite of pyraclostrobin) in the vehicle (0.5 w/v% carboxymethyl cellulose sodium salt aqueous solution) by high performance liquid chromatography with UV detection (HPLC-UV) according to control procedure number 14/0701_03-01. The study was performed by Biosafety Research Center, Shizuoka, Japan.

The vehicle samples are transferred into appropriate volumetric flasks with acetonitrile and filled up to the mark with acetonitrile to obtain concentrations within the tested calibration range. After filtering the samples (Millex-LH, PTFE, pore size: 0.45 µm) residues of Reg.No. 399379 are determined by HPLC-UV using a L-column ODS and an acetonitrile/water gradient with 0.1% formic acid as modifier. Detection is accomplished at a wavelength of 278 nm. The results are calculated by direct comparison of the sample peak responses to those of external standards.

The limit of quantification (LOQ) of the method is 0.5 mg/mL in aqueous 0.5% carboxymethyl cellulose for Reg. No. 399379.

Recovery findings

The method is suitable to determine residues of Reg.No. 399379 in 0.5 % carboxymethyl cellulose in aqueous solution (w/v). Samples are fortified with the analyte at the limit of quantification (0.5 mg/mL and at 100 mg/mL).

Mean recovery values (mean of five measurements per fortification level) are between 93 and 109% (see table below). Regarding the blank vehicle sample, no peak was observed in the chromatogram at the retention time of the test substance.

Table 4.1.2-13: Recoveries for Reg.No. 399379 (metabolite of pyraclostrobin) in aqueous 0.5% carboxymethyl cellulose (w/v)

Nominal concentration (mg/mL)	Sampling	Measured Concentration Individual		Found concentration Mean			
		(mg/mL)	(%)	(%)	RSD (%)	(%)	RSD (%)
0.5	Top	0.478	96	95	0.63	94	0.88
		0.473	95				
		0.475	95				
		0.472	94				
		0.471	94				
	Middle	0.467	93	93	0.24		
		0.465	93				
		0.466	93				
		0.465	93				
		0.467	93				
	Low	0.470	94	93	0.41		
		0.468	94				
		0.466	93				
		0.466	93				
		0.466	93				
100	Top	108	108	107	0.17	108	0.66
		108	108				
		107	107				
		107	107				
		107	107				
	Middle	109	109	109	0.29		
		109	109				
		109	109				
		109	109				
		109	109				
	Low	108	108	108	0.27		
		108	108				
		108	108				
		108	108				
		108	108				

Linearity Good linearity ($r^2 > 0.99$) is observed in the calibration curve covering the range of 0.01 mg/mL to 0.1 mg/mL using solvent standards.

Specificity There were no interferences at the corresponding retention time in any of the control specimens (blank value < 30 % LOQ).
Since UV-detection is used, no other signal suppression or depression can be expected.
As the method is only used for dose verification of known substances and known nominal concentrations, no additional confirmatory technique is necessary.

Matrix Effects	Since UV detection is used, no signal suppression or depression can be expected.
Limit of Quantification	The limit of quantification (LOQ) is defined by the lowest fortification level successfully tested, resulting in an LOQ of 0.5 mg/mL in 0.5% carboxymethyl cellulose in aqueous solution (w/v) for Reg.No. 399379.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels are below 20%.
Standard Stability	Standard solutions were measured after preparation and after 24-hour storage in the autosampler (10°C) 3 times repeatedly and were found to be stable over this time period.
Stability of Sample Solutions	Sample solutions of the test substance formulation were measured just after preparation and after 24-hour storage in the autosampler (10°C) and were found to be stable over this time period.
Reproducibility	Reproducibility of the method was not determined within this validation study.

Conclusion

The analytical method for the analysis of Reg.No. 399379 (500M106, metabolite of pyraclostrobin) in 0.5 % carboxymethyl cellulose in aqueous solution (w/v) uses HPLC UV for final determination, with a limit of quantification of 0.5 mg/mL.
It could be demonstrated that the method fulfills the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly and accurately determine residues of Reg.No. 399379 in 0.5 % carboxymethyl cellulose in aqueous solution (w/v).

(d) Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

No exposure studies were conducted with pyraclostrobin. Consequently, such methods of analysis are not required.

(e) Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

The analytical methods for the determination of pyraclostrobin in **foodstuffs of plant origin** were evaluated in the previous Annex I inclusion process and during more recent evaluations performed by EFSA in context of MRL applications. Pyraclostrobin residues in commodities of plant origin can be determined by LC-MS/MS with LOQs of 0.02 mg/kg or 0.01 mg/kg. The BASF methods 421/0, 445/0 and 535/1 (synonym: L0076/01) allow the determination of pyraclostrobin and its metabolite 500M07 (BF 500-3) in multiple crops. The metabolite 500M07 is not part of the residue definition in Europe. The data generation methods are fully validated in separate GLP studies; furthermore, an extensive set of concurrent fortification experiments exist from supervised field trials or processing studies.

The analytical methods for the determination of pyraclostrobin in **foodstuffs of animal origin** were evaluated in the previous Annex I inclusion process. Pyraclostrobin residues in commodities of animal origin can be determined by HPLC-UV methods with a validated LOQ of 0.01 mg/kg in milk and 0.05 mg/kg in muscle, liver, kidney, fat and eggs (BASF methods 439/0 and 446).

Information on the residue definition relevant for the EU is shown in Table 4.1.2-8.

Table 4.1.2-14: Residue definition - pyraclostrobin

End-Point	Active Substance: Pyraclostrobin	
	EU agreed endpoints (SANCO/1420/2001; Monograph 12945/ECCO/BBA/01, Vol. 1, list of endpoints)	Residue definitions proposed in context of this dossier
Residue definition in plant matrices for risk assessment	Pyraclostrobin (parent)	Pyraclostrobin (parent)
Residue definition in plant matrices for monitoring	Pyraclostrobin (parent)	Pyraclostrobin (parent)
Residue definition in animal matrices for risk assessment	Pyraclostrobin (parent) except: liver (except poultry liver) and milk fat only: pyraclostrobin and its metabolites analysed as the hydroxypyrazoles BF 500-5 * and BF 500-8 **, sum expressed as pyraclostrobin	Pyraclostrobin and its metabolites analysed as the hydroxypyrazoles BF 500-5 * and BF 500-8 **, sum expressed as pyraclostrobin
Residue definition in animal matrices for monitoring	Pyraclostrobin (parent)	Pyraclostrobin (parent)

* synonym: 500M04
** synonym: 500M85

The general suitability of the residue analytical methods provided for data generation and risk assessment has been confirmed by EFSA in the recently published Reasoned Opinion on MRLs (Review of established MRLs according to Reg. 396/2005 (Art. 12); EFSA Journal 2011;9(8):2344). They are supported by appropriate extractability investigations performed with sample material generated in course of the metabolism studies in crops and livestock.

Food of plant origin

Pyraclostrobin (BAS 500 F)

Due to the broad use of pyraclostrobin containing formulations, the scope of the methods has been considerably expanded over the past years. In 2011, EFSA re-evaluated the safety of the established EU MRLs according to Reg. 396/2005, Art. 12. In context of the evaluation the following data gap has been identified:

- a validated method for enforcement of pyraclostrobin in coffee beans.

The method summarized below has been used for the analysis of the samples generated during supervised field trials in Brazil. The independent laboratory validation study for method 421/0 in coffee is included in M-CA 4.2 (see CA 4.2/1).

Report:	CA 4.1.2/13 Leite R., 2005a Validation study of the SOP-PA.0243 for determination of Pyraclostrobin and its metabolite (BF 500-3) residues in coffee (grain), soybean (grain) and wheat (grain) 2005/1037978
Guidelines:	INMETRO NIT DICLA- 028 Rev. 01 (Sept. 2003), NBR ISO/IEC 17025/2001
GLP:	yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Principle of the method

SOP-PA.0243:

After extraction of the plant material with a methanol/water/hydrochloric acid mixture and subsequent centrifugation of an aliquot, an aliquot of the supernatant is transferred into a culture tube containing water. For purification, liquid/liquid partitioning with cyclohexane is performed. The cyclohexane is evaporated to dryness and the residue dissolved in methanol/water for LC-MS/MS quantification. For pyraclostrobin, the transition ions m/z = 388 -> 194 and m/z = 388-> 163 and for BF 500-3 m/z = 358 -> 164 and m/z = 358 -> 132 can be used for quantification.

Recovery findings

The method proved to be suitable for analysis of pyraclostrobin and BF 500-3 in coffee (grain), soybean (grain) and wheat (grain) to a limit of quantitation of 0.02 mg/kg for each analyte.
In all matrices tested, the mean recovery values were between 70 and 110%. The detailed results are given in Table 4.1.2-9.

Table 4.1.2-15: Recovery results of BAS 500 F and BF 500-3

Crop, Commodity	Test Substance	Fortification level (mg/kg)	No. of tests	Average Recovery (%)	Rel. Standard Deviation (%)
Coffee, grain	BAS 500 F	0.02, 2.0	10	101 [1] / 104 [2]	5.0 [1] / 4.0 [2]
	BF 500-3	0.02, 2.0	10	96 [3] / 96 [4]	5.0 [3] / 4.0 [4]
Soybean, grain	BAS 500 F	0.02, 2.0	10	92 [1] / 92 [2]	5.0 [1] / 4.0 [2]
	BF 500-3	0.02, 2.0	10	85 [3] / 85 [4]	4.0 [3] / 4.0 [4]
Wheat, grain	BAS 500 F	0.02, 2.0	10	103 [1] / 106 [2]	6.0 [1] / 5.0 [2]
	BF 500-3	0.02, 2.0	10	100 [3] / 100 [4]	7.0 [3] / 6.0 [4]

[1] For transition 388 → 194; [2] For transition 388 → 163; [3] For transition 358 → 164; [4] For transition 358 → 132

Linearity

Good linearity was observed in the range of 0.25 to 5.0 ng/mL for pyraclostrobin and BF 500-3 (external reference standard).

Specificity

The method determines parent pyraclostrobin and its metabolite BF 500-3 in coffee (grain), soybean (grain) and wheat (grain). There were no known interferences from plant components or from reagents, solvents and glassware used.

Limit of Quantification

The limit of quantification was defined by the lowest fortification level successfully tested, which was 0.02 mg/kg for each analyte in all sample materials.

Repeatability

The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%. The detailed values are shown in Table 4.1.2-9.

Reproducibility

The reproducibility of the method was not estimated as identical samples were not evaluated by an independent laboratory. However, based on the performance of the method, its reproducibility is expected to be good.

Conclusion

The method for analysis of pyraclostrobin and its metabolite BF 500-3 (500M07) uses LC-MS/MS for final determination, which is a modern and highly specific technique. The limit of quantitation is 0.02 mg/kg for each analyte.
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries.

Metabolite 500M04 and its glycoside conjugates

The method was developed in order to perform analysis of representative samples generated during supervised field trials. It is intended to support dietary risk assessment for the metabolite 500M04 and its conjugates as they were found in levels up to 5 – 10% TRR in the crop metabolism studies (mainly in leaf and green matter samplings). For proving the suitability of the enzymatic cleavage step applied, the glycoside 500M79 was selected; it was synthesized and exemplarily tested in fortification experiments.

Report:	CA 4.1.2/14 Courtois J., 2014a Validation of analytical method L0220/01 for the determination of metabolite 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS 2014/1001721
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/825/00 rev. 8.1 (16 November 2010), OECD-ENV/JM/MONO/(2007)17 (OECD No. 72)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 4.1.2/15 Eilers B. et al., 2014a Method for the determination of 500M79 (Reg.No. 5937091) in plant matrices by LC-MS/MS 2014/1001641
Guidelines:	not applicable
GLP:	no

Principle of the method

BASF Method No. L0220/01:

Metabolite 500M79 is extracted from plant matrices using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is evaporated to dryness and dissolved in water. Subsequently the analyte is hydrolyzed using enzymatic cleavage to 500M04 (BF 500-5). It is followed by reversed phase C18-column clean-up. The final determination of 500M04 is performed by UPLC-MS/MS. The results are expressed in 500M04. For 500M79 the ion transitions *m/z* = 195 → 153 and *m/z* = 195 → 150 can be used for quantification.

Recovery findings

The method proved to be suitable for analysis of metabolite 500M79 in plant matrices at a limit of quantitation of 0.01 mg/kg.
In all matrices tested (lettuce heads, white cabbage, leek), the mean recovery values were between 78 and 82%. The detailed results are given in Table 4.1.2-10.

Results of stability investigations of calibration and fortification solutions as well as stability investigations of the final volumes are summarized in Table 4.1.2-11, Table 4.1.2-12 and Table 4.1.2-13. The results show that fortification solutions in acetonitrile and calibration standards in acetonitrile/water (50/50, v/v) are stable over the investigated time of 30 days.

Table 4.1.2-16: Recovery results of 500M79

Matrix	Test Substance	Fortification level (mg/kg)	No. of tests	Average Recovery (%)	Rel. Standard Deviation (%)
Lettuce (head)	500M79	0.01	5	80.8 [1] / 82.5 [2]	3.0 [1] / 8.6 [2]
		0.1	5	81.8 [1] / 84.9 [2]	1.4 [1] / 4.5 [2]
		overall: 0.01 + 0.1	10	81.3 [1] / 83.7 [2]	2.3 [1] / 6.6 [2]
White cabbage	500M79	0.01	5	76.2 [1] / 74.1 [2]	6.3 [1] / 7.4 [2]
		0.1	5	80.3 [1] / 79.6 [2]	1.9 [1] / 3.0 [2]
		overall: 0.01 + 0.1	10	78.2 [1] / 76.9 [2]	5.1 [1] / 6.4 [2]
Leek (whole)	500M79	0.01	5	79.6 [1] / 88.9 [2]	1.6 [1] / 5.3 [2]
		0.1	5	83.6 [1] / 83.5 [2]	3.9 [1] / 4.1 [2]
		overall: 0.01 + 0.1	10	81.6 [1] / 86.2 [2]	3.8 [1] / 5.6 [2]

[1] For transition *m/z* 195 → 153; [2] For transition *m/z* 195 → 150

Table 4.1.2-17: Storage stability of fortification standards (at 4°C ± 2°C)

Concentration of the solution	Storage time (d)	Concentration [ng/mL]						
		Transition *m/z* 195 → 153 Sample 1			Transition *m/z* 195 → 153 Sample 2			Average Deviation compared to fresh sample [%]
0.5 ng/mL	0	0.53	0.54	0.51	0.53	0.54	0.54	--
	9	0.53	0.54	0.52	0.55	0.56	0.53	-2.2
	13	0.52	0.54	0.54	0.51	0.54	0.52	0.5
	29	0.49	0.51	0.52	0.50	0.51	0.51	4.6

Table 4.1.2-18: Storage stability of calibration standards (at 4°C ± 2°C)

Concentration of the solution	Storage time (d)	Concentration [ng/mL]						Average Deviation compared to fresh sample [%]
		Transition *m/z* 195 → 153 Sample 1			Transition *m/z* 195 → 153 Sample 2			
0.25 ng/mL	0	0.24	0.25	0.25	0.25	0.25	0.25	--
	9	0.24	0.25	0.24	0.25	0.25	0.25	0.4
	14	0.24	0.24	0.24	0.24	0.26	0.25	1.7
	29	0.25	0.24	0.24	0.24	0.25	0.24	3.4

Table 4.1.2-19: Final volume stability (sample extracts) at 4°C ± 2°C

Matrix	Storage time (days)	Concentration [ng/mL]									Average Deviation compared to fresh sample [%]
		Transition *m/z* 195 → 153 Sample 1			Transition *m/z* 195 → 153 Sample 2			Transition *m/z* 195 → 153 Sample 3			
Leek (whole)	0	0.139			0.126			0.138			--
	5	0.126	0.128	0.128	0.121	0.112	0.111	0.116	0.120	0.123	90
Lettuce (head)	0	0.114			0.113			0.107			--
	5	0.120	0.120	0.112	0.116	0.116	0.114	0.111	0.108	0.108	102
White cabbage	0	0.093			0.109			0.105			--
	5	0.098	0.097	0.102	0.113	0.113	0.115	0.103	0.105	0.105	103
		Overall final volume stability									98

Linearity	The linearity of the UPLC-MS/MS detector was tested using at least 5 standard solutions at concentrations between 0.025 and 2.5 ng/mL. Standards were injected and the response was plotted against concentration. Linear correlations with coefficients ≥0.999 were obtained for metabolite 500M79.
Specificity	The method L0220/01 determines residues of metabolite 500M79 in plant matrices. Significant interferences (> 20 or 30%) were not observed at the retention times and mass transitions considered for the analyte. UPLC-MS/MS, using two mass transitions, is a highly specific detection technique and therefore no additional confirmatory technique is required.
Limit of Quantification	The limit of quantification (LOQ) was defined as the lowest fortification level successfully tested. The limit of quantification was 0.01 mg/kg for the analyte (metabolite 500M79).
Repeatability	The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%. The detailed values are shown in Table 4.1.2-10.
Reproducibility	The reproducibility of the method was not estimated as identical samples were not evaluated by an independent laboratory. However, based on the performance of the method, its reproducibility is expected to be good.
Conclusion	**The results show that 500M79 can be hydrolyzed to 500M04 (BF 500-5) with BASF method No. L0220/01. The limit of quantitation was defined by the lowest fortification level successfully tested which was 0.01 mg/kg in all matrices. Validation criteria with mean recoveries between 70-110%, relative standard deviation ≤ 20% and blank values ≤ 30% were always met. No separate confirmation technique was necessary as two parent-daughter ion transitions were monitored during LC-MS/MS determination and evaluated.**

Report:	CA 4.1.2/16 Jose W.F.P. de, 2015 a Validation of BASF Method Number L0076/09 for the determination of BAS 500 F and its metabolite 500M07 in citrus (whole fruit), dry beans (seeds), tomato (whole fruit), soybeans (grain) and wheat (grain) using HPLC-MS/MS and UPLC-MS/MS 2015/3004795
Guidelines:	Resolucao RDC No. 4 - ANVISA (18/01/2012)
GLP:	yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Principle of the methods

Residues of pyraclostrobin and its metabolite 500M07 are extracted from plant matrices with a mixture of methanol / water / HCl (2 mol/L) (70/25/5, v/v/v). After centrifugation, an aliquot is alkalized and partitioned against cyclohexane. Then, after evaporation to dryness, residues are dissolved in methanol/water (50/50, v/v) and filtered before injection. Final determination is performed by HPLC- and UPLC-MS/MS monitoring ion transition at m/z 388→194 (for quantitation) and at m/z 388→163 (for confirmation) for pyraclostrobin and at m/z 358→164 (for quantitation) and at m/z 358→132 (for confirmation) for its metabolite 500M07 in ESI positive mode. Analysis by HPLC is accomplished on a Thermo Scientific, Betasil C18 column applying a methanol-pure water gradient and analysis by UPLC is accomplished on an Acquity BEH C18 column applying an acetonitrile-pure water gradient. For both analyses 0.1% formic acid is used as modifier.

Recovery findings

Method validation acceptance criteria were fully met with mean recovery values between 70 and 110%. The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%. Method validation data are summarized in the tables below.

Table 4.1.2-20: Recovery results from method validation of pyraclostrobin and its metabolite 500M07 using the analytical method L0076/09 - HPLC

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Dry beans	parent	0.01 (n=6)	92	3.6	Quantitation
		1.0 (n=6)	95	1.9	m/z 388→194
		0.01 (n=6)	86	6.7	Confirmation
		1.0 (n=6)	93	3.6	m/z 388→163
	500M07	0.01 (n=6)	91	6.7	Quantitation
		1.0 (n=6)	96	3.2	m/z 358→164
		0.01 (n=6)	86	5.3	Confirmation
		1.0 (n=6)	100	2.1	m/z 358→132
Soya bean, grain	parent	0.01 (n=6)	101	3.7	Quantitation
		1.0 (n=6)	82	2.3	m/z 388→194
		0.01 (n=6)	98	3.9	Confirmation
		1.0 (n=6)	83	3.6	m/z 388→163
	500M07	0.01 (n=6)	84	4.2	Quantitation
		1.0 (n=6)	82	3.3	m/z 358→164
		0.01 (n=6)	76	3.1	Confirmation
		1.0 (n=6)	78	6.3	m/z 358→132
Citrus	parent	0.01 (n=6)	94	2.8	Quantitation
		1.0 (n=6)	97	2.4	m/z 388→194
		0.01 (n=6)	92	3.0	Confirmation
		1.0 (n=6)	97	5.6	m/z 388→163
	500M07	0.01 (n=6)	94	2.0	Quantitation
		1.0 (n=6)	98	4.6	m/z 358→164
		0.01 (n=6)	91	4.3	Confirmation
		1.0 (n=6)	99	3.4	m/z 358→132
Wheat	parent	0.01 (n=6)	100	4.7	Quantitation
		1.0 (n=6)	92	2.6	m/z 388→194
		0.01 (n=6)	100	4.2	Confirmation
		1.0 (n=6)	91	3.4	m/z 388→163
	500M07	0.01 (n=6)	100	8.3	Quantitation
		1.0 (n=6)	95	2.8	m/z 358→164
		0.01 (n=6)	96	9.4	Confirmation
		1.0 (n=6)	95	1.8	m/z 358→132
Tomato	parent	0.01 (n=5)	86	13.0	Quantitation
		1.0 (n=6)	97	5.1	m/z 388→194
		10 (n=6)	85	3.1	
		0.01 (n=5)	87	13.0	Confirmation
		1.0 (n=6)	93	4.6	m/z 388→163
		10 (n=6)	86	3.5	
	500M07	0.01 (n=5)	86	12.0	Quantitation
		1.0 (n=6)	99	6.7	m/z 358→164
		0.01 (n=5)	83	15.0	Confirmation
		1.0 (n=6)	96	10.0	m/z 358→132

Table 4.1.2-21: Recovery results from method validation of pyraclostrobin and its metabolite 500M07 using the analytical method L0076/09 - UPLC

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Dry beans	parent	0.01 (n=6)	85	3.9	Quantitation
		1.0 (n=6)	89	6.7	m/z 388→194
		0.01 (n=6)	89	3.4	Confirmation
		1.0 (n=6)	91	5.2	m/z 388→163
	500M07	0.01 (n=6)	85	4.9	Quantitation
		1.0 (n=6)	93	5.0	m/z 358→164
		0.01 (n=6)	81	4.9	Confirmation
		1.0 (n=6)	92	4.1	m/z 358→132
Soya bean, grain	parent	0.01 (n=6)	90	7.8	Quantitation
		1.0 (n=6)	82	7.3	m/z 388→194
		0.01 (n=6)	90	6.6	Confirmation
		1.0 (n=6)	79	5.5	m/z 388→163
	500M07	0.01 (n=6)	76	2.0	Quantitation
		1.0 (n=6)	81	5.6	m/z 358→164
		0.01 (n=6)	77	5.1	Confirmation
		1.0 (n=6)	77	5.1	m/z 358→132
Citrus	parent	0.01 (n=6)	94	5.2	Quantitation
		1.0 (n=6)	95	1.6	m/z 388→194
		0.01 (n=6)	85	6.3	Confirmation
		1.0 (n=6)	93	4.2	m/z 388→163
	500M07	0.01 (n=6)	87	4.7	Quantitation
		1.0 (n=6)	99	5.9	m/z 358→164
		0.01 (n=6)	88	2.5	Confirmation
		1.0 (n=6)	103	6.1	m/z 358→132
Wheat	parent	0.01 (n=6)	109	7.2	Quantitation
		1.0 (n=6)	92	5.8	m/z 388→194
		0.01 (n=6)	108	2.4	Confirmation
		1.0 (n=6)	93	4.2	m/z 388→163
	500M07	0.01 (n=6)	102	5.9	Quantitation
		1.0 (n=6)	93	5.0	m/z 358→164
		0.01 (n=6)	101	7.7	Confirmation
		1.0 (n=6)	94	4.1	m/z 358→132
Tomato	parent	0.01 (n=6)	93	6.7	Quantitation
		1.0 (n=6)	101	7.5	m/z 388→194
		10 (n=6)	92	7.6	
		0.01 (n=6)	105	6.0	Confirmation
		1.0 (n=6)	102	6.6	m/z 388→163
		10 (n=6)	92	4.9	
	500M07	0.01 (n=6)	106	7.2	Quantitation
		1.0 (n=6)	108	4.3	m/z 358→164
		0.01 (n=6)	109	2.4	Confirmation
		1.0 (n=6)	107	4.2	m/z 358→132

Linearity	Calibration points were distributed over a concentration range of 0.04 to 2.0 ng/mL. Calibration standards were prepared in methanol/water (50/50, v/v). At least six calibration points were used and individual calibration data was presented. Good linearity was observed in the range of 0.04 to 2 ng/mL (external reference standard, injected in triplicate) with a correlation coefficient ≥0.99.
Specificity	Highly selective determination of pyraclostrobin and its metabolite 500M07 using LC-MS/MS monitoring two mass transitions. There were no interferences at the retention time corresponding to BAS 500 F and its metabolite 500M07 in any of the control specimens of citrus, dry beans, tomato, soya bean and wheat grain (blank value < 30 % LOQ).
Matrix Effects	Analysis of matrix-matched standards and solvent standards showed no significant matrix effects, except for pyraclostrobin in soya beans. Therefore, solvent-based calibration standard solutions were used for quantification, except for pyraclostrobin in soya beans, where matrix-matched calibration standard solutions were used.
Limit of Quantification	The limit of quantitation (LOQ) representing the lowest validated fortification level with sufficient recovery and precision was 0.01 mg/kg for pyraclostrobin and its metabolite 500M07.
Limit of Detection	The LOD is set at 20% of the LOQ, or 0.002 mg/kg, which corresponds to 0.04 ng/mL for the measurement solution.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels are below 20%.
Standard Stability	Pyraclostrobin and its metabolite 500M07 have been shown to be stable in methanol, the solvent used for preparation of stock solution, intermediate solutions and fortification standard solutions for up to 68 days, and in calibration solutions prepared by serial dilution of the intermediate solutions with methanol/water (50/50, v/v) for up to 34 days, when stored under refrigerator conditions.
Stability of Working Solutions	Pyraclostrobin and its metabolite 500M07 were shown to be stable after extraction with a mixture consisting of methanol/Milli-Q water/2 mol/L HCl solution (70/25/5, v/v/v) for a time interval of 0 to 7 days for all matrices, when stored under refrigerator conditions. The final volume dissolved with a mixture of methanol:Milli-Q water (50/50, v/v) was also investigated and pyraclostrobin and its metabolite 500M07 were shown to be stable for up to 7 days.
Reproducibility	Reproducibility of the method was not determined within this validation study.

Conclusion

The method L0076/09 uses highly specific LC-MS/MS for final determination of pyraclostrobin and its metabolite 500M07. The method is sufficiently validated for the commodities citrus (whole fruit), dry beans (seeds), tomato (whole fruit), soya bean (grain) and wheat (grain). Thereby, it could be demonstrated that the method fulfills the requirements with regard to linearity, specificity, repeatability, limit of quantitation and recoveries.

Food of animal origin

For proving the suitability of the method used in the cow feeding study, the common moiety method has been validated for further metabolites being identified in the livestock metabolism studies. Due to the high amounts found, specific attention was given to the metabolite 500M04 which occurs in amounts of max. 30% TRR (milk). The requirement to present validation data for key metabolites is included in the OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17). The principle of the common moiety approach from 2000 was kept; only the final quantitation technique (LC-MS/MS instead of GC-MS) was changed.

Report:	CA 4.1.2/17 Eilers B., Taraschewski I., 2014b Validation of analytical method 446/2 (L0058/03) for the determination of BAS 500 F (Reg.No. 304428) and its metabolites 500M04 (Reg.No. 298327) and 500M85 (Reg.No. 399530) in animal matrices by LC-MS/MS 2013/1400972
Guidelines:	SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), SANCO/825/00 rev. 8.1 (16 November 2010), EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, OECD-ENV/JM/MONO/(2007)17 (OECD No. 72)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 4.1.2/18 Tilting N. et al., 2014a Method for Determination of BAS 500 F (Reg.No. 304428) and its metabolites 500M04 (Reg.No. 298327) and 500M85 (Reg.No. 399530) in animal matrices by LC-MS/MS 2014/1138680
Guidelines:	not applicable
GLP:	no

Note: This reference was erroneously not included in the study list of the application.

Principle of the method

BASF Method No. 446/2 (L0058/03):

After a partition into acetonitrile/iso-hexane the total residues were cleaved by boiling in aqueous sodium hydroxide to yield hydroxpyrazole(s), which can be extracted using ethyl acetate. After acidification and phase separation, the organic layer was taken. The final determination of 500M04 (BF 500-5) and 500M85 (BF 500-8) is performed by HPLC-MS/MS. Pyraclostrobin was determined as 500M04; for 500M04 the ion transitions *m/z* = 195 → 117 and *m/z* = 195 → 153 and for 500M85 the transitions *m/z* = 211 → 138 and *m/z* = 211 → 166 can be used for quantification.

Recovery findings

The method proved to be suitable for analysis of pyraclostrobin and its metabolites 500M04 (BF 500-5) and 500M85 (BF 500-8) in animal matrices at a limit of quantitation of 0.01 mg/kg.
In all matrices tested, the mean recovery values were between 62 and 95%. The detailed results are given in Table 4.1.2-14.

Table 4.1.2-22: Recovery results of BAS 500 F, 500M04 and 500M85

Matrix	Test Substance	Fortification level (mg/kg)	No. of tests	Average Recovery (%)	Rel. Standard Deviation (%)
Muscle	BAS 500 F	0.01, 0.1	10 (5 per level)	77.7 [1] / 77.5 [2]	4.3 [1] / 4.2 [2]
	500M04	0.01, 0.1	10 (5 per level)	86.8 [3] / 86.6 [4]	7.5 [3] / 7.0 [4]
	500M85	0.01, 0.1	10 (5 per level)	62.1 [5] / 61.7 [6]	2.8 [5] / 3.2 [6]
Kidney	BAS 500 F	0.01, 0.2	10 (5 per level)	88.5 [1] / 84.8 [2]	3.8 [1] / 2.7 [2]
	500M04	0.01, 0.2	10 (5 per level)	81.1 [3] / 81.2 [4]	10.4 [3] / 8.9 [4]
	500M85	0.01, 0.2	10 (5 per level)	62.2 [5] / 62.2 [6]	8.2 [5] / 8.7 [6]
Liver	BAS 500 F	0.01, 1.0	10 (5 per level)	88.1 [1] / 92.1 [2]	6.7 [1] / 9.3 [2]
	500M04	0.01, 1.0	10 (5 per level)	83.5 [3] / 84.2 [4]	8.7 [3] / 9.1 [4]
	500M85	0.01, 1.0	10 (5 per level)	78.7 [5] / 80.0 [6]	7.8 [5] / 7.5 [6]
Fat	BAS 500 F	0.01, 0.1	10 (5 per level)	83.4 [1] / 83.6 [2]	7.8 [1] / 6.5 [2]
	500M04	0.01, 0.1	10 (5 per level)	73.8 [3] / 74.3 [4]	7.8 [3] / 7.6 [4]
	500M85	0.01, 0.1	10 (5 per level)	67.2 [5] / 67.4 [6]	8.2 [5] / 7.9 [6]
Milk	BAS 500 F	0.01, 0.1	10 (5 per level)	91.6 [1] / 90.1 [2]	4.6 [1] / 4.9 [2]
	500M04	0.01, 0.1	10 (5 per level)	89.7 [3] / 94.5 [4]	4.9 [3] / 6.9 [4]
	500M85	0.01, 0.1	10 (5 per level)	90.2 [5] / 90.1 [6]	8.2 [5] / 7.8 [6]
Egg	BAS 500 F	0.01, 0.1	10 (5 per level)	76.4 [1] / 76.9 [2]	7.4 [1] / 5.7 [2]
	500M04	0.01, 0.1	10 (5 per level)	87.5 [3] / 87.6 [4]	5.7 [3] / 6.3 [4]
	500M85	0.01, 0.1	10 (5 per level)	80.3 [5] / 79.6 [6]	6.7 [5] / 5.1 [6]

[1] For transition *m/z* 195 → 117; [2] For transition *m/z* 195 → 153; [3] For transition *m/z* 195 → 117;
[4] For transition *m/z* 195 → 153; [5] For transition *m/z* 211 → 138; [6] For transition *m/z* 211 → 166

Linearity

The linearity of the HPLC-MS/MS detector was tested using six standard solutions at concentrations between 0.05 and 2.5 ng/mL. Standards were injected in duplicate and the response plotted against concentration. Linear correlations with coefficients ≥0.99 were obtained for pyraclostrobin and its metabolites 500M04 and 500M85.

Specificity

The method L0058/03 determines residues of pyraclostrobin and its metabolites 500M04 and 500M85 in animal matrices. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions considered for each analyte. HPLC-MS/MS, using two mass transitions, is a highly specific detection technique and therefore a confirmatory technique is not required.

Limit of Quantification

The limit of quantification (LOQ) was defined as the lowest fortification level successfully tested. The limit of quantification was 0.01 mg/kg for all analytes.

Repeatability

The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%. The detailed values are shown in Table 4.1.2-14.

Reproducibility

The reproducibility of the method was not estimated as identical samples were not evaluated by an independent laboratory. However, based on the performance of the method, its reproducibility is expected to be good.

Conclusion

The method for analysis of pyraclostrobin and its metabolites in animal matrices uses LC-MS/MS for final determination, which is a modern and highly specific technique. The limit of quantitation is 0.01 mg/kg for each analyte.
It could be demonstrated that method L0058/03 fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of pyraclostrobin and its metabolites 500M04 (BF 500-5) and 500M85 (BF 500-8) in animal matrices.

Method L0151/01 for the determination of pyraclostrobin in food of animal origin is also provided for enforcement purposes. The independent laboratory validation study is included in M-CA 4.2 (see CA 4.2/3). In order to allow an efficient enforcement of pyraclostrobin parent residues using up-to-date technology (LC-MS/MS) method L0151/01 replaces the previously submitted and evaluated LC-UV method 439/0. It was also validated in blood. As required in SANCO 825/00 and the relevant OECD guidance document, the validation data include a confirmatory technique (second transition).

Report:	CA 4.1.2/19 Hopf B., 2010a Validation of the analytical method L0151/01: Method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices 2010/1018944
Guidelines:	EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 4.1.2/20 Hopf B., 2011a Technical procedure: Method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices - BASF Method Number L0151/01 2011/1018046
Guidelines:	not applicable
GLP:	no

Principle of the method

BASF method L0151/01:

Pyraclostrobin (BAS 500 F) is extracted with acetonitrile. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. An aliquot of the organic phase is evaporated to dryness and dissolved in a mixture of acetonitrile and water. The final determination of pyraclostrobin is performed by HPLC-MS/MS, monitoring two parent-daughter ion transitions.

The limit of quantitation (LOQ) of the method for cow liver, kidney, fat, muscle, milk, skim milk, cream and hen egg is 0.01 mg/kg. The LOQ for swine blood is 0.01 mg/L.

Recovery findings

The recoveries of pyraclostrobin in fortified cow muscle, kidney, fat, liver, milk, skim milk, cream, hen egg and swine blood samples are summarized in Table 4.1.2-15. The recoveries of pyraclostrobin ranged from 79.0 to 101.6% at the transition *m/z* 388 → *m/z* 194 and 79.8 to 101.1% at the transition *m/z* 388 → *m/z* 163. The CV ranged from 2.1 to 5.7% and 2.2 to 6.4%, respectively.

The recovery results in liver and egg after storage of extract solutions and final volumes (at 10x LOQ) are summarized in Table 4.1.2-16. Extract solutions, which were stored for 7 days and further worked-up thereafter, showed that the analyte was stable under refrigerated conditions. Also the solutions prepared for the final determination by HPLC-MS/MS had been kept in the refrigerator for 7 days. Their re-analysis after storage led to quite comparable recoveries.

Results of stability investigations of calibration and fortification solutions are summarized in Table 4.1.2-17. The results show that fortification solutions in acetonitrile and calibration standards in acetonitrile/water (50/50, v/v) are stable over the investigated time of 30 days.

Table 4.1.2-23: Recoveries of pyraclostrobin in animal matrices

Matrix	Fortification level (mg/kg)	n	Transition *m/z* 388 → 194*			Transition *m/z* 388 → 163		
			Mean recovery (%)	SD	CV (%)	Mean recovery (%)	SD	CV (%)
Cow, muscle	0.01	5	101.1	1.6	1.6	101.6	1.2	1.2
	0.1	5	97.0	1.7	1.7	95.8	1.4	1.5
	0.01 + 0.1	**10**	**99.1**	**2.7**	**2.7**	**98.7**	**3.3**	**3.4**
Cow, kidney	0.01	5	105.8	1.4	1.4	105.2	1.7	1.6
	0.1	5	97.3	1.8	1.8	97.0	0.8	0.8
	0.01 + 0.1	**10**	**101.6**	**4.7**	**4.7**	**101.1**	**4.5**	**4.4**
Cow, fat	0.01	5	102.7	1.6	1.5	102.4	1.3	1.3
	0.1	5	99.7	1.3	1.3	98.7	1.0	1.1
	0.01 + 0.1	**10**	**101.2**	**2.1**	**2.1**	**100.6**	**2.2**	**2.2**
Cow, liver	0.01	5	98.4	4.1	4.2	99.3	4.0	4.0
	0.1	5	96.7	2.0	2.1	95.1	1.3	1.4
	0.01 + 0.1	**10**	**97.6**	**3.2**	**3.2**	**97.2**	**3.6**	**3.7**
Cow, milk	0.01	5	102.7	1.9	1.8	101.1	1.6	1.6
	0.1	5	96.1	1.3	1.4	96.4	1.6	1.6
	0.01 + 0.1	**10**	**99.4**	**3.8**	**3.8**	**98.8**	**2.9**	**2.9**
Cow, skim milk	0.01	5	94.4	1.3	1.4	95.2	0.9	1.0
	0.1	5	90.9	2.1	2.3	90.6	1.3	1.5
	0.01 + 0.1	**10**	**92.7**	**2.5**	**2.7**	**92.9**	**2.7**	**2.9**
Cow, cream	0.01	5	99.3	4.9	5.0	99.8	3.5	3.5
	0.1	5	95.5	3.6	3.8	94.9	2.9	3.1
	0.01 + 0.1	**10**	**97.4**	**4.5**	**4.6**	**97.4**	**4.0**	**4.1**
Hen, egg	0.01	5	94.4	7.3	7.7	93.6	8.3	8.9
	0.1	5	98.5	1.8	1.8	98.0	1.5	1.6
	0.01 + 0.1	**10**	**96.5**	**5.5**	**5.7**	**95.8**	**6.1**	**6.4**
Swine, blood	0.01**	5	81.4	3.0	3.7	81.9	2.9	3.5
	0.1**	5	76.5	1.2	1.6	77.6	1.9	2.5
	0.01 + 0.1	**10**	**79.0**	**3.4**	**4.3**	**79.8**	**3.2**	**4.1**

* used for quantification; ** mg/L

Table 4.1.2-24: Recovery results of pyraclostrobin in liver and egg after storage of extract solutions and final volumes (at 10x LOQ)

Solution	Storage time (d)	n	Transition *m/z* 388 → 194			Transition *m/z* 388 → 163		
			Mean recovery (%)	SD	CV (%)	Mean recovery (%)	SD	CV (%)
Matrix: Cow liver								
Validation sample	0	5	96.7	2.0	2.1	95.1	1.3	1.4
Stored extract [1)]	7	5	99.7	0.9	0.9	100.6	1.3	1.3
Stored final volume [2)]	7	5	99.9	2.0	2.0	99.4	1.8	1.8
Matrix: Hen egg								
Validation sample	0	5	98.5	1.8	1.8	98.0	1.5	1.6
Stored extract [1)]	7	5	90.4	1.4	1.5	91.0	1.8	2.0
Stored final volume [2)]	7	5	102.4	1.4	1.4	103.6	1.4	1.4

[1)] extract stored in acetonitrile for 7 days
[2)] final volume stored in acetonitrile / water (50/50, v/v) for 7 days

Table 4.1.2-25: Standard Stability in Acetonitrile (100 ng/mL) and Acetonitrile/Water (0.25 ng/mL)

Storage time (days)	Concentration in refrigerator at dark (ng/mL)			
	Transition *m/z* 388 → 194		Transition *m/z* 388 → 163	
Stability of BAS 500 F in acetonitrile (100 ng/mL)				
0	104.4	104.6	104.0	103.8
8	97.2	101.2	96.6	102.2
14	104.8	105.0	101.8	103.2
30	100.6	98.2	98.6	100.2
Stability of BAS 500 F in acetonitrile/water (0.25 ng/mL)				
0	0.254	0.257	0.254	0.254
8	0.255	0.262	0.254	0.259
14	0.261	0.257	0.254	0.252
30	0.249	0.248	0.247	0.246

Linearity	Good linearity was observed in the range of 0.025 to 1.0 ng/mL pyraclostrobin.
Specificity	No significant interferences from the sample matrix were reported.
Limit of quantitation	The limit of quantitation was defined by the lowest fortification level successfully tested, which was 0.01 mg/kg for the analyte in cow liver, kidney, fat, muscle, milk, skim milk, cream and hen egg and 0.01 mg/L for swine blood.
Repeatability	The coefficients of variation with respect to recoveries following fortifications at the limit of quantification were between 0.8 and 8.9%. The values obtained are indicative of the method having satisfactory repeatability.
Reproducibility	The reproducibility of the study is proven with the use in an independent laboratory validation. The summary of the ILV study is included under M-CA 4.2/3. Due to the fact, that for the fortified matrices recoveries and RSDs provided in context of the original report and the related ILV were always well in the ranges specified in the current guidelines (e.g. SANCO/825/00 rev. 8.1), it can be stated, that BASF method L0151/01 shows a good reproducibility.
Conclusion	**The results show that BASF method No. L0151/01 is suitable to determine residues of pyraclostrobin in matrices of animal origin such as cow muscle, kidney, fat, liver, milk, skim milk, cream as well as swine blood and hen egg. In all matrices tested, the mean recovery values were between 70 and 110%. The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%.** **Furthermore, it could be proven in this study that the analyte was stable in the extract solvent and in the solution used as final volume. Also the fortification solutions and calibration standards proved to be stable over a period of 30 days.**

In order to support the poultry feeding study, the common residue analytical method used for sample analysis is provided as supplemental information. The poultry feeding study was submitted to US EPA. Main purpose of the submission within this dossier is to allow a comprehensive evaluation of all basic studies. Due to the favourable residue behaviour in hen tissues and eggs observed within the metabolism study, the poultry feeding study has not been submitted in Europe before.

Report: CA 4.1.2/21
Malinsky D.S.,Riley M.E., 2000a
Method validation of BASF analytical method D9902: Method for determination of residues of BAS 500F and its metabolite BF 500-16 in hen tissues using LC/MS/MS
2000/5004
Guidelines: EPA 860.1340
GLP: yes
(certified by United States Environmental Protection Agency)

Principle of the method: *Method D9902*

Analytical Method D9902 was developed in order to determine residues of pyraclostrobin, metabolite BF 500-16 and related metabolites in hen tissues. Validation of the method was conducted on four hen tissues (egg, liver, muscle and fat). The method involves a common moiety approach for total residue determination. Pyraclostrobin and its metabolite BF 500-16 (500M77) are base hydrolyzed into metabolite BF 500-5 (500M04) and BF 500-9 (Reg.No. 402733, a derivative used for determination only and therefore not included in the metabolite list), respectively, so BF 500-5 and BF 500-9 are the final analytes of the method. Other compounds sharing the BF 500-5 and BF 500-9 moieties are also expected to undergo the same hydrolytic conversion to the analytes mentioned.

As a first step, egg, liver, and muscle samples are extracted as well as hydrolyzed in a sodium hydroxide solution. Fat samples are handled somewhat differently. Fat is first partitioned with hexane and acetonitrile before hydrolysis. After hydrolysis, an aliquot of the fat extract is purified by partitioning with ethyl acetate. For egg, liver and muscle aliquots after hydrolysis, samples are purified by ENV and silica solid phase extraction column chromatography. For all matrices, the final analytes BF 500-5 and BF 500-9 are measured by LC-MS/MS. The limit of quantitation of the method is 0.05 mg/kg for each analyte and for all matrices.

Recovery Findings

Good recoveries of pyraclostrobin were obtained in the four hen matrices over the fortification range tested (0.05 mg/kg and 0.10 mg/kg). Generally, recoveries for the parent were higher, averaging near 100% for all matrices. For the BF 500-16, recoveries were lower, averaging between 58-71% for the various matrices, but the deviation between samples was quite low. The overall average recoveries of pyraclostrobin and BF 500-16 in hen matrices were 103%±17 (n=32) and 65%±10 (n=32), respectively. Detailed results are shown in Table 4.1.2-18.

Table 4.1.2-26: Recovery results from method validation of BAS 500 F and BF 500-16 in animal matrices

Animal	Commodity	Test Substance	Fortification level (mg/kg)	No. of tests	Average recovery (%)	Rel. Standard Deviation (%)
Hen	Egg	BAS 500 F	0.05; 1.0	8 (4 per level)	104	16
		BF 500-16	0.05; 1.0	8 (4 per level)	62	11
	Liver	BAS 500 F	0.05; 1.0	8 (4 per level)	104	24
		BF 500-16	0.05; 1.0	8 (4 per level)	71	8
	Muscle	BAS 500 F	0.05; 1.0	8 (4 per level)	110	12
		BF 500-16	0.05; 1.0	8 (4 per level)	58	7
	Fat	BAS 500 F	0.05; 1.0	8 (4 per level)	103	17
		BF 500-16	0.05; 1.0	8 (4 per level)	65	15

Linearity

Good linearity was observed in the range of 2.5 to 25 pg/µL for BF 500-5 and BF 500-9 (external reference standard).

Specificity

The method determines parent pyraclostrobin and its metabolite BF 500-16 in hen tissues and eggs. There were no known interferences from plant components or from reagents, solvents and glassware used.

Limit of Quantification

The limit of quantification was defined by the lowest fortification level successfully tested, which was 0.05 mg/kg for each analyte in all sample materials.

Repeatability

The relative standard deviations for pyraclostrobin and BF 500-16 obtained from fortified egg, liver, muscle and fat samples were between 12 - 24% and 7 -15%, respectively. The detailed values are shown in Table 4.1.2-18.

Reproducibility	The reproducibility of the method was not estimated as identical samples were not evaluated by an independent laboratory. However, based on the performance of the method, its reproducibility is expected to be good.
Conclusion	**The method D9902 for analysis of pyraclostrobin and its metabolite BF 500-16 (500M77) uses HPLC-MS/MS for final determination, which is a modern and highly specific technique. The limit of quantitation is 0.05 mg/kg for each analyte.** **It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantitation and recoveries.**

(f) Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Methods for concentration control are reported, where necessary, along with the respective ecotoxicological studies.

(g) Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Where necessary, these methods are reported along with the respective studies.

CA 4.2 Methods for post-approval control and monitoring purposes

(a) Methods for the determination of all components included in the monitoring residue definition as submitted in accordance with the provision of point 6.7.1 in order to enable Member States to determine compliance with established maximum residue levels (MRLs); they shall cover residues in or on food and feed of plant and animal origin

Based on the residue definition for MRL setting and enforcement, residue analytical methods are required for the parent molecule pyraclostrobin in both, food of plant and animal origin.

The enforcement methods evaluated in the previous Annex I inclusion process were considered as suitable. They are supported by appropriate extractability investigations performed with sample material generated in course of the metabolism studies in crops and livestock.

The following conclusion on residue analytical methods for food of plant and animal origin was derived from the EFSA Reasoned Opinion on MRLs (European Food Safety Authority; Review of the existing maximum residue levels (MRLs) for pyraclostrobin according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(8):2344. [92 pp.] doi:10.2903/j.efsa.2011.2344. Available online: www.efsa.europa.eu/efsajournal).

Analytical methods for residues (Annex IIA, point 4.2, enforcement purposes)

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	LC-MS/MS	0.02 mg/kg (hops, high oil content, high water content, acidic and dry commodities)
	HPLC-UV	0.02 mg /kg (high fat content, acidic and dry commodities)
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	HPLC-UV	0.01 mg/kg (milk) 0.05 mg/kg (muscle, liver, kidney, fat, egg)

In addition, the QuEChERS method (determination using GC-MS and/or LC-MS/MS) is sufficiently validated at the LOQ of 0.01 mg/kg for the determination of pyraclostrobin in plant matrices. The use of the multi residue method for the determination of pyraclostrobin residues is widely published in the Internet.
In order to demonstrate the use of the QuEChERS method as additional enforcement method, two articles from literature are exemplarily summarized in this chapter. The first publication is describing the application of the method in coffee (CA 4.2/2). In the second publication comparative extractability investigations are described (CA 4.2/4). Both publications were not mentioned in the application for the renewal of approval.

Food of plant origin

Pyraclostrobin (BAS 500 F)

In context of the re-evaluation of existing MRLs, EFSA identified the lack of an enforcement method for coffee as data gap. For proving that the residue analytical method 421/0, which is recommended as enforcement method in other plant matrices, is suitable for coffee and tea, the subsequent independent laboratory validation study has been performed. Due to their matrix constituents, coffee and tea are considered as matrices which are difficult to analyze.

Report:	CA 4.2/1 Scherthan D., 2011a Independent laboratory validation of the BASF analytical method 421/0: Method for determination of BAS 500 F and its metabolite BF 500-3 residues in plant matrices using LC/MS/MS 2011/1268146
Guidelines:	EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5), EEC 96/46 (16.07.1996)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

BASF method 421/0

Pyraclostrobin and its metabolite BF 500-3 (500M07) were extracted from plant matrices (green coffee and green tea) using a mixture of methanol/water 70/30. A 0.5% aliquot of the extract was removed and cleaned by C18; Polar Plus micro-column. The final determination of BAS 500 F and its metabolite BF 500-3 was performed by HPLC-MS/MS.

Recovery findings

The recoveries of pyraclostrobin and its metabolite BF 500-3 in fortified green tea and green coffee are summarized in Table 4.2-1 and Table 4.2-2. The recoveries of pyraclostrobin ranged from 79.3 to 86.6% at the transition *m/z* 388 → *m/z* 194 and at the transition *m/z* 388 → m/z 163. The CV ranged from 2.9 to 5.6% and 3.0 to 5.9%, respectively.

Table 4.2-1: Recoveries of pyraclostrobin in plant matrices

Matrix	Fortification level (mg/kg)	Transition *m/z* 388 → *m/z* 194		Transition *m/z* 388 → *m/z* 163	
		Mean recovery (%)	CV (%)	Mean recovery (%)	CV (%)
Green tea	0.02 (n=5)	79.9	8.6	78.9	8.9
	0.2 (n=5)	80.3	2.6	79.6	2.8
	Overall Mean (n=10)	**80.1**	**5.6**	**79.3**	**5.9**
Green coffee	0.02 (n=5)	87.7	4.0	87.0	4.4
	0.2 (n=5)	85.4	1.8	85.2	1.6
	Overall Mean (n=10)	**86.6**	**2.9**	**86.1**	**3.0**

Table 4.2-2: Recoveries of BF 500-3 (500M07) in plant matrices

Matrix	Fortification level (mg/kg)	Mean recovery (%)	CV (%)
Transition *m/z* 358 → *m/z* 164			
Green tea	0.05 (n=5)	72.6	4.5
	0.5 (n=5)	97.0	7.2
	Overall Mean (n=10)	**84.8**	**5.9**
Green coffee	0.05 (n=4)	84.8	5.7
	0.5 (n=5)	84.2	8.6
	Overall Mean (n=9)	**84.5**	**7.2**
Transition *m/z* 358 → *m/z* 132			
Green tea	0.05 (n=5)	87.5	9.6
	0.5 (n=5)	86.8	1.3
	Overall Mean (n=10)	**87.2**	**5.5**
Green coffee	0.05 (n=5)	81.7	10.8
	0.5 (n=5)	89.4	1.4
	Overall Mean (n=10)	**85.6**	**6.1**

Linearity	Good linearity was observed in the range of 0.5 to 5.0 ng/ml pyraclostrobin. Coefficients of determination (R^2) were in the linearity experiments always higher than 0.999.
Specificity	Due to matrix effects, metabolite BF 500-3 was tested by 0.05 mg/kg at transition *m/z* 358 → *m/z* 132.
Limit of quantitation	The limit of quantitation was defined by the lowest fortification level successfully tested which was 0.02 mg/kg except of the metabolite BF 500-3, which was tested by 0.05 mg/kg at transition *m/z* 358 → *m/z* 132 due to matrix effects.
Repeatability	The coefficients of variation with respect to recoveries following fortifications at the limit of quantification were between 2.9 and 6.1%. The values obtained are indicative of the method having satisfactory repeatability.
Reproducibility	In context of this ILV study, the reproducibility of the BASF method 421/0 was tested by a laboratory not involved in method development. As can be seen from the results, a high reproducibility was achieved.
Conclusion	**The results show that BASF method No. 421/0 is suitable to determine pyraclostrobin and its metabolite BF 500-3 (500M07) in the plant matrices green coffee and green tea at a limit of quantitation of 0.02 mg/kg (except for the metabolite BF 500-3, which was tested by 0.05 mg/kg at transition 358 → 132 due to matrix effects).**

Report: CA 4.2/2
Dias C.M. et al., 2013a
Multi-residue method for the analysis of pesticides in arabica coffee using liquid chromatography/tandem mass spectrometry
2014/1145909
Guidelines: none
GLP: no

This entry is taken from public literature and was not included in the study list of the application.

Principle of the method A multi-residue method for the determination of 123 pesticides (including isomers) in green coffee beans was developed.
The method includes a modified acetonitrile-based extraction procedure (modified QuEChERS) and C18 d-SPE clean-up. The final determination was performed by UPLC-MS/MS with a triplequadrupole instrument in the MRM mode. The column used was a Shim-Pack ODS XR-II, the eluent system consisted of 2 mobile phases (A: 10 mmol/L ammonium acetate containing 0.01% formic acid & B: methanol) which were used applying gradient elution. The oven temperature reported was 60°C.

Recovery findings For all analytes, the recoveries were in the range of 70.7 and 108.8% at fortification levels between 0.01 and 0.1 mg/kg. No detailed information was given for pyraclostrobin.

Linearity Good linearity ($R^2 \geq 0.992$) was observed in the range of 0.01 to 0.1 mg/kg for all analytes.

Specificity The method was excellent, allowing unequivocal identification of the target pesticides in green coffee bean extracts. However, despite the clean-up step applied, high matrix effects were still observed, thus requiring matrix-matched standard calibration for reliable quantification in routine analysis.

Limit of Quantification The limits of quantification ranged from 0.11 to 40.12 µg/kg for all analytes. For pyraclostrobin the limit of quantification was at 0.62 µg/kg $[M + H]^+$ and at 1.36 µg/kg $[M + NH_4]^+$.

Repeatability The relative standard deviations (RSD, %) for all analytes analysed and all fortification levels were well below 20%.

Reproducibility The reproducibility of the method was not estimated as identical samples were not evaluated by an independent laboratory.

Conclusion **The method for analysis, representing a modified QuEChERS method, uses LC-MS/MS for final determination, which is a modern and highly specific technique. Considering the good results obtained in this study with regard to all parameters studied, the proposed method could be used for the quantification of multi-residue pesticides including pyraclostrobin in coffee and is promising for use in routine monitoring.**

Food of animal origin

Pyraclostrobin (BAS 500 F)

In order to allow an efficient enforcement of pyraclostrobin parent residues using up-to-date technology (LC-MS/MS) method L0151/01 replaces the previously submitted LC-UV method 439/0. It was also validated in blood. As required in SANCO 825/00 and the relevant OECD guidance document, the validation data include a confirmatory technique (second transition).

Report:	CA 4.2/3 Schacherl A., 2010a Independent laboratory validation (ILV) of an analytical method for the determination of BAS 500 F (Reg.No. 304428) in animal matrices 2010/1123694
Guidelines:	SANCO/825/00 rev. 7 (17 March 2004), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07
GLP:	yes (certified by Umweltministerium Baden-Wuerttemberg, Stuttgart)

Principle of the method

BASF method L0151/01 (ILV):

Pyraclostrobin (BAS 500 F) is extracted with acetonitrile. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. An aliquot of the organic phase is evaporated to dryness and dissolved in a mixture of acetonitrile and water. The final determination of pyraclostrobin is performed by HPLC-MS/MS, monitoring two parent-daughter ion transitions.

The limit of quantitation (LOQ) of the method for cow liver, kidney, fat, muscle, milk, skim milk, cream and hen egg is 0.01 mg/kg. The LOQ for swine blood is 0.01 mg/L.

Recovery findings

The recoveries of pyraclostrobin in fortified bovine muscle, cow fat, kidney, milk, hen egg and swine blood samples are summarised in Table 4.2-3. The recoveries of pyraclostrobin ranged from 72 to 104% at the transition *m/z* 388 → *m/z* 194 and at the transition *m/z* 388 → *m/z* 163. The CV ranged from 3 to 5% and 1 to 5%, respectively.

The results of the matrix effect evaluation are summarized in Table 4.2-4, and indicate that no significant matrix effect was present for meat, fat, kidney, milk and blood. For egg the matrix effect was significant. To compensate ion enhancement or suppression effects for the analysis of egg, bracketed standards prepared in blank matrix were used.

The measured concentration of the stored matrix pyraclostrobin standards was for fat 98% and for egg 102% of the initial amount. Therefore, pyraclostrobin can be regarded as stable in final egg and fat sample extracts for at least 3 days.

Table 4.2-3: Recoveries of pyraclostrobin in animal matrices

Matrix	Fortification level (mg/kg)	n	Transition 388 → 194*		Transition 388 → 163**	
			Mean recovery (%)	CV (%)	Mean recovery (%)	CV (%)
Meat (bovine, muscle)	0.01	5	70	2	76	4
	0.1	5	73	5	72	3
	0.01 + 0.1	**10**	**72**	**4**	**74**	**5**
Cow, fat	0.01	5	71	4	71	4
	0.1	5	73	3	72	3
	0.01 + 0.1	**10**	**72**	**3**	**72**	**3**
Cow, kidney	0.01	5	71	6	73	1
	0.1	5	72	2	73	2
	0.01 + 0.1	**10**	**72**	**4**	**73**	**1**
Cow, milk	0.01	5	77	4	77	3
	0.1	5	82	2	83	3
	0.01 + 0.1	**10**	**80**	**5**	**80**	**5**
Hen, egg	0.01	5	104	3	104	4
	0.1	5	105	3	105	3
	0.01 + 0.1	**10**	**104**	**3**	**104**	**3**
Swine, blood	0.01**	5	71	4	72	2
	0.1**	5	73	4	73	5
	0.01 + 0.1	**10**	**72**	**4**	**73**	**4**

* used for quantification; ** used for confirmation

Table 4.2-4: Evaluation of matrix effects for pyraclostrobin analysis

Matrix	Nominal concentration (ng/mL)	Mean determined concentration (ng/mL)	% of nominal	Matrix effect
Meat	0.5	0.511	102.2	2.2
	5	5.06	101.1	1.1
Fat	0.5	0.522	104.4	4.4
	5	4.94	98.8	-1.2
Kidney	0.5	0.480	96.0	-4.0
	5	5.01	100.1	0.1
Milk	0.5	0.512	102.4	2.4
	5	5.09	101.8	1.8
Egg	0.5	0.362	72.4	-27.6
	5	3.15	63.0	-37.0
Blood	0.5	0.504	100.8	0.8
	5	4.99	99.8	-0.2

Linearity

Good linearity was observed in the range of 0.02 to 100 ng/mL pyraclostrobin. Coefficients of determination (R^2) were in the linearity experiments always higher than 0.999.

Specificity

No significant interferences from the sample matrices were reported.

Limit of quantitation

The limit of quantitation was defined by the lowest fortification level successfully tested which was 0.01 mg/kg for the analyte in meat, fat, kidney, milk and hen egg and 0.01 mg/L for swine blood.

Repeatability

The coefficients of variation with respect to recoveries following fortifications at the limit of quantification were between 1 and 5%. The values obtained are indicative of the method having satisfactory repeatability.

Reproducibility

The reproducibility of the BASF method L0151/01 was estimated in context of this ILV. Due to the fact, that for the fortified matrices recoveries and RSDs provided in context of the original report and the related ILV were always well in the ranges specified in the current guidelines (e.g. SANCO/825/00 rev. 8.1), it can be stated, that BASF method L0151/01 shows a good reproducibility.

Conclusion

The results show that BASF method No. L0151/01 is suitable to determine residues of pyraclostrobin in animal matrices such as muscle, fat, kidney, milk, hen egg and swine blood at a limit of quantitation of 0.01 mg/kg (for swine blood 0.01 mg/L).
No significant matrix effect was present for meat, fat, kidney, milk and blood. For egg the matrix effect was significant. It was compensated using bracketed standards.

Comparison of extractability using different extraction methods

Report: CA 4.2/4
Lagunas-Allue L. et al., 2012a
Comparison of four extraction methods for the determination of fungicide residues in grapes through gas chromatography-mass spectrometry
2012/1366723
Guidelines: none
GLP: no

This entry is taken from public literature and was not included in the study list of the application.

Four extraction methods were assessed and compared for the analysis of pyraclostrobin in grape samples. Red grapes (variety: Tempranillo) were chopped into small pieces, homogenized using a crusher and spiked with pyraclostrobin prior to extraction.

Principle of the methods

Matrix solid phase dispersion (MSPD):
The sample was mixed with C_{18} and transferred to a column filled with adsorbent (silica). Dichloromethane/ethyl acetate (1:1, v:v) was added for elution. The eluent was concentrated and subjected to GC–MS, SIM analysis where Tetradifon (used as internal standard) was added.

Microwave assisted extraction (MAE):
MAE experiments were performed using an MARS-microwave sample preparation unit equipped with a solvent detector. Samples were extracted with a hexane/acetone (1:1, v:v) mixture in a Teflon-lined extraction vessel under high temperature. Tetradifon was added after extraction and samples were analyzed by GC–MS, SIM.

Soli-liquid extraction (SLE) using ethyl acetate:
The samples were extracted with ethyl acetate and then centrifuged. The organic phase was dried and the extract was concentrated in the TurboVap®. Tetradifon was added and the sample was analyzed by GC–MS, SIM.

	QuECheRS method: Homogenized samples were extracted with acetonitrile (including 1.0% HAc, v:v). An aliquot of the extract was centrifuged and clean-up was achieved with dispersive solid-phase extraction (d-SPE) method. Tetradifon was added and the sample was analyzed by GC–MS, SIM. All samples obtained after applying the different extraction procedures were analyzed by GC-MS, working in the selected ion monitoring mode (SIM). The GC column used was a HP-5MS with a length of 30 m, an inner diameter of 0.25 mm and a film thickness of 0.25 µm. Injection temperature was at 250°C, Helium was used as carrier gas at constant flow rate of 1.5 mL/min. For pyraclostrobin, the target ion was *m/z* 132 and qualifier ions were *m/z* 164 and *m/z* 325.
Recovery findings	For pyraclostrobin, average recoveries were > 80% at fortification levels of 0.010 mg/kg and 0.05 mg/kg for all methods tested.
Linearity	Good linearity (≥0.994) was observed in the range of the calculated LOQ to 0.1 mg/kg for all analytes and all methods tested.
Specificity	There were significant differences between the slopes of the regression lines obtained in solvent and grape extracts for pyraclostrobin except for QuEChERS and SLE method. Matrix effects were not reported.
Limit of Quantification	The limits of quantification for pyraclostrobin were in the same dimension for all methods tested (MAE: 0.0058 mg/kg, MSPD: 0.0087 mg/kg, QuEChERS: 0.0143 mg/kg and SLE: 0.0136 mg/kg).
Repeatability	The relative standard deviations (RSD, %) for pyraclostrobin were similar and well below 20% for all the methods tested (MAE: 6.6%, MSPD: 6.7%, QuEChERS: 10.1%, Ethyl acetate: 8.3% at a fortification level of 0.05 mg/kg).

Comparison of the four methods

Regression lines

There were no significant differences between the extraction methods for pyraclostrobin confirmed by the slopes and intercepts of the regression line (each point on this graph represented a single sample analyzed by two separate methods) close to 0 and 1 at 10 different fortification levels. Pyraclostrobin showed a good agreement between all the methods. The coefficients of determination (R^2) for pyraclostrobin ranged from 0.97 to 0.99 mg/kg, indicating that after applying one of the four extraction methods only slightly different residues will be measured.

Analysis of real samples

Statistical tests showed that there were no significant differences between the methods for pyraclostrobin, when grape samples collected from the local markets were analyzed.

Conclusion

For pyraclostrobin, the methods showed good results with regard to the parameters studied. No significant differences among the four methods for the extraction of pyraclostrobin were found.
It can be stated, that QuEChERS method is a suitable method to determine pyraclostrobin in plant matrices.

(b) Methods for the determination of all components included for monitoring purposes in the residue definitions for soil and water as submitted in accordance with the provisions of point 7.4.2

According to M-CA 7.4.2 the proposal for the definition of residues for environmental matrices for pyraclostrobin is parent-only. No special analytical methods for monitoring purposes were developed. The methods described under M-CA 4.1.2 for soil and water for risk assessment can be used also for monitoring purposes.

According to SANCO/825/00 rev. 8.1, an independent laboratory validation was performed for pyraclostrobin and its metabolites in drinking- and surface water.

Water

Pyraclostrobin (BAS 500 F)

The purpose of the study was to demonstrate that BASF Analytical Method L0182/02, "Determination of BAS 500 F (Pyraclostrobin) and its metabolites BF 500-5 (Reg. No. 298327), BF 500-12 (Reg. No. 412053), BF 500-11 (Reg. No. 411847), BF 500-13 (Reg. No. 412785), BF 500-14 (Reg. No. 413038) and BF 500-15 (Reg. No. 377613) in drinking- and surface- water by LC/MS/MS", could be performed successfully at an outside facility with no prior experience using the method.

~~**Report:**~~	~~CA 4.2/5~~ ~~Rutt D.,Jones G., 2014b~~ ~~Independent Laboratory Validation of BASF Method Number L0182/02: Determination of BAS 500 F (Pyraclostrobin) and its metabolites BF 500-5 (Reg. No. 298327), BF 500-12 (Reg. No. 412053), BF 500-11 (Reg. No. 411847), BF 500-13 (Reg. No. 412785), BF 500-14 (Reg. No. 413038) and BF 500-15 (Reg. No. 377613 in ground- and surface- water by LC/MS/MS~~ ~~2014/7000022~~
~~**Guidelines:**~~	~~EPA 850.6100, SANCO/825/00 rev. 8.1 (16 November 2010)~~
~~**GLP:**~~	~~yes~~ ~~(certified by United States Environmental Protection Agency)~~

Note: This report is already covering metabolite BF 500-5 (500M04). A separate report as included in the study list of the application is consequently not submitted.

Report:	CA 4.2/5 Bianca C.M., 2015 a Independent laboratory validation of BASF method L0182/02: BAS 500 F (Pyraclostrobin) and its metabolites BF 500-5 (Reg.No. 298327), BF 500-12, BF 500-11, BF 500-13, BF 500-14, BF 500-15 in ground- and surface-water by LC/MS/MS 2015/7001873
Guidelines:	EPA 850.6100, SANCO/825/00 rev. 8.1 (16 November 2010)
GLP:	yes (certified by United States Environmental Protection Agency)

Note: After dossier submission in July 2014 a new amended report became available, which is replacing BASF DocID 2014/7000022.

Principle of the method

A 50 mL water sample aliquot is extracted by acidification with formic acid followed by solid phase extraction on a C18 SPE column. Residues are then eluted twice with ethyl acetate. The extracts are concentrated to dryness and reconstituted in acetonitrile/water (20/80, v/v).
Final determination is performed by LC-MS/MS using an Atlantis T3 analytical column and a gradient mixture of water – acetonitrile with formic acid as modifier at a flow rate of 500 µL/min. Detection is accomplished using positive ion electrospray ionization tandem mass spectrometry (MS/MS-ESI+) monitoring two mass transitions for quantification and confirmation: 389 → 195 and 389 → 164 for pyraclostrobin, 196 → 154 and 196 → 118 for 500M04, 370 → 194 and 370 → 278 for 500M59, 278 → 194 and 278 → 149 for 500M60, 248 → 132 and 248 → 164 for 500M62, 389 → 242 and 389 → 301 for 500M76 and 177 → 135 and 177 → 132 for 500M78.

Recovery findings

Method L0182/02 was proven to be suitable to determine residues of pyraclostrobin and its metabolites 500M04, 500M59, 500M60, 500M62, 500M76 and 500M78 to the respective limit of quantification in surface water. For drinking water, the method was validated as well for all analytes, with the exception of analyte 500M78. However, as only the parent molecule is relevant for the residue definition for enforcement in drinking water for Europe, 500M78 could be eliminated in the ILV study in the drinking water. The mean recovery values of the validation experiments were between 70 and 120%, which fulfills the legal requirements for mean recovery values. The detailed results are given in Table 4.2-5.

Table 4.2-5: Results of the method validation for the determination of pyraclostrobin and its metabolites in drinking water

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water	pyraclostrobin	0.003 (n = 5)	88.9	10.8	389 → 195
		0.03 (n = 5)	102	5.4	Quantifier
		0.003 (n = 5)	89.8	10.5	389 → 164
		0.03 (n = 5)	105	7.8	Qualifier
	500M04	0.03 (n = 5)	100	9.3	196 → 154
		0.3 (n = 5)	101	8.8	Quantifier
		0.03 (n = 5)	87.0	19.8	196 → 118
		0.3 (n = 5)	98.2	5.4	Qualifier
	500M59	0.03 (n = 5)	86.6	9.2	370 → 194
		0.3 (n = 5)	98.3	2.9	Quantifier
		0.03 (n = 5)	83.8	10.6	370 → 278
		0.3 (n = 5)	94.6	8.9	Qualifier
	500M60	0.03 (n = 5)	94.7	3.0	278 → 194
		0.3 (n = 5)	101	4.2	Quantifier
		0.03 (n = 5)	90.5	5.0	278 → 149
		0.3 (n = 5)	96.5	2.7	Qualifier
	500M62	0.03 (n = 5)	95.4	3.8	248 → 132
		0.3 (n = 5)	97.4	5.3	Quantifier
		0.03 (n = 5)	95.9	3.0	248 → 164
		0.3 (n = 5)	98.9	2.1	Qualifier
	500M76	0.03 (n = 5)	86.3	19.4	389 → 242
		0.3 (n = 5)	91.0	7.9	Quantifier
		0.03 (n = 5)	83.6	8.8	389 → 301
		0.3 (n = 5)	103	8.9	Qualifier

Table 4.2-6: Results of the method validation for the determination of pyraclostrobin and its metabolites in surface water

Matrix	Analyte	Fortification level (µg/L) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Surface water	pyraclostrobin	0.003 (n = 5)	103	6.6	389 → 195
		0.03 (n = 5)	104	3.3	Quantifier
		0.003 (n = 5)	107	5.2	389 → 164
		0.03 (n = 5)	106	3.3	Qualifier
	500M04	0.03 (n = 5)	78.2	13.6	196 → 154
		0.3 (n = 5)	90.8	12.1	Quantifier
		0.03 (n = 5)	95.2	16.6	196 → 118
		0.3 (n = 5)	95.0	11.0	Qualifier
	500M59	0.03 (n = 5)	86.7	3.9	370 → 194
		0.3 (n = 5)	93.1	3.4	Quantifier
		0.03 (n = 5)	85.4	3.1	370 → 278
		0.3 (n = 5)	88.1	1.0	Qualifier
	500M60	0.03 (n = 5)	101	2.9	278 → 194
		0.3 (n = 5)	103	3.0	Quantifier
		0.03 (n = 5)	101	4.5	278 → 149
		0.3 (n = 5)	101	2.2	Qualifier
	500M62	0.03 (n = 5)	95.6	3.1	248 → 132
		0.3 (n = 5)	99.8	3.2	Quantifier
		0.03 (n = 5)	97.0	3.1	248 → 164
		0.3 (n = 5)	99.9	2.7	Qualifier
	500M76	0.03 (n = 5)	99.5	7.7	389 → 242
		0.3 (n = 5)	104	11.9	Quantifier
		0.03 (n = 5)	108	7.1	389 → 301
		0.3 (n = 5)	104	6.2	Qualifier
	500M78	0.03 (n = 5)	91.2	8.4	177 → 135
		0.3 (n = 5)	116	2.9	Quantifier
		0.03 (n = 5)	94.2	7.5	177 → 132
		0.3 (n = 5)	114	5.7	Qualifier

Linearity	Linear regression was used for calibration using six calibration levels. Matrix-matched standards were used for calibration. For pyraclostrobin, good linearity ($r^2 \geq 0.99$) was observed in matrix-matched standards in the range of 0.005 ng/mL to 0.1 ng/mL in drinking and surface water. For metabolites 500M04, 500M59, 500M60, 500M62 and 500M76, good linearity ($r^2 \geq 0.99$) was observed in the range of 0.05 ng/mL to 1.0 ng/mL in drinking and surface water. For metabolite 500M78, good linearity ($r^2 \geq 0.98$) was observed in matrix-matched standards in the range of 0.05 ng/mL to 1.0 ng/mL in surface water.
Specificity	The method L0182/02 allows the specific determination of pyraclostrobin and its metabolites 500M04, 500M59, 500M60, 500M62, 500M76 and 500M78 in water. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. The tested untreated water samples showed no significant interferences (< 30%) at the retention time of the analytes.
Matrix Effects	Matrix-matched standards were used for the calibration of validation experiments.
Limit of Quantification	The method has a limit of quantification (LOQ) of 0.003 µg L^{-1} for pyraclostrobin and 0.03 µg L^{-1} for each metabolite.
Limit of Detection	The limit of detection (LOD) is estimated as 20% of the LOQ with 0.0006 µg/L for pyraclostrobin and 0.006 µg/L for each metabolite.
Standard Stability	The stability has been investigated within the original validation study and has not been considered within the ILV.
Stability of Working Solutions	The stability has been investigated within the original validation study and has not been considered within the ILV.
Repeatability	The relative standard deviations (RSD, %) for all fortification levels were below 20%.
Reproducibility	In context of this ILV study, the reproducibility of this analytical method was estimated. As can be seen from the results, a high reproducibility was determined.

Conclusion

The method L0182/02 for the analysis of pyraclostrobin (BAS 500 F) and its metabolites 500M04, 500M59, 500M60, 500M62, 500M76 and 500M78 in surface water and drinking water used HPLC-MS/MS for final determination, with a limit of quantification of 0.003 µg L^{-1} for pyraclostrobin and 0.03 µg L^{-1} for each metabolite.
It was demonstrated that the method L0182/02 fulfills the requirements with regards to specificity, repeatability, limit of quantification, and recoveries and is therefore applicable to correctly determine pyraclostrobin and metabolites 500M04, 500M59, 500M60, 500M62 and 500M76 in drinking and surface water. Additionally, method L0182/02 was proven to be suitable to determine residues of metabolite 500M78 in surface water.

(c) Methods for the analysis in air of the active substance and relevant breakdown products formed during or after application, unless the applicant shows that exposure of operators, workers, residents or bystanders is negligible

A validated analytical method for air is described in CA 4.1.2/6.

However, as pyraclostrobin has a very low vapour pressure of 2.6 x 10^{-8} Pa (for details see M-CA 2.2), it is considered to be non-volatile. Consequently, inhalation exposure of operators, workers, residents or bystanders to vapours of pyraclostrobin is negligible. Further information can be found in M-CP 7.2.

(d) Methods for the analysis in body fluids and tissues for active substances and relevant metabolites

Independent laboratory validation data for the determination of pyraclostrobin in blood are provided above (see CA 4.2/3, BASF Doc ID 2010/1123694).

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT M-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162246 (version 1)
27-Feb-2017	Chapter 5.1: addition of new metabolites in table 5.1-1; inclusion of additional studies in 5.1.1 and 5.1.2 (including a revised overall conclusion); Chapter 5.2: revision of 5.2.1 to reflect an additional study provided in 5.4.2 addition of report amendments in 5.2.4 and 5.2.5 Chapter 5.4: addition of two studies in 5.4.2 addition of three studies in 5.4.3 and accordingly update of the text Chapter 5.6: addition of a study in 5.6.1 Chapter 5.8: update of the listed endpoints (pages 3-5); addition of new studies: KCA 5.8.1/27 and KCA 5.8.1/39-49 re-numbering of already contained studies and tables to the extent necessary; update of the genotox evaluation of metabolite 500M62 at the end of 5.8.1. New or changed text is marked in yellow.	BASF DocID 2017/1032922 (version 2)
30-May-2017	Chapter 5.8 was amended by KCA 5.8.1/53 and the text was updated where necessary (new or changed text is marked in blue).	BASF DocID 2017/1146981 (version 3)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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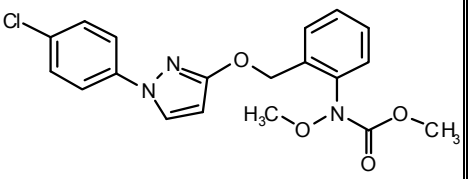
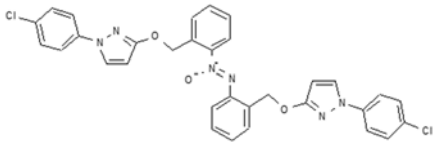
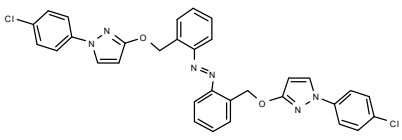
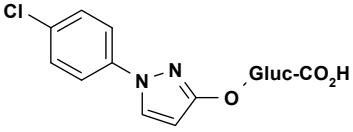
CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

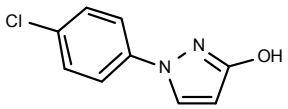
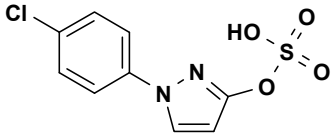
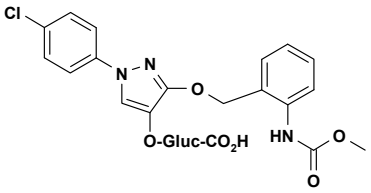
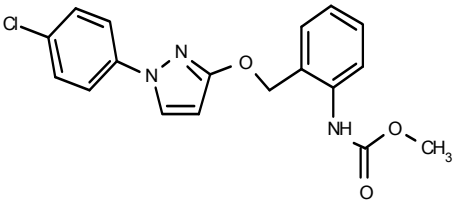
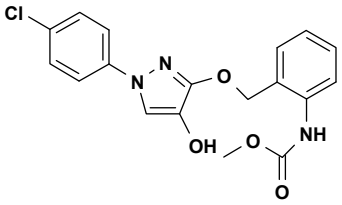
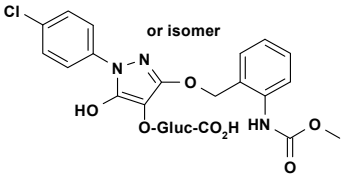
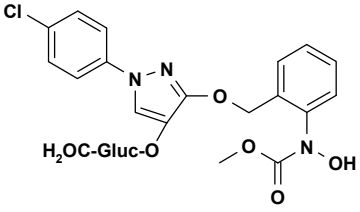
CA 5.1 Studies on Absorption, Distribution, Metabolism and Excretion in Mammals

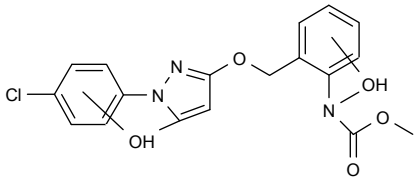
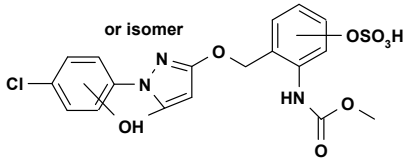
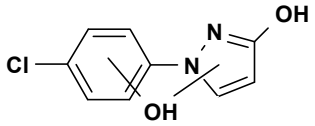
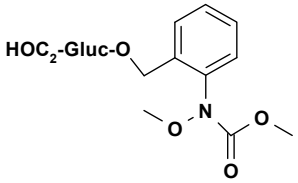
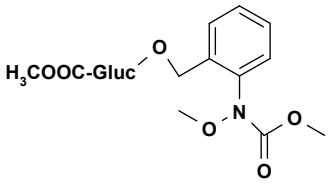
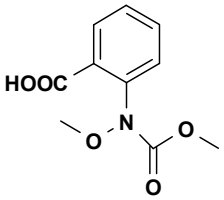
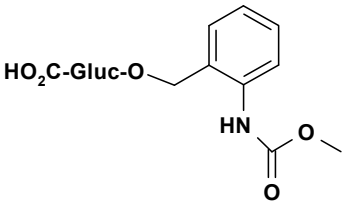
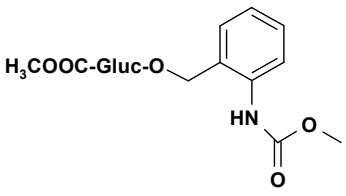
An overview on the metabolites being identified in the rat metabolism studies from 1999 and 2014 is provided in the table below. Additionally, the table includes also those metabolites which have been detected in the *in-vitro* comparison study (BASF DocID 2014/1001562) and the rat metabolism study where metabolite 500M106 was administered to rats (BASF DocID 2015/1198492).

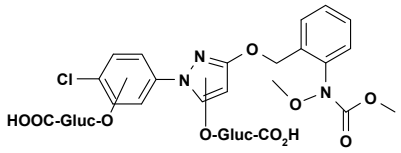
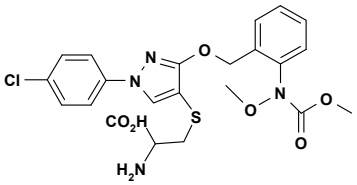
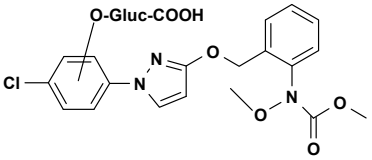
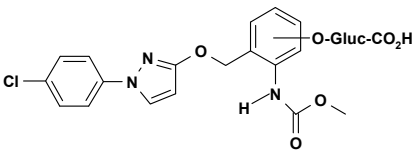
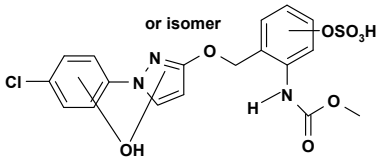
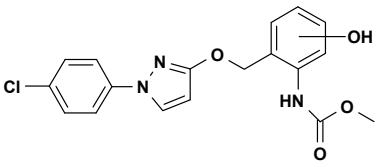
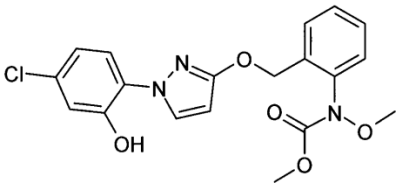
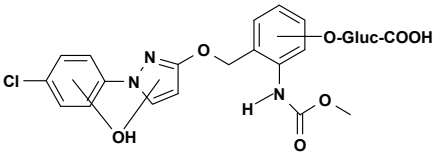
In order to increase the readability of the table, information from other test systems have been deleted. If not stated otherwise in the occurrence column, the metabolites indicated for rats have been found in the corresponding *in-vivo* study (BASF DocID 1999/11781). Metabolites which were (re)confirmed in the new *in-vivo*-plasma study (BASF DocID 2014/1136557) are specifically indicated. If not indicated otherwise, pyraclostrobin was used as test item.

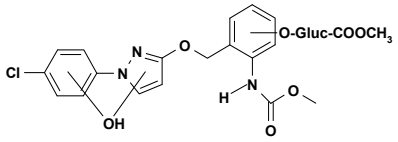
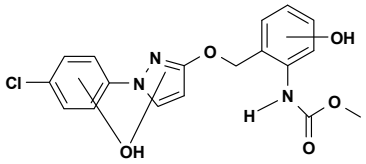
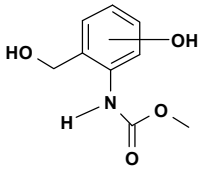
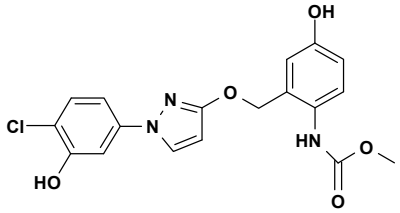
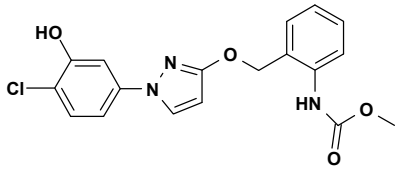
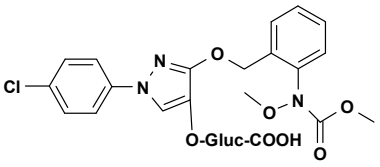
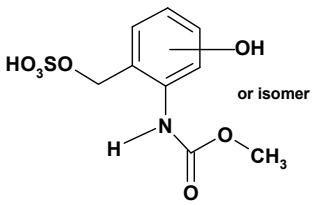
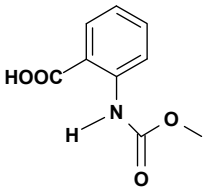
Table 5.1-1: Notations of parent and metabolites of pyraclostrobin

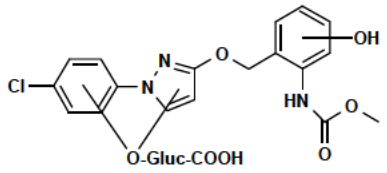
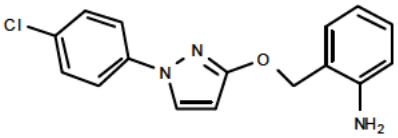
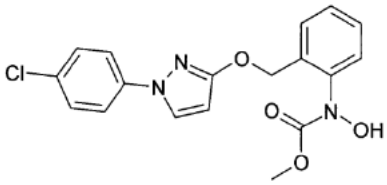
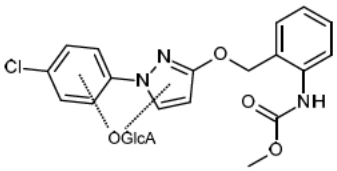
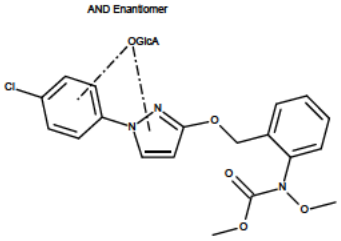
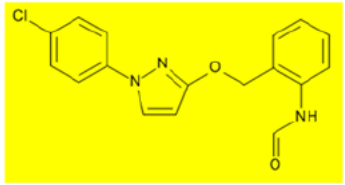
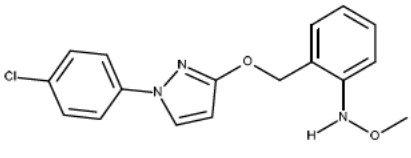
Substance/ Metabolite Code	Reg No.	Synonyms	CAS-No.	Compound found in	Structure
BAS 500 F	304428	500M00	175013-18-0		
500M01	364380	BF 500-6	not assigned	rat after dosing with metabolite 500M106	
500M02	369315	BF 500-7	not assigned	human, dog, rabbit (*in-vitro*) rat after dosing with metabolite 500M106	
500M03	not assigned		not assigned	rat human, rabbit (*in-vitro*) rat after dosing with metabolite 500M106	

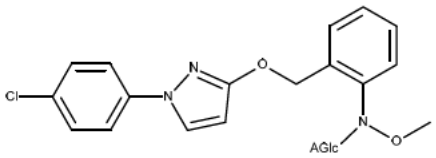
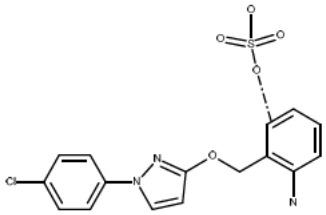
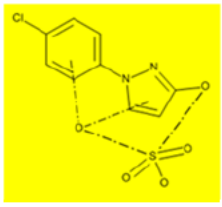
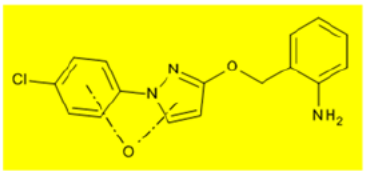
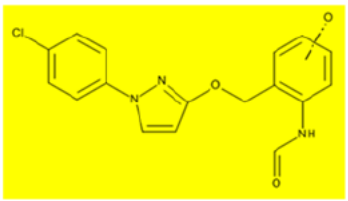
Substance/ Metabolite Code	Reg No.	Synonyms	CAS-No.	Compound found in	Structure
500M04	298327	BF 500-5	76205-19-1	rat human, rat, rabbit, dog (*in-vitro*) rat after dosing with metabolite 500M106	
500M05	not assigned		not assigned	rat rat after dosing with metabolite 500M106	
500M06	not assigned		not assigned	rat (*in-vivo*, plasma) human, rabbit, rat (*in-vitro*)	
500M07	340266	BF 500-3	512165-96-7	rat	
500M08	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M13	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M15	not assigned		not assigned	rat (*in-vivo*, plasma)	

Substance/ Metabolite Code	Reg No.	Synonyms	CAS-No.	Compound found in	Structure
500M18	not assigned		not assigned	rat	
500M19	not assigned		not assigned	rat	
500M21	not assigned		not assigned	rat rat after dosing with metabolite 500M106	
500M22	not assigned		not assigned	rat	
500M23	not assigned		not assigned	rat	
500M24	5916421		not assigned	rat	
500M25	not assigned		not assigned	rat	
500M26	not assigned		not assigned	rat	

Substance/ Metabolite Code	Reg No.	Synonyms	CAS-No.	Compound found in	Structure
500M29	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M30	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M31	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M32	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M33	not assigned		not assigned	rat	
500M34	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M35	412040		not assigned	rat (*in-vivo*, plasma)	
500M37	not assigned		not assigned	rat (*in-vivo*, plasma)	

Substance/ Metabolite Code	Reg No.	Synonyms	CAS-No.	Compound found in	Structure
500M38	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M39	not assigned		not assigned	rat	
500M40	not assigned		not assigned	rat	
500M44	not assigned		not assigned	rat	
500M45	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M46	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M48	not assigned		not assigned	rat	
500M51	78810		6268-38-8	rat	

Substance/ Metabolite Code	Reg No.	Synonyms	CAS-No.	Compound found in	Structure
500M52	not assigned		not assigned	rat	
500M73	358672	BF 500-4	not assigned	human, rat, rabbit, dog (*in-vitro*) rat after dosing with metabolite 500M106	
500M88	322410	BF 500-1	220897-76-7	human, rat, rabbit, dog (*in-vitro*)	
500M103	not assigned		not assigned	human, rat, rabbit (*in-vitro*)	
500M104	not assigned		not assigned	rat (*in-vivo*, plasma) human, rat, rabbit (*in-vitro*)	
500M105	not assigned		not assigned	rat after dosing with metabolite 500M106	
500M106	399379		not assigned	rat (*in-vivo*, plasma) human, rabbit (*in-vitro*)	

Substance/ Metabolite Code	Reg No.	Synonyms	CAS-No.	Compound found in	Structure
500M107	not assigned		not assigned	rat (*in-vivo*, plasma) human, rabbit (*in-vitro*)	AND Enantiomer 
500M108	not assigned		not assigned	rat (*in-vivo*, plasma) human, rat, rabbit (*in-vitro*)	
500M109	not assigned		not assigned	rat after dosing with metabolite 500M106	
500M112	not assigned		not assigned	rat after dosing with metabolite 500M106	
500M117	not assigned		not assigned	rat after dosing with metabolite 500M106	

CA 5.1.1 Absorption, distribution, metabolism and excretion by oral exposure

Studies submitted in context of the Annex I inclusion according to Directive 91/414

In order to provide information on the adsorption, distribution, metabolism and excretion in rats, two studies were provided for the previous Annex I listing process and are considered as peer reviewed and still valid. Due to the importance of the studies for dietary exposure assessment, but also for allowing a direct comparison between these "old" and newly performed studies, relevant information of these studies is summarized below based on the previous Annex II dossier.

- In study BASF DocID 1998/10997, the absorption, distribution, elimination and biokinetics of ^{14}C-BAS 500 F in male and female Wistar rats were investigated at dose levels of 5 and 50 mg/kg bw. The experiments were mainly performed with ^{14}C tolyl-labelled BAS 500 F. An additional experiment investigating the balance and excretion pattern was performed with ^{14}C-BAS 500 F labelled in the chlorophenyl-ring. Samples from this study were transferred to the metabolism laboratory for pathway elucidation.
- The purpose of the second study (BASF DocID 1999/11781) was the identification and characterization of metabolites in rats. In context of this study, further dosing experiments were performed.

Dosing and dose groups

In Table 5.1.1-1, details on dosing are provided for those experiments, which were finally used for metabolism investigations. Besides these experiments, animals of both sexes were dosed at 5 and 50 mg/kg bw for the determination of pharmacokinetic parameters in blood and plasma (10 time points after dosing: 0.5 – 120 hours) and for tissue distribution (four time points).
Additional samples were also collected from animals of dose groups B, C and D for deriving information on the potential presence of volatile metabolites (only D), but also radioactivity levels in further organs (heart, liver, spleen, bone, skin, lung, ovaries, bone marrow, carcass, muscle, kidney, testes, brain, pancreas, uterus, adipose tissue, stomach, thyroid glands, adrenal glands, blood, GIT).

Table 5.1.1-1: Details on dosing in studies BASF DocID 1998/10997 and 1999/11781

Dose group (Designation)	Nominal dose [mg/kg bw]	Label	Number of doses	Number of animals [per label] *	Samples investigated in the study
B	5	tolyl	1	4 + 4	urine, feces
C	5	tolyl	14 +1	4 + 4	urine, feces
D	50	tolyl, chlorophenyl	1	4 + 4	urine, feces
DX	50	tolyl, chlorophenyl	1	10 + 10	urine, feces
R	5	tolyl	1	7 + 5	bile
S	50	tolyl	1	4 + 8	bile
V	5	tolyl, chlorophenyl	1	4 + 4	plasma, liver, kidney (8 hours after dosing)
W	50	tolyl, chlorophenyl	1	4 + 4	plasma, liver, kidney (8 hours)

* male + female

Excretion balance

During the first 48 hours after single oral dosing with 5 or 50 mg/kg bw (tolyl-label, dose groups B and D), 10 - 13% of the administered radioactivity was excreted in urine and 74 - 91% in feces. After 120 hours, the total amount of radioactivity excreted in urine was in the range of 11 - 15% and in feces in the range of 81 - 92% of the administered dose. Radioactivity remaining in tissues and organs 120 hours post dosing was less than 1 µg eq/g at a dose level of 50 mg/kg bw and less than 0.1 µg eq/g at a dose level of 5 mg/kg bw.

An excretion pattern as described above was also obtained after repeated oral administration (14 x unlabelled at 50 mg/kg bw, 1 x labelled at 5 mg/kg bw, dose group C) and after single oral administration of 50 mg/kg bw of the chlorophenyl-labelled test substance (dose group D). After single oral administration of 50 mg/kg bw, no radioactivity was detectable in the exhaled air with either label used. The overall recovery of radioactivity was in the range of 91.4 - 105.0% in all experiments (see Table 5.1.1-2 and Table 5.1.1-3).

Within 48 hours after administration of 5 or 50 mg/kg bw of ^{14}C-BAS 500 F (dose groups R and S), approximately 35 - 38% of the administered radioactivity was excreted via bile. As compared to the radioactivity excreted in the feces 48 hours after single oral administration (about 74 - 91%), the 0 - 48 hours biliary excretion (about 35 - 38%) is considerably lower. From this comparison, it may be concluded that not all of the administered test substance becomes systemically available, i.e. absorption is incomplete. If it is assumed that the amount of radioactivity excreted via bile and urine represents the bioavailable amount of ^{14}C-BAS 500 F, the **bioavailability is approximately 50%**.

Table 5.1.1-2: Excretion balance at 48 h post dosing, including biliary excretion (in % of dose)

Dose [mg/kg bw] Label Application site Application mode	**50** tolyl oral single	**5** tolyl oral single	**5** tolyl oral repeated	**50** chlorophenyl oral single
Males				
Urine 0-48 *	13.23	12.29	13.38	14.87
Feces 0-48 *	73.72	91.20	76.34	67.67
Subtotal	86.95	103.49	89.72	82.54
Bile 0-48 **	36.81	37.72	---	---
Females				
Urine 0-48 *	10.01	10.93	11.86	10.74
Feces 0-48 *	82.36	82.79	77.05	85.07
Subtotal	92.37	93.72	88.91	95.81
Bile 0-48 **	34.51	35.82	---	---

* dose groups B, C and D
** dose groups R and S

Table 5.1.1-3: Excretion balance at 120 h post dosing (in % of dose)

Dose [mg/kg bw] **Dose group** Label Application site Application mode	**50** **D** tolyl oral single	**5** **B** tolyl oral single	**5** **C** tolyl oral repeated	**50** D chlorophenyl oral single
Males				
Urine 0-120	14.52	12.61	13.83	16.01
Cage wash	0.67	0.13	1.30	0.63
Feces 0-120	81.27	92.04	79.04	74.32
Carcass + organs	0.23	0.19	0.17	0.43
Total	96.68	104.96	94.35	91.38
Females				
Urine 0-120	10.78	11.32	12.29	11.54
Cage wash	0.71	0.60	0.48	1.99
Feces 0-120	89.92	83.71	81.40	88.95
Carcass + organs	0.22	0.28	0.18	0.46
Total	101.60	95.91	94.33	102.92

Pharmacokinetics

In rats exposed to a single oral dose of 50 mg/kg bw of ^{14}C-BAS 500 F, the plasma concentration/time curve showed 2 peaks. The first plasma peak was reached 0.5 hour post dosing with peak levels of 1.96 µg eq/g in males and 2.62 µg eq/g in females. At the second plasma peak occurring after 8 hours in males and after 24 hours in females, plasma levels were 2.04 and 1.77 µg eq/g in males and females, respectively. After the second peak, plasma concentrations declined to levels of 0.08 µg eq/g in males and 0.05 µg eq/g in females at 120 hours post dosing. Plasma levels declined monophasically with half-lives of 20.7 and 19.7 hours in males and females, respectively.

In rats exposed to a single oral dose of 5 mg/kg bw of ^{14}C-BAS 500 F, the plasma concentration/time curve also showed 2 peaks. The first plasma peak was reached after 1.0 hour in males and 0.5 hour in females with peak levels of 0.432 µg eq/g and 0.537 µg eq/g, respectively. At the second plasma peak occurring after 8 hours, plasma levels were 0.458 and 0.353 µg eq/g in males and females, respectively. After the second peak, plasma concentrations declined biphasically to levels of 0.006 µg eq/g in males and 0.005 µg eq/g in females at 120 hours post dosing. The initial half-life was found to be 9.0 hours in males and 10.5 hours in females. Terminal half lives in male and female rats were 37.4 and 31.6 hours, respectively.

Increasing the dose level by a factor of about 10 resulted in an increase of the AUC-values by a factor of 9.9 in males and 7.6 in females. At both dose levels, a similar course of the radioactivity with time is found for blood and for plasma. During the first 24 hours post dosing, lower concentrations of radioactivity were found in blood indicating that major parts of the radioactivity are in plasma and not bound to cellular blood constituents.

Table 5.1.1-4: Pharmacokinetic parameter

	5 mg/kg bw		50 mg/kg bw	
	males	**females**	**males**	**females**
1st Cmax (µg eq/g)	0.432	0.537	1.96	2.62
1st Tmax (h)	1.0	0.5	0.5	0.5
2nd Cmax (µg eq/g)	0.458	0.353	2.04	1.77
2nd Tmax (h)	8	8	8	24
Initial T1/2 (h)	9.0	10.5	-	-
Terminal T1/2 (h)	37.4	31.6	20.7	19.7
AUC (µg eq x h / g)	9.46	8.74	93.97	66.41
Total clearance (g/min)	8.81	9.54	8.87	12.4

Tissue distribution
Following one single oral dose of ^{14}C-BAS 500 F at a dose level of 50 mg/kg bw, tissue radioactivity concentration was measured 0.5, 24, 36 and 72 hours after dosing. At the low dose level of 5 mg/kg bw, the corresponding radioactivity measurements were done 0.5, 8, 20 and 42 hours after application. In general, tissue radioactivity levels in both sexes were in the same range at the respective time points and dose levels. The pattern of distribution and elimination in various organs and tissues was also similar. Throughout the time course of the experiments, highest radioactivity concentrations were found in the GI tract. Liver (factor: 4 – 5) and to a lesser extent kidneys (factor: 2) had higher values than the plasma. Most other organs and tissues had values similar to the ones of the plasma. Radioactivity concentrations were lowest in bone and brain. All other tissues had values comparable to or less than the ones for the plasma. There is no evidence of a cumulative potential of BAS 500 F.

Proposed metabolic pathway in rats
After oral application, BAS 500 F is rapidly and almost quantitatively metabolized. Only minor amounts of parent were detected in plasma (below 0.01% of dose) and liver (below 0.4% of dose) whereas in kidney BAS 500 F formed the major part of radioactivity identified. BAS 500 F was metabolised by N-desmethoxlyation, various hydroxylation reactions, cleavage of the ether bond and further oxidation of the two resulting molecule parts. Combinations of these reactions and the conjugation of the resulting OH-groups with glucuronic acid or sulphate led to a large number of observed metabolites. The metabolic pathway is presented below (Figure 5.1.1-1).

Grouping of metabolites for supporting the dietary risk asessment
Pyraclostrobin is intensively metabolized in rats, but also in plant and livestock matrices. In order to perform an indicative dietary exposure assessment, the metabolites occurring in plants, succeeding crops and animal tissues (plus milk and eggs) were grouped in total into 6 groups (see M-CA 6.7 and 6.9). The groups are based on substructural elements.

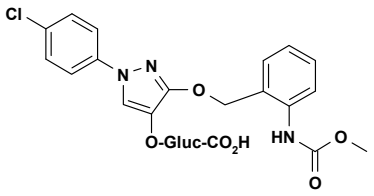
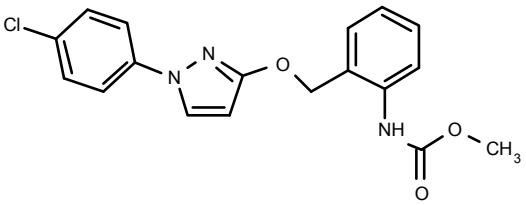
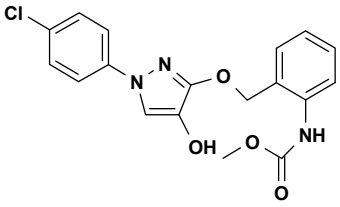
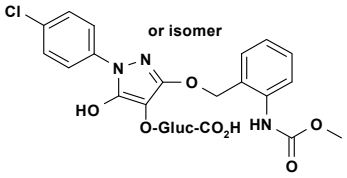
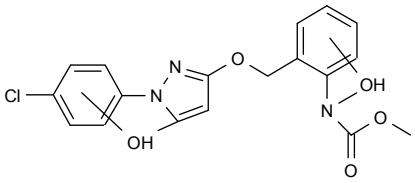
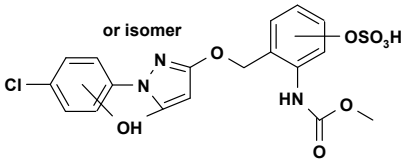
- Group 1: Desmethoxy metabolite 500M07 (synonym: BF 500-3)
- Group 2: Chlorphenyl pyrazole derivatives
- Group 3: Anthranilic acid derivatives
- Group 4: Hydroxylated metabolites (chlorphenyl pyrazole moiety)
- Group 5: Hydroxylated metabolites (tolyl moiety)
- Group 6: Photo metabolite 500M76

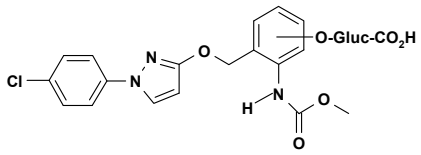
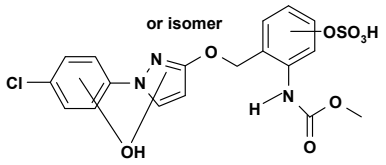
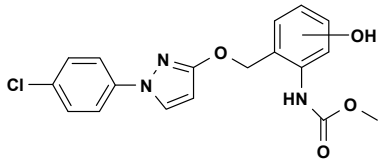
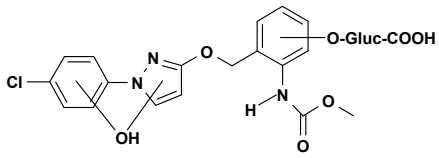
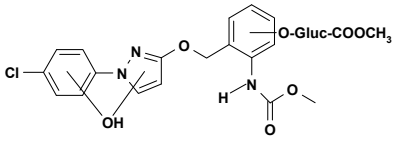
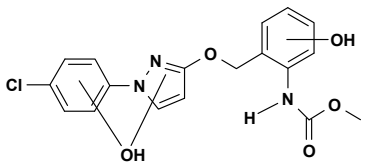
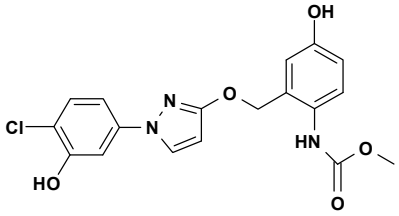
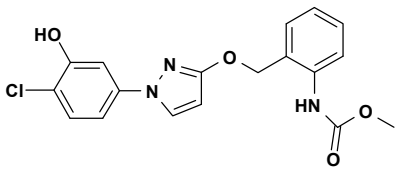
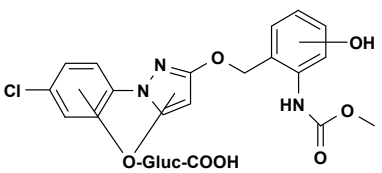
In order to decide for which group the toxicological endpoints (ADI, ARfD) of the parent molecule are applying, the metabolites being identified in the *in-vivo* rat metabolism study were grouped following the same principles. In cases where metabolites show more than one substructural element, assignment occurred to all relevant groups. In a next step, the average amounts of the metabolites across all dose groups were roughly estimated; overlapping peaks are indicated. Depending on the estimated quantities (expressed in % of dose), either the weight of evidence approach was selected or additional toxicological investigations were initiated (see section M-CA 5.8). As supplemental information, the evaluation on toxification / detoxification reactions which was done by Austrian AGES was considered. (T. Coja, presentation at the Fresenius Conference Mainz, March 2012).

Group1: Desmethoxy metabolite 500M07

The desmethoxylation reaction is an important metabolic degradation step in rats. The metabolite 500M07 is metabolic precursor of multiple metabolites identified in rats. In total, 50 - 60 % of dose can be assigned to the metabolite 500M07 and its subsequent degradation products. Due to these findings, the endpoints of pyraclostrobin are also applying to the desmethoxy metabolite 500M07. This conclusion is corroborated by the evaluations of AGES within an EFSA project on the definition of the relevant residue for risk assessment purposes. Simple demethylation of a ring or side chain did not result in any toxification.

Table 5.1.1-5: Grouping of metabolites: Metabolites resulting from desmethoxylation

Substance/ Metabolite Code	Structure	Occurrence [mean, % of dose]
500M06		Urine: 2%* Bile: 4%* Plasma: < 0.01% Liver: 0.1%
500M07		Feces: 7%*
500M08		Urine: < 1%* Feces: 40%
500M13		Urine: 1%*
500M18		Urine: 1%*
500M19		Urine: 1%*

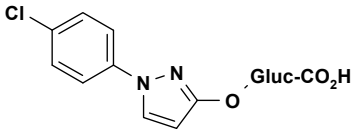
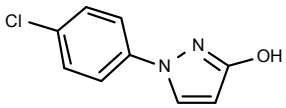
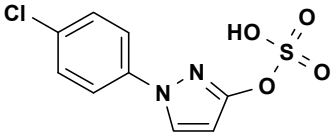
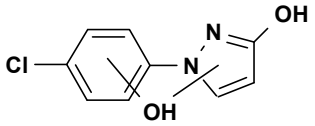
Substance/ Metabolite Code	Structure	Occurrence [mean, % of dose]
500M32		Bile: 4%*
500M33		Bile: < 0.5%
500M34		Bile: 1%*
500M37		Bile: < 0.5%*
500M38		Bile: < 0.5%*
500M39		Bile: < 0.5%*
500M44		Feces: 1.5%
500M45		Feces: 4.5%
500M52		Urine: 1.5%*

* sum of several, co-eluting metabolites

Group 2: Chlorphenyl pyrazole derivatives

Chlorphenyl pyrazole derivatives were almost exclusively found in urine. No bile experiment has been performed with the chlorophenyl ring label. In rats, the cleavage step resulting in the chlorophenyl pyrazole derivatives is by far less pronounced than desmethoxylation or hydroxylation. As in plant and livestock matrices metabolites as 500M04, 500M05 and 500M79 are clearly exceeding 10% TRR, additional tox testing was performed (see M-CA 5.8). The metabolites cannot be considered as fully covered by the investigations performed with the parent compound.

Table 5.1.1-6: Grouping of metabolites: Metabolites resulting from cleavage reactions of the ether bond (chlorophenyl pyrazole derivatives)

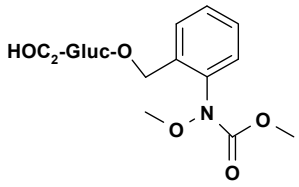
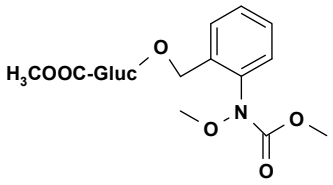
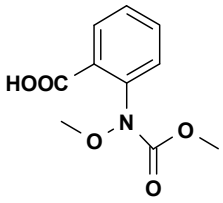
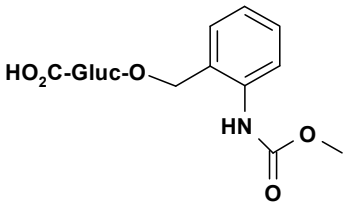
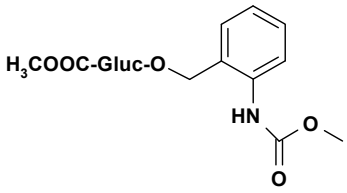
Substance/ Metabolite Code	Structure	Occurrence [mean, % of dose]
500M03		Urine: 1.5%*
500M04		Urine: 1.5%*
500M05		Urine: 1.5%*
500M21		Feces: 1%

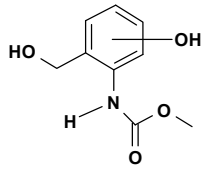
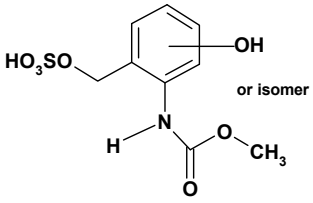
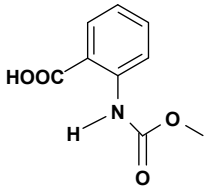
* sum of several, co-eluting metabolites

Group 3: Anthranilic acid derivatives

Anthranilic acid derivatives were almost exclusively found in urine. In rats, the cleavage step of the ether bond is by far less pronounced than any desmethoxylation or hydroxylation step. As in plant and livestock matrices metabolites as 500M24, 500M49 and 500M51 are close or slightly above 10% TRR, additional tox testing was performed (see M-CA 5.8). The metabolites cannot be considered as fully covered by the investigations performed with the parent compound.

Table 5.1.1-7: Grouping of metabolites: Metabolites resulting from cleavage reactions of the ether bond (anthranilic acid derivatives)

Substance/ Metabolite Code	Structure	Occurrence [mean, % of dose]
500M22		Urine: 1.3%* Bile: 1.6%
500M23		Urine: 1.3%*
500M24		Urine: 1.6%
500M25		Urine: 0.6%*
500M26		Urine: 0.6%*

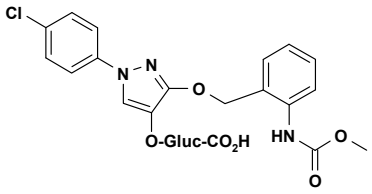
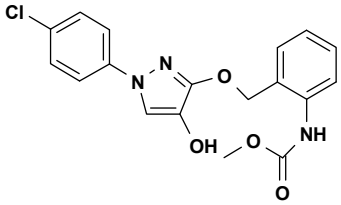
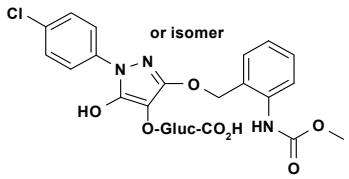
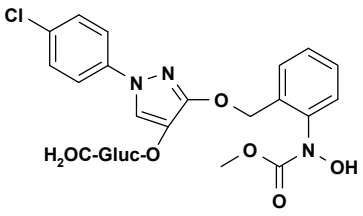
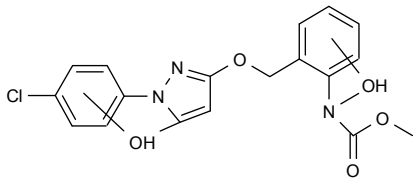
Substance/ Metabolite Code	Structure	Occurrence [mean, % of dose]
500M40		Urine: 0.3%*
500M48		Urine: 0.3%*
500M51		Urine: 0.3%

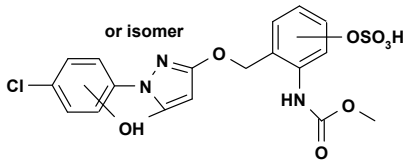
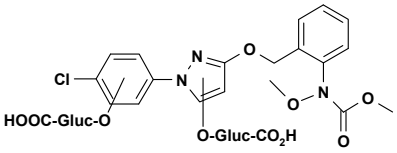
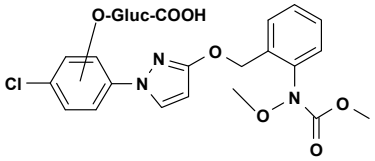
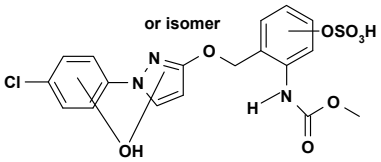
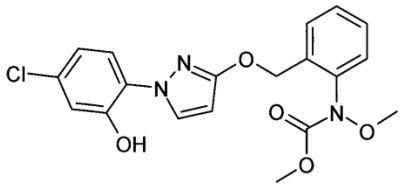
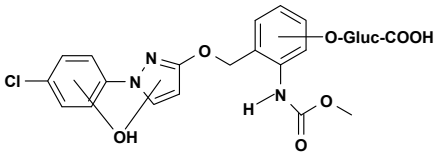
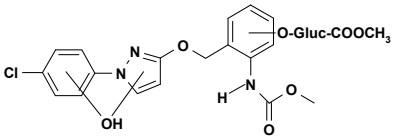
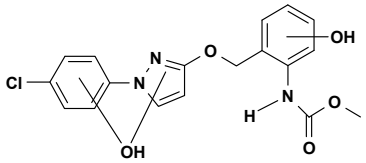
* sum of several, co-eluting metabolites

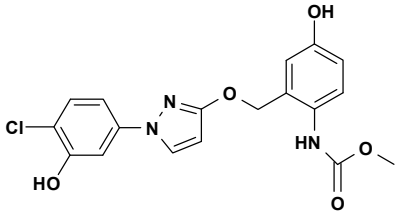
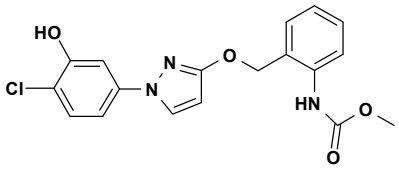
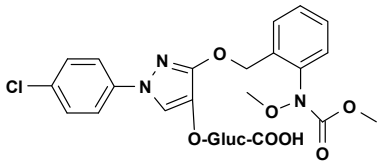
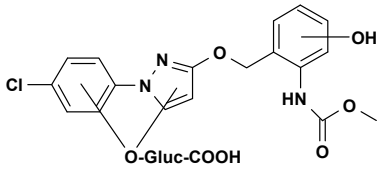
Group 4: Hydroxylated metabolites (chlorphenyl pyrazole moiety)

Hydroxylation reactions are an important metabolic degradation step in rats. In total, about 20 - 30 % of dose can be assigned to metabolites hydroxylated in the chlorphenyl pyrazole moiety. Due to these findings, the endpoints of pyraclostrobin are also applying to group 4 metabolites. This conclusion is corroborated by the evaluations of AGES within an EFSA project on the definition of the relevant residue for risk assessment purposes. Simple hydroxylation of a ring or side chain without any further cleavage did not result in any toxification.

Table 5.1.1-8: Grouping of metabolites: Metabolites resulting from hydroxylation reactions

Substance/ Metabolite Code	Structure	Occurrence [mean, % of dose]
500M06		Urine: 2%* Bile: 4%* Plasma: < 0.01% Liver: 0.1%
500M08		Urine: < 1%* Feces: 40%
500M13		Urine: 1%*
500M15		Bile: 1% Plasma: < 0.01%
500M18		Urine: 1%*

Substance/ Metabolite Code	Structure	Occurrence [mean, % of dose]
500M19		Urine: 1%*
500M29		Bile: 1%
500M31		Bile: 4%*
500M33		Bile: < 0.5%
500M35		Bile: 1%*
500M37		Bile: < 0.5%*
500M38		Bile: < 0.5%*
500M39		Bile: < 0.5%*

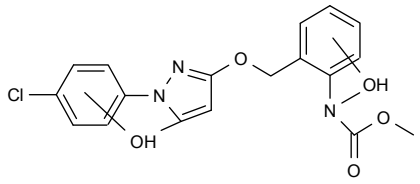
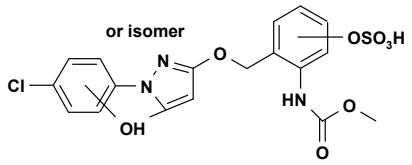
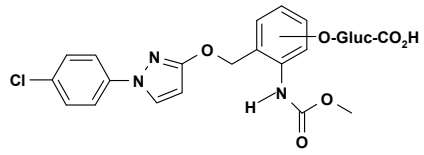
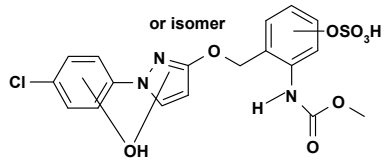
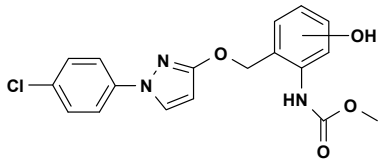
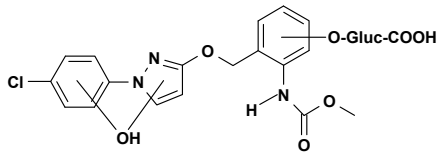
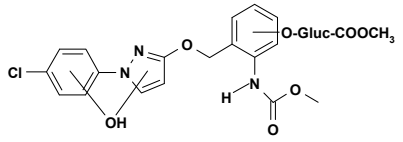
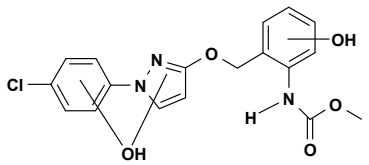
Substance/ Metabolite Code	Structure	Occurrence [mean, % of dose]
500M44		Feces: 1.5%
500M45		Feces: 4.5%
500M46		Bile: 22%, Plasma: < 0.015% Liver: 0.1%
500M52		Urine: 1.5%*

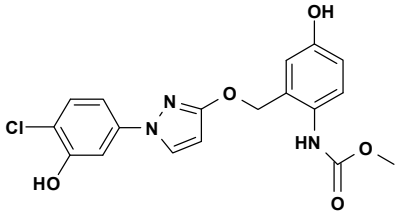
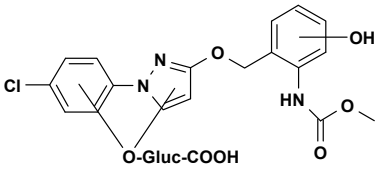
* sum of several, co-eluting metabolites

Group 5: Hydroxylated metabolites (tolyl moiety)

Hydroxylation reactions are an important metabolic degradation step in rats. In total, about 10% of dose can be assigned to metabolites hydroxylated in the tolyl moiety. Due to these findings, the endpoints of pyraclostrobin are also applying to group 5 metabolites. This conclusion is corroborated by the evaluations of AGES within an EFSA project on the definition of the relevant residue for risk assessment purposes. Simple hydroxylation of a ring or side chain without any further cleavage did not result in any toxification.

Table 5.1.1-9: Grouping of metabolites: Metabolites resulting from hydroxylation reactions

Substance/ Metabolite Code	Structure	Occurrence [mean, % of dose]
500M18		Urine: 1%*
500M19		Urine: 1%*
500M32		Bile: 4%*
500M33		Bile: < 0.5%
500M34		Bile: 1%*
500M37		Bile: < 0.5%*
500M38		Bile: < 0.5%*
500M39		Bile: < 0.5%*

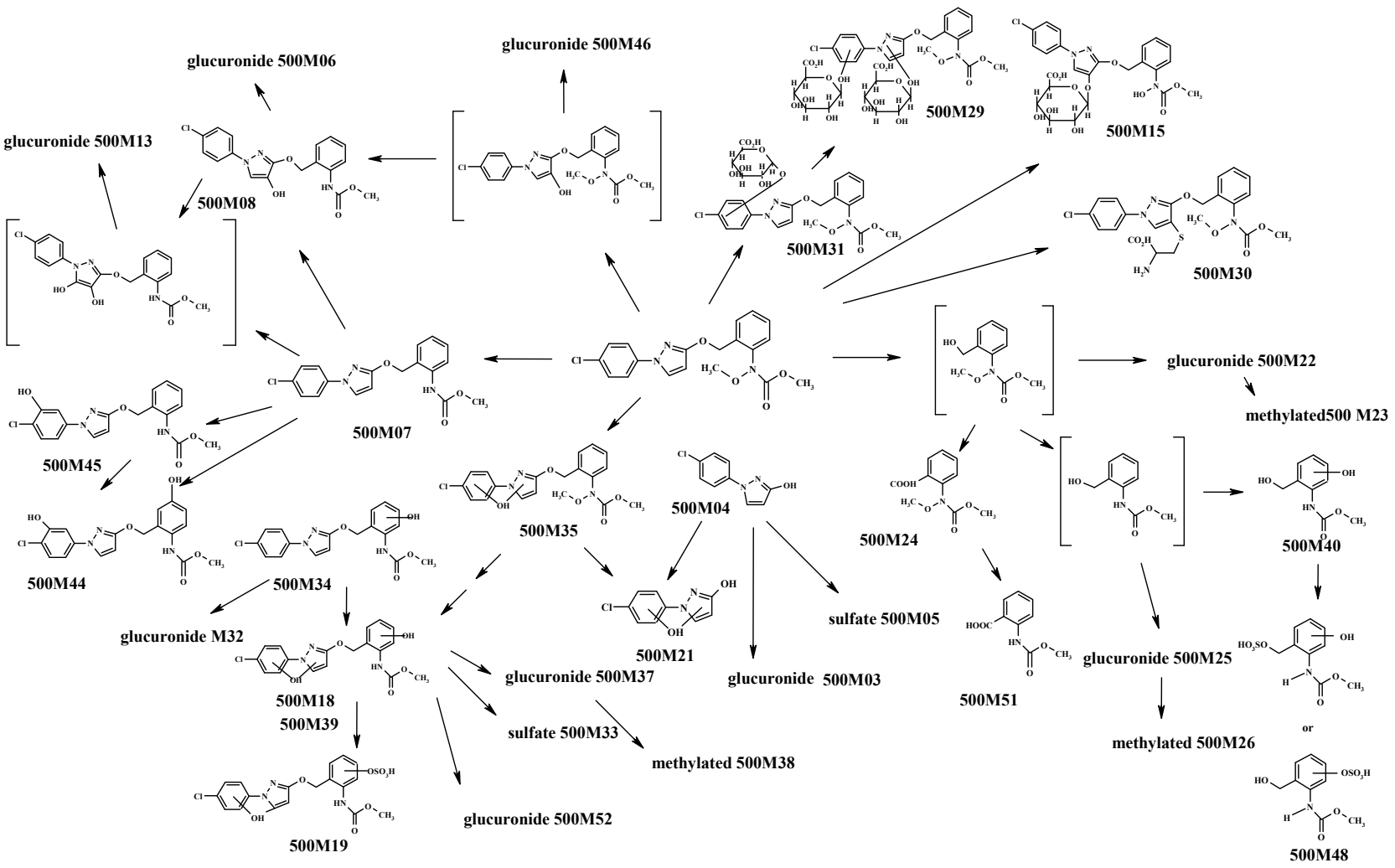
Substance/ Metabolite Code	Structure	Occurrence [mean, % of dose]
500M44		Feces: 1.5%
500M52		Urine: 1.5%*

* sum of several, co-eluting metabolites

Group 6: Photo metabolite 500M76

The metabolite 500M76 was only found in wheat forage samples. As it is formed by a photolytic re-arrangement reaction on leaf surfaces, its presence in the rat metabolism study can be excluded. Due to this fact and for supporting an assessment according to the TTC approach, genotoxicity investigations have been performed (see M-CA 5.8).

Figure 5.1.1-1: Proposed metabolic pathway of pyraclostrobin in rats



New study

The objective of the new study was to further investigate metabolites in rat plasma samples after oral dosing of pyraclostrobin (BAS 500 F) in order

- to analyse rat plasma samples at different time points after dosing
- to specifically address metabolites being present at short time intervals after dosing for covering the time point of the first plasma peak; the previously submitted study provides mainly information on the second plasma peak (tmax of both peaks: see Table 5.1.1-4)
- to compare the results with those obtained in the previous rat metabolism study

In July 2014 only a GLP interim report was available for dossier submission. At this time the primary focus was to qualify the metabolites in plasma. Further investigations including peak assignment and quantification of metabolites being identified by MS have been carried out in the second half of 2014. The final report containing also these data is now included in the updated dossier. In addition to the study summary of the interim report, the OECD summary of the final report is provided below as well.

Study summary of interim report

Report:	CA 5.1.1/1 [redacted] et al., 2014a Further investigations of metabolites in rat plasma samples after dosing with 14C-Pyraclostrobin 2014/1136557
Guidelines:	EPA Health Effects Test Guidelines OPPTS 870.7485: Metabolism and Pharmacokinetics (USA), EPA Residue Chemistry Test Guidelines OPPTS 860.1000: Background (USA), MAFF Testing Guidelines for Toxicology Studies: Metabolism Animals (Japan), OECD Guideline for Testing of Chemicals No. 417: Toxicokinetics (OECD), EU Commission Directive 87/302/EEC of 18 November 1987, Part B, Toxicokinetics (EU)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 F (pyraclostrobin, Reg No. 304428)
Description: ^{14}C-BAS 500 F: tolyl-ring-U-C14, specific activity 6.68 MBq/mg
^{13}C-BAS 500 F: Pyrazole-3-C-13
Batch/purity #: ^{14}C-BAS 500 F: 566-5011 / 99.3 % (radiochemical), 95.7 % (chemical)
^{13}C-BAS 500 F: 1026-1018 / 99.8 % (chemical)
Stability of test compound: stable for 33 hours in dosing solution

2. Vehicle and/or positive control:
1 % (w/w) aqueous solution of carboxymethyl cellulose mixed with Cremophor EL suspension in a ratio of 9+1 (w/w)

3. Test animals: Rat
Species: Wistar rats
Strain: Crl:WI(Han)
Sex: female and male
Age: 10 – 12 weeks
Weight at dosing: female: 200 g (mean), male: 312 g (mean)
Source: Charles River Laboratories, Sulzfeld, Germany
Acclimation period: 7 days
Diet: Kliba 3433 diet (Provimi Kliba SA) *ad libitum*
Water: Tap water *ad libitum*
Housing: Makrolon cages type 3
Environmental conditions:
Temperature: 21-24 °C
Humidity: 40-54 %
Air changes: not reported
Photo period: Day/night rhythm of 12 h

B. STUDY DESIGN AND METHODS

1. Dates of work: May 13, 2014 – June 12, 2014 (for structural identification part)
Further investigations are ongoing.

2. Study design

All experiments were performed with Wistar rats of strain Crl:WI(Han). For each of the 8 exsanguination time points, 1 female (PFx) and 1 male (PMx) rat were dosed once orally. The rats were dosed with a mixture of unlabelled, ^{13}C- and ^{14}C-labelled pyraclostrobin in an approximate ratio of 9+9+2 resulting in a specific activity of approx. 40.000 dpm /µg in the dose solution. The application formulation was administered orally via gavage.

The nominal dose level was 50 mg/kg body weight (corresponding to the high dose in the previous studies).

Figure 5.1.1-2: Structural formula of unlabelled pyraclostrobin

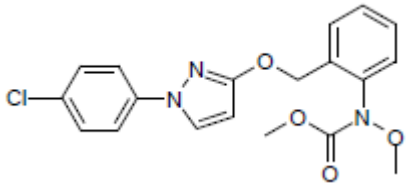


Figure 5.1.1-3: Structural formula of ^{13}C-labelled pyraclostrobin

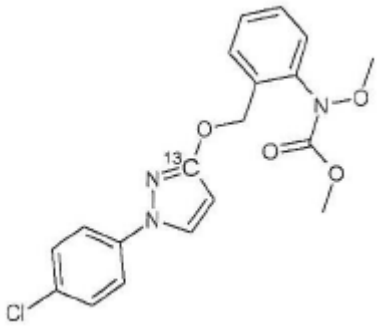
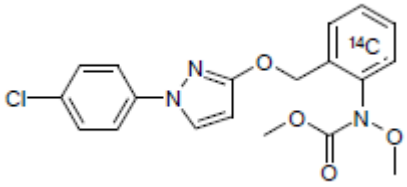


Figure 5.1.1-4: Structural formula of ^{14}C-pyraclostrobin labelled at the tolyl ring



Sampling and Sample Storage

For each designated time point (0.25, 0.5, 1, 2, 3, 4, 8 and 16 h) blood was sampled from 1 female (PFx) and 1 male (PMx) rat. Blood sampling was done by exsanguination under isoflurane anaesthesia.

The blood was collected in tubes containing either EDTA or lithium heparin (LiHep) as anticoagulant. Subsequently, blood samples were split into two aliquots (1/4 as a blood aliquot and 3/4 for the generation of plasma). The aliquot for the generation of plasma was centrifuged at 3000 rpm for 10 min. The supernatant (plasma) and pellet (clot) were collected. Blood, plasma and clot from the individual time points were stored under frozen conditions.

Workup Procedure of EDTA and LiHep Plasma Samples

Prior to processing of the plasma, the radioactivity was measured by LSC. Thereafter, the plasma samples were mixed with acetonitrile and centrifuged in order to precipitate the proteins. The supernatants were analysed by radio-HPLC.

Workup of the Blood Pool Sample (EDTA)
For HPLC method development, but also recovery determinations and investigations on non-released radioactivity, a pool sample was prepared. For this purpose, aliquots of 0.5 mL blood of each time point (0.25, 0.5, 1, 2, 3, 4, 8 and 16 h) were pooled.
The pooled blood sample was centrifuged twice to obtain plasma. The radioactivity in plasma was analysed by LSC and set to 100 % TRR. Thereafter, the plasma was mixed with acetonitrile and subsequently centrifuged to obtain the supernatant and the protein pellet. This work-up step was performed twice. In both cases the supernatants were concentrated to dryness and dissolved in CH_3CN/H_2O/Triton-X100 (700/300/25, v/v/v) to obtain concentrated plasma supernatants. The samples were analysed with HPLC methods LC04 and LC02, respectively, but also subjected to mass spectroscopic investigations. The protein pellet was washed with acetonitrile and sonicated. The resulting wash fractions were pooled and filled up to the mark with acetonitrile.
The protein pellet was finally cut with a scalpel and dried in a nitrogen stream. The dried protein pellet was dissolved in Tris buffer. Protease (*S. griseus*) was added and the mixture was incubated overnight at 37 °C. Then, additional protease and Tris buffer were added. After 4 h of incubation (37 °C), the sample was centrifuged. The supernatant was transferred into a volumetric flask and the volume was adjusted with acetonitrile for LSC measurement. The supernatant was concentrated, dissolved in CH_3CN/H_2O/Triton-X100 (700/300/25, v/v/v) and analysed by HPLC.

HPLC method LC02 (used for the identification of metabolites)
The column used was a Gemini C18, the eluent system consisted of 2 mobile phases (A: H_2O + CH_3CN + HCOOH, 950+50+1 & B: CH_3CN + H_2O + HCOOH, 950+50+1) which were used applying gradient elution.

HPLC method LC04 (used for the identification and quantification of metabolites)
The column used was a Synergi Hydro-RP, the eluent system consisted of 2 mobile phases (A: H_2O + CH_3CN + HCOOH, 900+100+1 & B: CH_3CN + H_2O + HCOOH, 900+100+1), which were used applying gradient elution.

II. RESULTS AND DISCUSSION

1. Determination of the recovered radioactivity in plasma supernatant

In order to determine the radioactivity, which was recovered in the plasma supernatant after acetonitrile precipitation, an EDTA blood pool sample over all time points was generated. As next step, plasma was obtained and precipitation carried out. Thereby, 84.0 % of the TRR was recovered in the plasma supernatant. This recovery was taken to calculate the concentrations of the plasma supernatants of the individual time points.

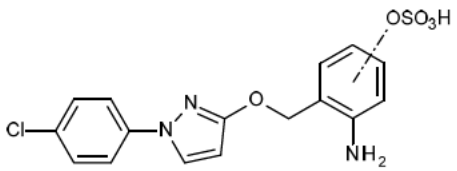
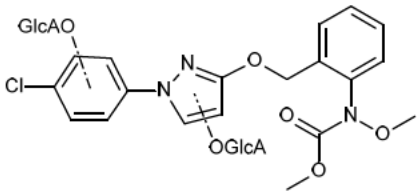
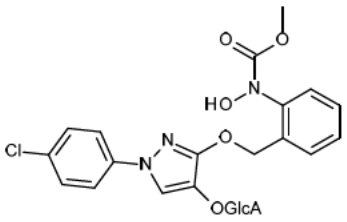
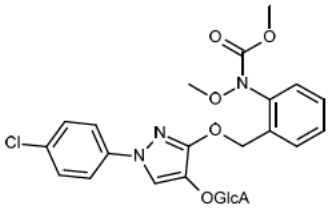
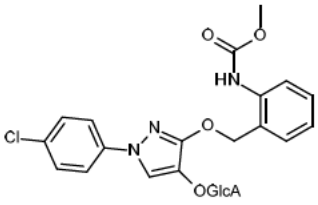
2. Radioactive residues in plasma and plasma supernatants

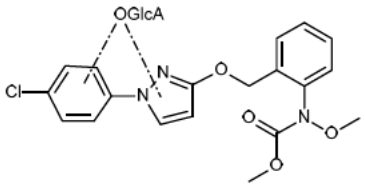
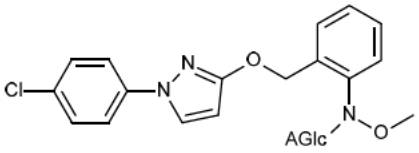
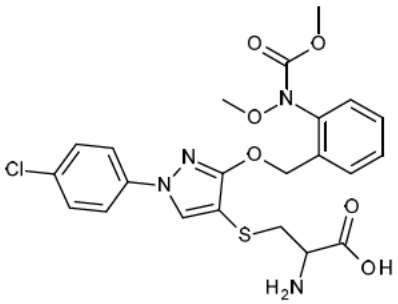
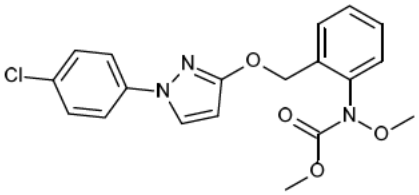
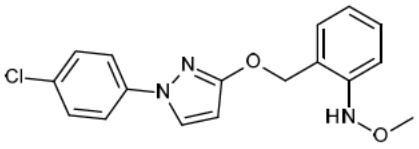
For time points 0.25 and 0.5 h of the EDTA plasma, the radioactivity accounted for 1.429 and 1.343 mg/kg, respectively. Thereafter, the concentration reached almost a steady state (from time point 1 to 16 h) and was generally higher (from 3 h: 2.615 mg/kg to 16 h: 3.030 mg/kg). Only at time point 4 h, the concentration decreased to 1.288 mg/kg. The plasma concentrations are in accordance to the previous rat metabolism study of pyraclostrobin. The results of the LiHep plasma were similar to those of the EDTA plasma.

3. Mass spectroscopic investigations

High resolution HPLC-ESI-MS was used to confirm the identity of the active substance BAS 500 F and for the assignment of the metabolites obtained from plasma supernatants after acetonitrile precipitation. The parent compound and several metabolites were detected. All these metabolites are either described in the previous *in-vivo* metabolism in rats (BASF DocID 1999/11781) or in a new comparative *in-vitro*-metabolism study (BASF DocID 2014/1001562). The metabolites 500M104, 500M106, 500M107 and 500M108 have been newly identified in this *in-vivo* study.

Table 5.1.1-10: Summary of metabolites identified in plasma samples of a new rat study (BASF DocID 2014/1136557)

Component	Structure	Metabolite also identified in
500M108		2014/1001562 (*in-vitro*)
500M29		1999/11781 (*in-vivo* rat)
500M15		1999/11781 (*in-vivo* rat)
500M46		1999/11781 (*in-vivo* rat)
500M06		1999/11781 (*in-vivo* rat)

Component	Structure	Metabolite also identified in
500M104		2014/1001562 (*in-vitro*)
500M107		2014/1001562 (*in-vitro*)
500M30		1999/11781 (*in-vivo* rat)
BAS 500 F (500M00)		1999/11781 (*in-vivo* rat)
500M106		2014/1001562 (*in-vitro*)

4. HPLC investigations: Identification of pyraclostrobin and its metabolites in plasma samples

Analysis of the EDTA plasma supernatants of the different time intervals by radio HPLC, led to comparable metabolite patterns. The unchanged parent compound pyraclostrobin was identified for all time points. Thereby, the concentration of pyraclostrobin ranged from 0.068 mg/kg (0.5 h) to 0.290 mg/kg (16 h). HPLC analysis of the LiHep plasma supernatants led to comparable results as those of the EDTA samples.

5. Proposed metabolic pathway in rat plasma

In rat plasma, the following metabolic transformation steps have been observed:

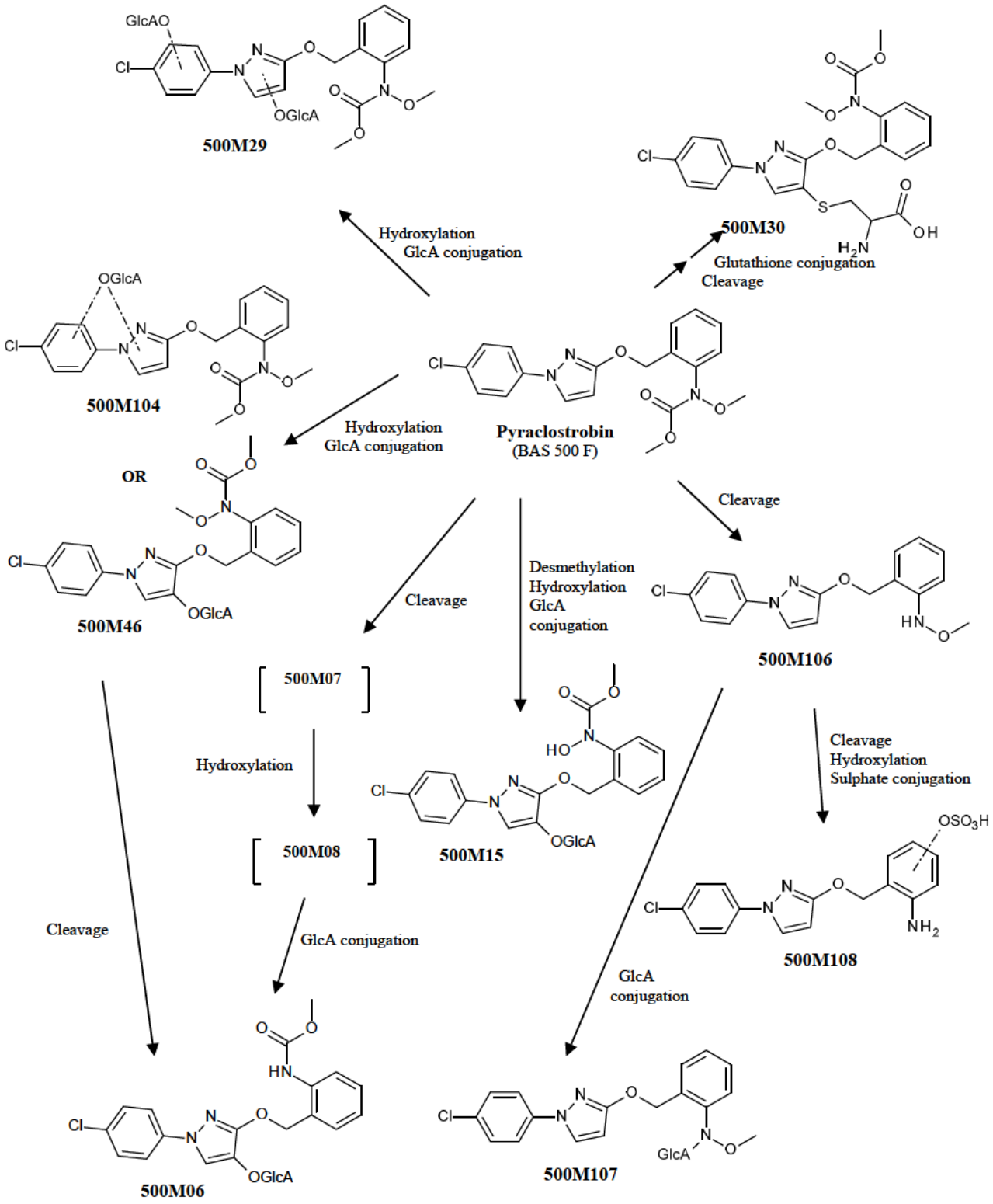
- Desmethoxylation of the side chain
- Hydroxylation of the chlorophenyl pyrazole ring system
- Hydroxylation of the tolyl ring system
- Desmethylation of the side chain
- Cleavage of the amide bond in the side chain

The combination of these reactions followed by conjugation steps results in the formation of several metabolites (Table 5.1.1-10). The proposed pathway in plasma is shown in the figure below.

III. CONCLUSION

The metabolism is accordance with previous investigations, but also with the findings of the *in-vitro* metabolism study summarized in 5.1.2/1. In addition to already known metabolites, 500M104, 500M106, 500M107 and 500M108 have been newly identified in rat plasma samples by high resolution MS.

Figure 5.1.1-5: Proposed metabolic pathway of pyraclostrobin in rat plasma



Study summary of final report

Report:	CA 5.1.1/2 [redacted] et al., 2014 b Further investigations of metabolites in rat plasma samples after dosing with 14C-Pyraclostrobin 2014/1315930 (Final Report)
Guidelines:	EPA 870.7485, EPA 860.1000: EPA Residue Chemistry Test Guidelines, MAFF Testing Guidelines for Toxicology Studies: Metabolism Animals (Japan), OECD 417, EEC 87/302 B
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	BAS 500 F (pyraclostrobin, Reg No. 304428)
	Description:	^{14}C-BAS 500 F: tolyl-ring-U-C14, specific activity 6.68 MBq/mg ^{13}C-BAS 500 F: pyrazole-3-C13
	Batch/purity #:	^{14}C-BAS 500 F: 566-5011 / 99.3 % (radiochemical), 95.7 % (chemical) ^{13}C-BAS 500 F: 1026-1018 / 99.8 % (chemical)
	Stability of test compound:	stable for 33 hours in dosing solution

2. Vehicle and/or positive control: 1 % (w/w) aqueous solution of carboxymethyl cellulose mixed with Cremophor EL suspension in a ratio of 9+1 (w/w)

3.	**Test animals:**	Rat
	Species:	Wistar rats
	Strain:	Crl:WI(Han)
	Sex:	female and male
	Age:	not reported
	Weight at dosing:	female: 200 g (mean), male: 312 g (mean)
	Source:	Charles River Laboratories, Sulzfeld, Germany
	Acclimation period:	7 days
	Diet:	Kliba 3433 diet (Provimi Kliba SA) *ad libitum*
	Water:	Tap water *ad libitum*
	Housing:	Makrolon cages type 3
	Environmental conditions:	
	Temperature:	21-24°C
	Humidity:	40-54%
	Air changes:	not reported
	Photo period:	Day/night rhythm of 12 h

B. STUDY DESIGN AND METHODS

1. Dates of work: Experimental starting date (in-life phase): May 12, 2014
Experimental completion date (in-life phase): May 14, 2014

Experimental starting date (analytical phase): May 19, 2014
Experimental completion date (analytical phase): Aug 25, 2014

Experimental starting date (structure elucidation phase): June 12, 2014
Experimental completion date (structure elucidation phase): June 25, 2014

2. Study design

All experiments were performed with Wistar rats of strain Crl:WI(Han). For each of the 8 exsanguination time points, 1 female (PFx) and 1 male (PMx) rat were dosed once orally. The rats were dosed with a mixture of unlabelled, ^{13}C- and ^{14}C-labeled pyraclostrobin in an approximate ratio of 9+9+2. The application formulation was administered orally via gavage.

The nominal dose level was 50 mg/kg body weight (corresponding to the high dose in the previous studies).

Figure 5.1.1-6: Structural formula of unlabelled pyraclostrobin

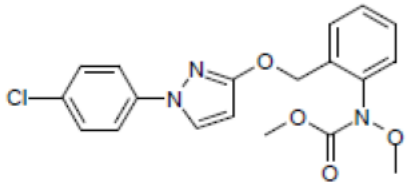


Figure 5.1.1-7: Structural formula of ^{13}C-labelled pyraclostrobin

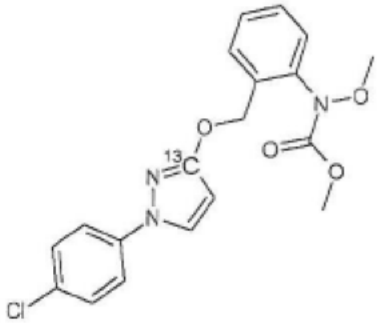
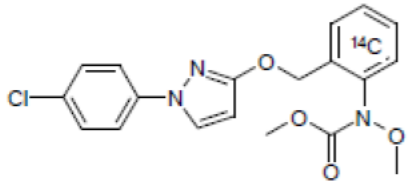


Figure 5.1.1-8: Structural formula of ^{14}C-pyraclostrobin labelled at the tolyl ring



Sampling and Sample Storage

For each designated time point (0.25, 0.5, 1, 2, 3, 4, 8 and 16 h) blood was sampled from 1 female (PFx) and 1 male (PMx) rat. Blood sampling was done by exsanguination under isoflurane anaesthesia.

The blood was collected in tubes containing either EDTA or lithium heparin (LiHep) as anticoagulant. Subsequently, blood samples were split into two aliquots (1/4 as a blood aliquot and 3/4 for the generation of plasma). The aliquot for the generation of plasma was centrifuged at 3000 rpm for 10 min. The supernatant (plasma) and pellet (clot) were collected. Blood, plasma and clot from the individual time points were eventually stored at ≤ -10°C.

Workup Procedure of EDTA and LiHep Plasma Samples

Prior to processing of the plasma, the radioactivity was measured by LSC. Thereafter, the plasma samples were mixed with acetonitrile and centrifuged in order to precipitate the proteins. The supernatants were analysed by radio-HPLC.

The concentrations of the EDTA and LiHep plasma supernatants were calculated as [Concentration of corresponding plasma] * [% TRR of plasma supernatant of blood pool sample] / 100.

The plasma supernatants were concentrated to dryness and dissolved upon sonication in CH_3CN/H_2O/Triton-X100 (700/300/25). Afterwards, the supernatants were analysed by radio-HPLC with methods LC04 and LC02. The HPLC runs of the LiHep plasma supernatants are only exemplarily shown for time points 0.25 h and 0.5 h. For the summary of the quantitative HPLC runs, the metabolite concentrations were re-calculated with the calculated plasma supernatant concentrations. The EDTA supernatant (0.25 h to16 h and 3 h) and the LiHep supernatants (0.25 h and 0.5 h) were additionally analysed with HPLC method LC05, in order to estimate metabolite quantities.

Workup of the Blood Pool Sample (EDTA)

For HPLC method development, but also recovery determinations and investigations on non-released radioactivity, a pool sample was prepared. For this purpose, aliquots of 0.5 mL blood of each time point (0.25, 0.5, 1, 2, 3, 4, 8 and 16 h) were pooled.

The pooled blood sample was centrifuged twice to obtain plasma. The radioactivity in plasma was analysed by LSC and set to 100% TRR. Thereafter, the plasma was mixed with acetonitrile and subsequently centrifuged to obtain the supernatant and the protein pellet.

1.0 mL of the supernatant were concentrated to dryness and dissolved in CH_3CN/H_2O/Triton-X100 (700/300/25, v/v/v) to obtain concentrated plasma supernatants. The samples were analysed with HPLC methods LC04 and LC02, respectively. The concentrated plasma supernatant was additionally analysed with HPLC method LC05, in order to estimate metabolite quantities. The protein pellet was washed with acetonitrile and sonicated. The resulting wash fractions were pooled and filled up to the mark with acetonitrile.
The protein pellet was finally cut with a scalpel and dried in a nitrogen stream. The dried protein pellet was dissolved in Tris buffer. Protease (*S. griseus*) was added and the mixture was incubated overnight at 37 °C. Then, additional protease and Tris buffer were added. After 4 h of incubation (37 °C), the sample was centrifuged. The supernatant was transferred into a volumetric flask and the volume was adjusted with acetonitrile for LSC measurement. The supernatant was concentrated, dissolved in CH_3CN/H_2O/Triton-X100 (700/300/25, v/v/v) and analysed by HPLC method LC02.

HPLC method LC02 (used for confirmation of metabolites)
The column used was a Phenomenex Synergi 4 µm Hydro-RP, the eluent system consisted of 2 mobile phases (A: H_2O + CH_3CN + HCOOH, 900+100+1 & B: CH_3CN + H_2O + HCOOH, 900+100+1) which were used applying gradient elution.

HPLC method LC04 (used for the characterization and quantification of metabolites)
The column used was a Phenomenex Gemini 5 µm C18, the eluent system consisted of 2 mobile phases (A: H_2O + CH_3CN + HCOOH, 950+50+1 & B: CH_3CN + H_2O + HCOOH, 950+50+1), which were used applying gradient elution.

HPLC method LC05 (used for quantification)
The column used was a Thermo Scientific Accucore aQ 2.6 µm, the eluent system consisted of 2 mobile phases (A: H_2O + HCOOH, 1000+1 & B: CH_3CN + HCOOH, 1000+1), which were used applying gradient elution.

II. RESULTS AND DISCUSSION

Determination of the recovered radioactivity in plasma supernatant
In order to determine the radioactivity which was recovered in the plasma supernatant after acetonitrile precipitation, an EDTA blood pool sample over all time points was generated, plasma was obtained and the precipitation was carried out. Thereby, 84.0% of the TRR was recovered in the plasma supernatant. This recovery was taken to calculate the concentrations of the plasma supernatants of the individual time points.
Since the concentrations of the LiHep plasma samples were similar to those of the EDTA samples and the metabolite patterns in the plasma supernatants were also comparable, the determined recovery of 84.0% was also taken to calculate the concentrations of the LiHep plasma supernatants of the individual time points.

Radioactive residues in plasma and plasma supernatants

A summary of the radioactive residues in plasma and plasma supernatants of EDTA and LiHep samples is shown in Table 5.1.1-11. The radioactive residues in plasma were measured, while the radioactive residues in the EDTA plasma supernatants were calculated based on the measurements of the EDTA blood pool sample. For time points 0.25 h and 0.5 h of the EDTA plasma, the radioactivity accounted for 1.429 mg/kg and 1.343 mg/kg, respectively. Thereafter, the concentration reached almost a steady state (from time point 1 h to 16 h) and was generally higher compared to time points 0.25 h and 0.5 h (from 3 h: 2.615 mg/kg to 16 h: 3.030 mg/kg). Only at time point 4 h, the concentration decreased to 1.288 mg/kg. The plasma concentrations are in accordance to the previous rat metabolism study of pyraclostrobin (BASF DocID 1999/11781). The results of the LiHep plasma were similar to those of the EDTA plasma.

Table 5.1.1-11: Summary of metabolites identified in plasma samples

Anticoagulant	Time Interval [h]	Plasma (Measured) [mg/kg]	Plasma Supernatant (Calculated)[1] [mg/kg]
EDTA	0.25	1.429	1.201
	0.5	1.343	1.129
	1	2.953	2.482
	2	2.775	2.332
	3	2.615	2.197
	4	1.288	1.083
	8	2.780	2.336
	16	3.030	2.547
LiHep	0.25	1.385	1.164
	0.5	1.401	1.177
	1	3.069	2.579
	2	3.144	2.643
	3	3.165	2.660
	4	1.329	1.117
	8	3.077	2.568
	16	3.909	3.286

[1] Calculation explained in the study

Mass spectroscopic investigations

In order to identify metabolites in plasma, a concentrated plasma supernatant sample of the EDTA blood pool sample of time points 0.25 h to 16 h was subjected to HPLC-MS analysis. Accurate masses were obtained via high resolution HPLC-MS analysis. Due to a high matrix load, MS/MS analysis was possible only in one case. Generally, more than one possible metabolite or isomer could be assigned to one accurate mass. Hence, for the analysis of the pool plasma supernatant a metabolite was regarded as identified when its accurate mass was detected upon HPLC-MS analysis, the metabolite was identified in rat plasma of a previous study (BASF DocID 1999/11781) and / or the metabolite was identified in an *in-vitro* study with human hepatocytes (BASF DocID 2014/1001562). These assumptions complied with the unchanged parent compound pyraclostrobin and metabolites 500M108, 500M29, 500M15, 500M46, 500M06, 500M104, 500M107 and 500M30. Moreover, MS/MS data confirmed the structure of 500M46.

HPLC analysis of the LiHep plasma supernatant (0.25 h) with HPLC method LC02 led to a peak at 72.1 mm, which was approximately the retention time of reference item 500M106. Metabolite 500M106 was identified in an in vitro study with human hepatocytes (BASF DocID 2014/1001562). In the same study, metabolite 500M107 was also identified, which is a conjugate of 500M106. Therefore, the LiHep plasma supernatant (0.25 h) was screened via HPLC-MS for corresponding accurate masses. Thereby, the accurate masses of 500M106 and 500M107 were detected (see Table 5.1.1-12).

Table 5.1.1-12: Summary of metabolites identified in plasma samples

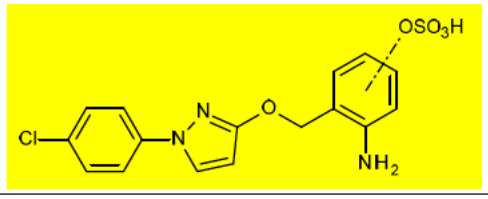
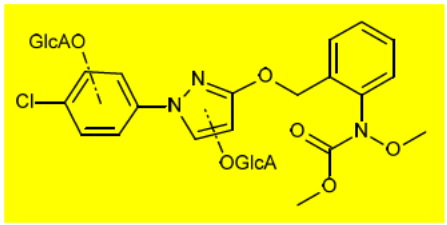
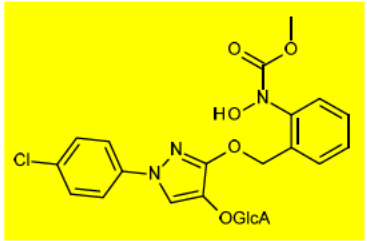
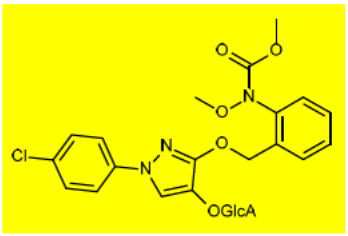
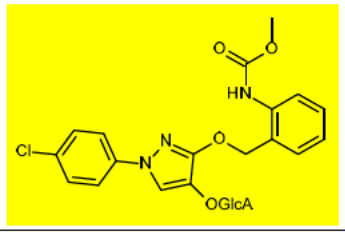
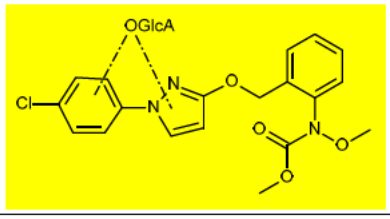
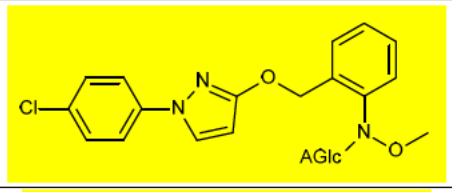
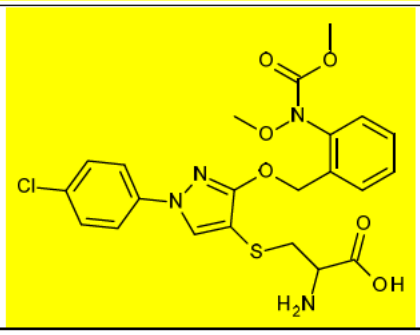
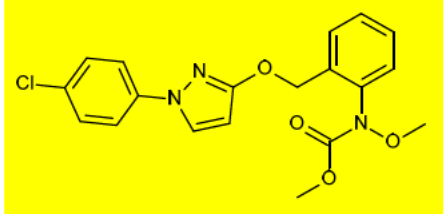
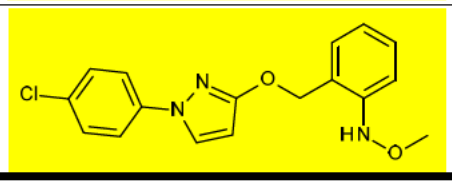
Component	Structure	Metabolite also identified in
500M108		2014/1001562 (*in-vitro*)
500M29		1999/11781 (*in-vivo* rat)
500M15		1999/11781 (*in-vivo* rat)

Table 5.1.1-12: Summary of metabolites identified in plasma samples

Component	Structure	Metabolite also identified in
500M46		1999/11781 (*in-vivo* rat)
500M06		1999/11781 (*in-vivo* rat)
500M104		2014/1001562 (*in-vitro*)
500M107		2014/1001562 (*in-vitro*)
500M30		1999/11781 (*in-vivo* rat)
BAS 500 F (500M00)		1999/11781 (*in-vivo* rat)
500M106		2014/1001562 (*in-vitro*)

Identification and Quantification of pyraclostrobin and its metabolites in plasma samples

Plasma supernatants (both EDTA and LiHep) were analysed with HPLC method LC04 for characterization of the radioactive residues and quantification of pyraclostrobin and metabolite 500M106. HPLC method LC02 was used for confirmation of the metabolite pattern. The peak assignment for pyraclostrobin and metabolite 500M106 was done by comparing the retention times with those of the corresponding reference items.

Analysis of EDTA plasma supernatants of the different time intervals with HPLC method LC04, led to comparable metabolite patterns. The unchanged parent compound pyraclostrobin was identified for all time points. Thereby, the concentration of pyraclostrobin ranged from 0.068 mg/kg (0.5 h) to 0.290 mg/kg (16 h). Analysis of the EDTA plasma supernatants with HPLC method LC02 confirmed the presence of the parent compound for time points 0.25, 1, 2, 3, 4 and 16 h. For time points 0.5 and 8 h the signal of pyraclostrobin was not confirmed, which was probably due to relatively low concentrations of the compound.

Comparable with the results of the EDTA samples, the parent compound pyraclostrobin was identified with HPLC method LC04 and LC02 for time point 0.25 h. For time point 0.5 h pyraclostrobin was only identified with HPLC method LC02. Additionally, for time point 0.25 h a peak at 72.1 mm was detected with HPLC method LC02, which corresponded approximately to the retention time of reference item 500M106. Therefore, the same sample was analysed via HPLC-MS, whereby the accurate mass of 500M106 was detected. Hence, the peak at 72.1 mm was assigned to metabolite 500M106.

The concentrated plasma supernatant of the pool blood sample was also analysed with HPLC methods LC04 and LC02. Thereby, the metabolite pattern was generally comparable with the metabolite patterns of the plasma supernatants of the individual time points.

The protease solubilizate of the pellet (0.25 h - 16 h) was analysed with HPLC method LC02, which resulted in a pattern of 5 peaks.

Since HPLC method LC04 and LC02 did not correspond to the method of the HPLC-MS analyses, peak assignment of the MS-identified metabolites was not possible. Therefore, four selected samples (EDTA plasma supernatant 3 h, concentrated plasma supernatant of the EDTA blood pool sample 0.25 h - 16 h, LiHep plasma supernatants 0.25 h and 0.5 h) were additionally analysed with HPLC method LC05, which is identical to the method of HPLC-MS.

Peak assignment was based on comparison of the ^{14}C pattern and retention times of the HPLC-MS analysis of the EDTA blood pool sample (0.25 h - 16 h) with the ^{14}C pattern of the same sample analysed with HPLC method LC05. Similar to HPLC method LC04, analysis of the EDTA blood pool sample (0.25 h. - 16 h) resulted in broad peaks, which were not baseline-separated. Generally, more than one metabolite could be assigned to such a broad peak. Hence, the peaks were classified as regions (Region 1 to 4), to which the identified components were assigned. Region 1 included 500M108 and 500M29, Region 2 500M15 and 500M108, Region 3 500M46 and 500M06 and Region 4 500M104, 500M107 and 500M30. Since the resolution of pyraclostrobin and metabolite 500M106 was better, pyraclostrobin and metabolite 500M106 were assigned to discrete peaks.

In all four samples, the relative amounts of Region 1, Region 3 and Region 4 were similar. The amounts of Region 1, Region 3 and Region 4 ranged for the EDTA plasma supernatant 3 h from 0.337 mg/kg to 0.560 mg/kg, for the concentrated plasma supernatant of the EDTA blood pool sample 0.25 h - 16 h from 0.172 mg/kg to 0.268 mg/kg, for the LiHep plasma supernatant 0.25 h from 0.158 mg/kg to 0.227 mg/kg and for the LiHep plasma supernatant 0.5 h from 0.227 mg/kg to 0.331 mg/kg. In all four samples, Region 2 was the least abundant region and accounted from 0.044 mg/kg (LiHep 0.5 h) to 0.111 mg/kg (EDTA 3 h).

Pyraclostrobin was identified in all four samples and the quantities corresponded well to the results of the analyses of the EDTA plasma supernatant samples with HPLC method LC04. Metabolite 500M106 was only detected in the LiHep plasma supernatant of 0.25 h, where it was the least abundant component.

Proposed metabolic pathway of pyraclostrobin in rat plasma samples

The metabolites of pyraclostrobin which were detected in rat plasma can be deduced from the typical phase I and II reactions:

- Desmethoxylation of the side chain
- Hydroxylation of the chlorophenyl pyrazole ring system
- Hydroxylation of the tolyl ring system
- Desmethylation of the side chain
- Cleavage of the amide bond in the side chain
- The combination of these reactions followed by conjugation steps results in a huge number of metabolites.

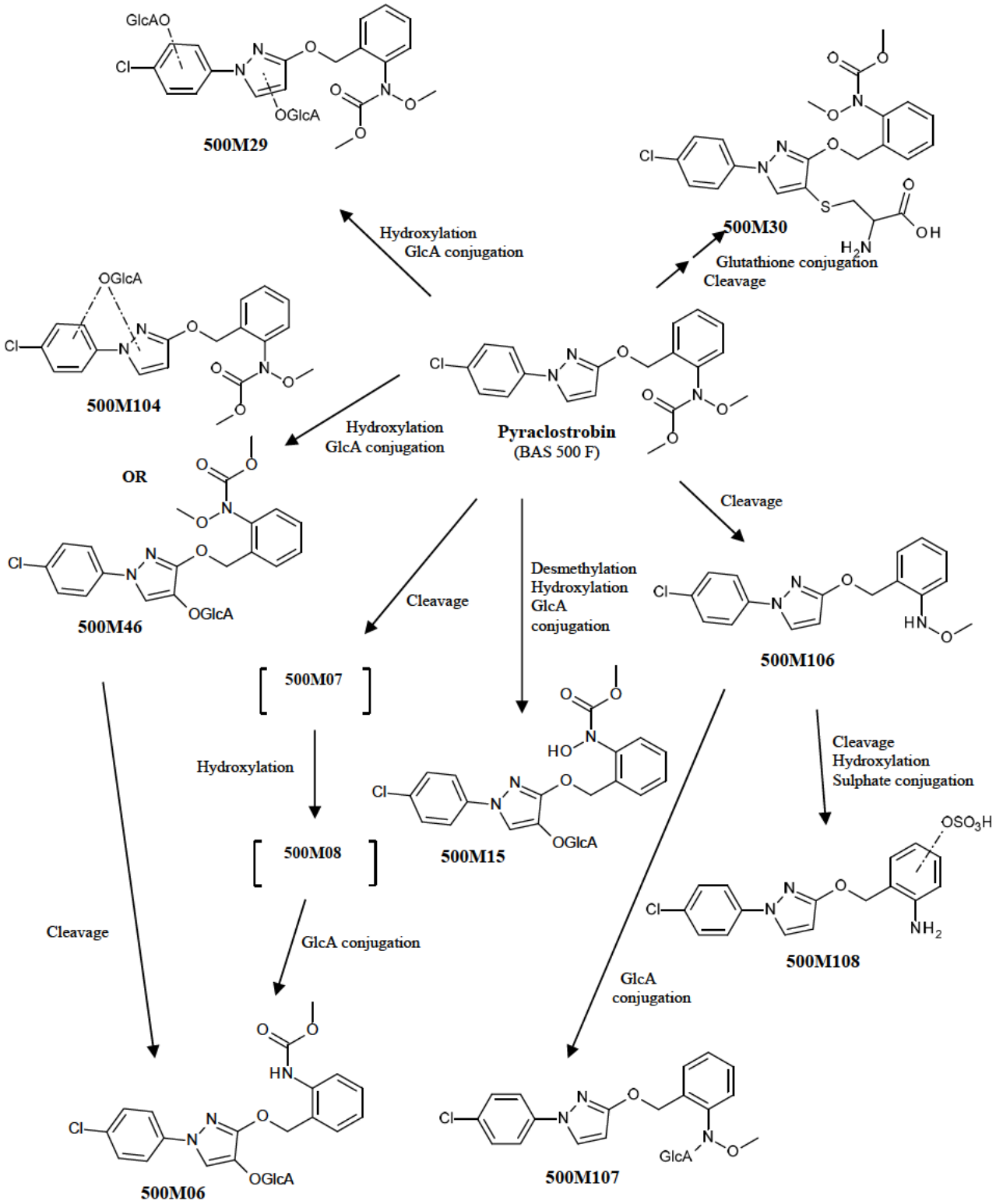
Hydroxylation and glucuronic acid conjugation of the chlorophenyl and the pyrazole ring of pyraclostrobin yield metabolite 500M29. Hydroxylation and glucuronic acid conjugation of only the pyrazole ring result in metabolite 500M46 and 500M104. Metabolite 500M30 is a cysteine conjugation product of pyraclostrobin, wherein cysteine is bound via its sulphur to the pyrazole ring. 500M30 is probably generated via conjugation of pyraclostrobin with glutathione, followed by enzymatic cleavage of the glutamic acid and the glycine of glutathione.
Desmethoxylation of pyraclostrobin yields the intermediate 500M07. Hydroxylation of the pyrazole ring of 500M07 results in the intermediate 500M08. Both, 500M07 and 500M08 were previously identified in a rat metabolism study of pyraclostrobin (BASF DocID 1999/11781). Glucuronic acid conjugation of 500M08 results in metabolite 500M06. 500M06 might also be generated via N-desmethoxylation of 500M46.
Desmethylation of the N-methoxy side chain of pyraclostrobin, followed by hydroxylation of the pyrazole ring and glucuronic acid conjugation results in metabolite 500M15. Cleavage of the methylcarboxy group of pyraclostrobin yields metabolite 500M106. Conjugation of the secondary amine group of 500M106 with glucuronic acid results in metabolite 500M107. Cleavage of the methoxy moiety of 500M106, followed by hydroxylation and sulphate conjugation of the tolyl moiety results in metabolite 500M108.

III. CONCLUSION

The objective of this study was to further investigate the fate and kinetic behavior of pyraclostrobin (BAS 500 F) after a single oral application of 50 mg/kg. For designated time points, one female (PFx) and one male (PMx) rat were dosed with a mixture of ^{14}C-labelled, ^{13}C and unlabeled pyraclostrobin. Blood was sampled in presence of EDTA or lithium heparin (LiHep) and plasma was obtained.
For time points 0.25 h and 0.5 h of the EDTA plasma, the radioactivity accounted for 1.429 mg/kg and 1.343 mg/kg, respectively. Thereafter, the concentration reached almost a steady state (from time point 1 h to 16 h) and was generally higher compared to time points 0.25 h and 0.5 h (from 3 h: 2.615 mg/kg to 16 h: 3.030 mg/kg). Only at time point 4 h, the concentration decreased to 1.288 mg/kg. The results of the LiHep plasma were comparable to those of the EDTA plasma. The plasma concentrations are in accordance to the previous rat metabolism study of pyraclostrobin.

Analysis of the EDTA plasma supernatants of the different time intervals with HPLC, led to comparable metabolite patterns. The unchanged parent compound pyraclostrobin was identified for all time points. Thereby, the concentration of pyraclostrobin ranged from 0.068 mg/kg (0.5 h) to 0.290 mg/kg (16 h). HPLC analysis of the LiHep plasma supernatants led to similar results as those of the EDTA samples.
Via HPLC-MS analysis of plasma supernatant samples the accurate masses corresponding to the parent compound pyraclostrobin and the following metabolites were detected: 500M108, 500M29, 500M15, 500M46, 500M06, 500M104, 500M107, 500M30 and 500M106. Thereby, all metabolites which were identified in plasma of the previous rat metabolism study were confirmed (pyraclostrobin, 500M06, 500M15 and 500M46). Moreover, metabolites which were identified in the *in-vitro* comparison study were also detected in rat plasma (500M108, 500M104, 500M107 and 500M106).
The identified metabolites were quantified exemplarily in four samples (EDTA plasma supernatant 3 h, concentrated plasma supernatant of the EDTA blood pool sample 0.25 h - 16 h and LiHep plasma supernatants 0.25 h and 0.5 h). HPLC peaks which contained more than one component were classified as region (Region 1 to 4). Region 1 included 500M108 and 500M29, Region 2 500M15 and 500M108, Region 3 500M46 and 500M06 and Region 4 500M104, 500M107 and 500M30. In all four samples, the relative amounts of Region 1, Region 3 and Region 4 were similar. The amounts of Region 1, Region 3 and Region 4 ranged for the EDTA plasma supernatant 3 h from 0.337 mg/kg to 0.560 mg/kg, for the concentrated plasma supernatant of the EDTA blood pool sample 0.25 h - 16 h from 0.172 mg/kg to 0.268 mg/kg, for the LiHep plasma supernatant 0.25 h from 0.158 mg/kg to 0.227 mg/kg and for the LiHep plasma supernatant 0.5 h from 0.227 mg/kg to 0.331 mg/kg. In all four samples, Region 2 was the least abundant region and accounted from 0.044 mg/kg (LiHep 0.5 h) to 0.111 mg/kg (EDTA 3 h). Pyraclostrobin was identified in all four samples and the quantities corresponded well to the analyses of the EDTA plasma supernatant samples of the different time intervals (0.25 h to 16 h). Metabolite 500M106 was only detected in the LiHep plasma supernatant of 0.25 h, where it was the least abundant component (0.030 mg/kg).

Figure 5.1.1-9: Proposed metabolic pathway of pyraclostrobin in rat plasma samples



In the *in-vitro* comparison study (see M-CA 5.1.2), the metabolites 500M106 (plus its conjugate 500M107) and 500M02 were predominantly found in human cell cultures and in rabbit, but only in minor amounts in species as rat, mouse and dog for which chronic studies are available.
Based on these results, a metabolism study has been conducted, where metabolite 500M106 was dosed. The study was not part of the supplemental dossier provided in July 2014, but is added to this updated dossier. The OECD study summary can be found below.
The investigations had the following goals:

- To get a comparison to the *in-vitro* situation
- To investigate whether the rat is an appropriate species for further toxicological testing.

Report:	CA 5.1.1/3 [redacted] 2016 a 14C-Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Study on kinetics in Wistar rats after oral administration 2015/1241735
Guidelines:	OECD 417 (July 2010), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, EPA 870.7485, JMAFF Guidelines on the Compiling of Test Results on Toxicity - Tests on In Vivo Fate in Animals (2001)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 5.1.1/4 [redacted] 2016 a Metabolism investigation of 14C-Reg.No. 399379 (metabolites of BAS 500 F, Pyraclostrobin) in faeces and urine of male Wistar rats 2015/1198492
Guidelines:	EPA 870.7485, EPA 860.1000, JMAFF, MAFF Testing Guidelines for Toxicology Studies: Metabolism Animals (Japan), OECD 417, EEC 87/302 B
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: ^{14}C-Reg. No. 399379 (Metabolite of BAS 500 F, Pyraclostrobin)
Reg. No. 399379 (Metabolite of BAS 500 F, Pyraclostrobin)

Description:	pyrazole-3-C14 label, unlabelled Reg. No. 399379
Chemical name:	N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) Omethylhydroxylamine (IUPAC-Name)
Test substance No:	15/0106-2 (^{14}C-labelled), 14/0701-1 (unlabelled)
Lot/Batch #:	1166-1010 (^{14}C-labelled), L83-166 (unlabelled)
CAS#:	not indicated by the sponsor
Development code:	Pyraclostrobin: BAS 500 F Reg. No. 399379 : 500M106 (synonym: M500F106)
Purity:	Radiochemical purity of labelled test item: > 95-99.7% Purity of unlabelled test item: 97.1% (tolerance ± 1.0%)
Specific activity:	6.42 MBq/mg
Stability of test compound:	The stability of the test substance in the test-substance preparation over the test period was verified by analyses.

2. Vehicle and/or positive control: see "preparation of dosing solutions"

3. Test animals:

Species:	Rat
Strain:	Crl: WI (Han) (SPF)
Sex:	male
Age:	10 weeks old at start of acclimatization
Weight at dosing:	Average: 353 g
Source:	Charles River, Sulzfeld, Germany
Acclimation period:	5 days
Diet:	Kliba lab diet (mouse / rat "GLP"), meal Origin: Provimi Kliba SA, 4303 Kaiseraugst, Switzerland ad libitum prior to and during the experiment.
Water:	Tap water *ad libitum*
Housing:	During acclimatization animals housed in groups (up to five animals in Polysulfonate Cages (2000P; H Temp (PSU), 2065 qcm, Tecniplast). During the balance experiment animals were kept individually in Plastic Metabolism Cages (Tecniplast, Italy), labelled with the project number, animal number, dose and time of administration.

Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	15 per hour
Photo period:	12 h light / 12 h dark

4. Preparation of dosing solutions

Stock solutions in acetonitrile were prepared for the radio-labelled and non-labelled test substance, yielding nominal concentrations of 13.2 and 35.2 mg/g, respectively.
In order to achieve the required specific activity, appropriate amounts of the radio-labelled and non-labelled test-substance solutions were taken and the organic solvent (acetonitrile) was evaporated to dryness. Respective amounts of the aqueous vehicle [0.5% carboxymethylcellulose (CMC) in tap water] were added. Based on the current data, the nominal concentration of the test substance in the test-substance preparation was 1.0 mg/mL and the nominal specific activity of the test-substance preparation was 3.4 MBq/mL.
The preparation was stirred and sonicated in order to produce a homogeneous preparation.
Before start and at the end of the administration, samples were taken to determine the amount of radioactivity in the preparation and to demonstrate the correct concentration of the test substance, its homogeneity and its radiochemical purity.

B. STUDY DESIGN AND METHODS

1. Dates of experimental work March 11, 2015 to March 08, 2016

2. Study design

Sampling, pooling, combining and storage
Four male rats received a single oral dose of a mixture of ^{14}C-labelled and unlabelled 500M106. The test item preparation was applied at a nominal rate of 10 mg/kg bw. Urine was sampled in time intervals of 0-6, 6-12, 12-24 hours and afterwards in 24 hours intervals for up to 168 hours after treatment. Faeces was sampled in 24 hour intervals for up to 168 hours after treatment. After 168 hours, animals were sacrificed. In addition to the excreta samples the total amount of radioactive residue was measured in the carcass of each animal. For balance estimates the cage wash was also checked for radioactivity.
Samples were stored in a freezer, and all samples were transferred to the Agricultural Center of BASF SE (Limburgerhof, Germany) under frozen conditions.
At the Agricultural Center, samples were pooled (urine samples from several time intervals of individual animals) or combined (faeces samples of several animals of one time interval). For urine, equal portions, i.e. 30%, were taken per sampling interval and pooled to obtain the time interval 0-48 hours for each animal. This time interval was in focus as the major fraction of radioactive residues (% dose) was excreted within 48 hours and simultaneously the fraction was not diluted with urine sampled at later time intervals containing less than 1% of the administered dose. Additionally, equal portions of urine samples, i.e. 10%, were taken per sampling interval and animal. These aliquots were pooled and combined (for all four animals) to obtain one single sample for the time interval 48-168 hours. For faeces, equal portions, i.e. 50%, were taken per animal and combined for each sampling interval.

Table 5.1.1-13: Summary of dose groups and dosing parameters

Test Item	Reg. No. 399379 (500M106, metabolite of pyraclostrobin)
No. of doses and route of administration	Single oral dose[1]
Number of treated animals (gender)	4 (male)
Animals used for evaluation	1-4
Nominal dose [mg/kg bw]	10
Mean dose achieved [mg/kg bw]	10.3
Label	Pyrazole-3C-14 label, unlabelled (C12)
Ratio of isotopes ^{14}C :^{12}C in application formulation	1:1
Specific radioactivity used [dpm/g]	187307[2]
Radioactive dose [MBq/animal]	11.34
Duration of experiment	168 h
Sampling and analysis	Urine and faeces

1 Application was performed at Department of Experimental Toxicology and Ecology of BASF SE (GV/TB, Ludwigshafen, Germany

2 Corrected value is 187140 and shows an abbreviation of 0.09%, which has no influence on the outcome of the study. Therefore, the uncorrected value was further used for calculations.

Determination of radioactivity

Appropriate aliquots (generally five) of homogenized solid samples were weighed and combusted by means of an automatic sample oxidiser. The $^{14}CO_2$ formed during combustion was trapped by an absorption liquid, and the collected radioactivity was measured by Liquid Scintillation Counting (LSC). For the quantification of radioactivity in liquid samples, a liquid scintillation counter was used. Aliquots of liquid samples were mixed with a sufficient volume of a suitable scintillator prior to measurements. All data were corrected using appropriate quench curves and are expressed in disintegrations per minute (dpm).

Each cage side was washed with 10% RBS® cleaner in drinking water and acetone at the end of the experiment. After weighing the urine and cage wash samples they were mixed with scintillation cocktail and analyzed for radioactivity without any additional treatment.

Carcass was suspended in deionized water and was homogenized using a WARING Blender. Aliquots of the suspension was dried by lyophilisation, dissolved with Soluene®-350, filled up with isopropanol, bleached with perhydrol solution (30% H_2O_2) and Hionic Fluor was added before measurement of radioactivity by LSC.

HPLC analysis / spectroscopy

For the detection of metabolites in urine and faeces samples were analysed by two analytical methods (Radio-HPLC / HPLC-UV). One used for quantitative determination, and the other used for confirmation. Metabolites of 500M106 were identified by HPLC-MS/MS analysis of a urine sample of male rat 2 (0 - 48 h) and an acetonitrile extract of combined faeces (rat 1-4, 24-48 h). Based on retention time comparison known for the metabolite standards, the MS signals/peaks were assigned to the selected radioactive peaks. For confirmation, semi-quantitative comparison of the radioactive and MS peaks was performed, if feasible. Metabolite patterns (radioactive and MS) were compared between matrices.

Sample preparation for analysis

Homogenization
Faeces was homogenized along with dry ice using an analytical mill. The TRR values for faeces were calculated as the sum of extracted radioactive residues (ERR) and residual radioactive residues (RRR) after solvent extraction.

Extraction and Calculation of the Total Radioactive Residue (TRR)
The TRR in urine was determined by direct liquid scintillation counting of three aliquots.
Homogenized individual faeces samples were extracted three times with sufficient amounts of acetonitrile using a dispersing tube. The extracts were separated from the solid by centrifugation. The acetonitrile extracts of the three steps were pooled and aliquots were radioassayed. The results of the acetonitrile extractions referred to extractable radioactive residues (ERR). The solid residue was homogenized using a spatula, aliquots were combusted for the determination of the residual radioactive residues (RRR). The TRR was calculated as the sum of the ERR and RRR.
Homogenized, combined faeces samples were also extracted three times with acetonitrile. After each extraction step, the extract was separated and the solid residue was subjected to the next extraction step. The acetonitrile extracts of the three steps were pooled, adjusted to a defined volume with acetonitrile and appropriate aliquots were measured by LSC. The solid residue was further extracted two times with sufficient amounts of water. The water extracts of the two steps were also centrifuged, filtered, pooled, adjusted to a defined volume with water, and aliquots were radioassayed (LSC). The solid residue after extraction with water was dried, homogenized and additionally extracted three times with a mixture of acetonitrile and acetone (4/1, v/v). After each extraction step, the extract was separated from the solid by centrifugation and filtration, and the solid residue was subjected to the next extraction step. The solid residue was transferred with methanol into a crystal bowl for drying.
The results of the acetonitrile, water and acetonitrile / acetone extractions referred to ERR. The residues after solvent extraction were dried, homogenized and weighed aliquots were measured by LSC for the determination of the RRR. The TRR was calculated as the sum of the ERR and RRR.

Investigation of the Residual Radioactive Residues after Solvent Extraction
Combined faeces samples with residual radioactive residues above 1% of the dose after solvent extraction of combined faeces samples were subjected to a sequential solubilization procedure. Various enzyme incubations (protease, pepsin and pancreatin) were applied in order to analyse the residues under physiological conditions and to subsequently categorize the released radioactive residues as bioavailable. In addition, an artificial solubilization method was applied (alkaline treatment) in order to release radioactive residues being categorized as non-bioavailable.

The residue after solvent extraction was dried, a sufficient amount of protease was added, and the mixture was re-suspended in 0.1 M Tris buffer (at pH 7) and incubated at 37°C upon shaking for 20 hours. Thereafter the sample was centrifuged. The supernatant was filtered, adjusted to a defined volume with an appropriate solvent and aliquots were analyzed by LSC. Additionally, supernatants containing sufficient amounts of radioactive residues were subjected to HPLC analysis. The solid residue was dried and subjected to pepsin solubilization, simulating an artificial gastric juice. Therefore, the dried residue after protease incubation was mixed with an artificial gastric juice (6.4 g pepsin, 4 g NaCI, 84 mL 2 M HCl, 2 L water) incubated at 37°C upon shaking for approximately 24 hours. Thereafter the sample was centrifuged. The supernatant was filtered, adjusted to a defined volume with an appropriate solvent and aliquots were analyzed by LSC. The solid residue was dried and subjected to pancreatin solubilization. Therefore, the dried residue after pepsin incubation was mixed with an artificial intestinal juice (10 g pancreatin per 1 L potassium dihydrogen phosphate buffer at pH 7.5) and incubated at 37°C upon shaking overnight. Thereafter the sample was centrifuged. The supernatant was filtered, adjusted to a defined volume with acetonitrile and aliquots were analyzed by LSC. The solid residue was dried, homogenized, and aliquots were combusted for the determination of the radioactive residues after enzyme solubilization. Thereafter, the solid residue was subjected to alkaline treatment, where 2-4 g of the dried sample was mixed with 30 mL 2N sodium hydroxide. The mixture was boiled at 100°C for 1.5 hours under reflux. The sample was centrifuged, and the supernatant was filtered. The volume was determined and aliquots were analyzed by LSC. The solid residue was dried, homogenized using an analytic mill and aliquots were combusted for the determination of the radioactive residues.

Investigation of Metabolite Patterns and Identification of Metabolites

In general, urine samples were diluted with water prior to HPLC analysis. Pooled extracts and protease solubilizates of combined faeces were either injected directly, concentrated to dryness and re-dissolved in a mixture of acetonitrile, water and Triton X-100 or diluted with water or a mixture of acetonitrile, water and Triton X-100 prior to HPLC analyses (only for faeces extracts).

- *Urine*

Pooled urine samples (interval 0-48 hours) from rats were analyzed by radio-HPLC, in order to investigate the metabolic pattern and quantities. Therefore, appropriate urine aliquots were analyzed using HPLC method LC01 for quantitative evaluation and LC02 or LC05 for confirmatory analysis after dilution with water.

For identification of metabolites, an aliquot of urine sampled from rat 3 within 48 hours after treatment was subjected to HPLC-MS/MS without prior sample preparation. Peak assignment was conducted by comparison of the metabolic profile and the retention times obtained by radio-HPLC and HPLC-MS/MS analyses.

- *Faeces*

Pooled acetonitrile, water and acetonitrile/acetone extracts of combined faeces samples as well as protease solubilizates, containing sufficient amounts of radioactive residues, were analyzed by radio-HPLC, in order to investigate the metabolic pattern and quantities.
For identification of metabolites, an aliquot of the pooled acetonitrile extract of combined faeces sampled 24-48 hours after treatment was subjected to HPLC-MS/MS after concentration to dryness and dissolving in a mixture of water and acetonitrile (1/1, v/v). Additionally, aliquots of the pooled acetonitrile/acetone extracts of combined faeces sampled 0-24 hours after treatment were fractionated. In total seven fractions were separated and filled up to a defined volume with acetonitrile. Aliquots of fraction 3, comprising peaks at 26.1 min and 26.8 min, and fraction 5, comprising peaks at 33.8 min and 34.9 min, were concentrated to dryness, dissolved in a mixture of water and acetonitrile (1/1, v/v) supported by ultra-sonication and subjected to HPLC-MS/MS analysis.
The HPLC method used for metabolite identification was identical to HPLC method LC01. Peak assignment was conducted by comparison of the metabolic profile and the retention times obtained by radio-HPLC and HPLC-MS/MS analyses.

II. RESULTS AND DISCUSSION

1. Excretion of Radioactive Residues of 500M106

The portions of radioactive residues, which were excreted via urine or faeces after application of a single oral dose of 10 mg/kg bw 500M106 to male rats are summarized in Table 5.1.1-14.
In general, the excretion was completed within the observation period of 168 hours, ranging from for 101.83 to 105.82% of the dose. Thereby, the main excretion route was via faeces and detected portions of radioactive residues ranged from 84.61 to 89.89% of the dose within 168 hours, where the major fraction was excreted within 24 hours (approximately 61-71% of the dose) and being completed within 48 h (approximately 82-85 % of the dose within 0-48 h). The excretion via urine ranged from 15.93 to 17.84% of the dose within 0-168 hours and the main portion was excreted within 48 h (approximately 14 to 17% of the dose).

Table 5.1.1-14: Excretion of Radioactive Residues via Urine and Faeces after a Single Oral Administration of 10 mg/kg bw 500M106 to Male Rats

Matrix	Time interval [h]	Animals			
		Rat 1	Rat 2	Rat 3	Rat 4
		% of the dose			
Urine	0-6	3.61	2.77	1.64	3.15
	6-12	3.32	4.22	2.99	3.87
	12-24	6.53	6.76	6.88	6.96
	24-48	2.55	2.23	2.77	2.90
	48-72	0.57	0.38	0.91	0.44
	72-96	0.32	0.19	0.37	0.22
	96-120	0.16	0.15	0.19	0.13
	120-144	0.09	0.09	0.09	0.10
	144-168	0.07	0.07	0.08	0.07
Total urine	***0-168***	***17.22***	***16.87***	***15.93***	***17.84***
Faeces	0-24	60.76	70.83	68.19	61.12
	24-48	20.78	14.19	15.16	22.30
	48-72	2.43	0.99	3.71	1.54
	72-96	0.35	0.20	2.37	0.28
	96-120	0.13	0.10	0.35	0.12
	120-144	0.12	0.07	0.08	0.08
	144-168	0.04	0.05	0.04	0.07
Total faeces	***0-168***	***84.61***	***86.44***	***89.89***	***85.51***
Total	**0-168**	**101.83**	**103.31**	**105.82**	**103.35**

2. Extractability and Solubilization of Radioactive Residues from Faeces

For the determination of the extractability of the radioactive residues in faeces, the combined samples were extracted three times with acetonitrile and twice with water. The residue after acetonitrile and water extraction was further extracted three times with a mixture of acetonitrile and acetone. The results are summarised in Table 5.1.1-15.
The ERR from faeces was moderate ranging from 52.0 to 74.2% TRR, where most of the radioactive residues were extracted with acetonitrile ranging from 42.0 to 69.2% TRR, and only low amounts were subsequently extracted with water (up to 12.7% TRR) and with the mixture of acetonitrile and acetone (up to 2.6% TRR). Due to the high residue levels (above or equal to 21.7% TRR), the RRR after solvent extraction was subjected to sequential solubilization procedure (Table 5.1.1-16). Portions of radioactive residues were released by protease incubation (up to 4.2% TRR), pepsin incubation (up to 1.1% TRR) and pancreatin incubation (up to 3.7% TRR). In total, up to 8% TRR was released by enzyme incubations. In addition, alkaline treatment (at 100°C) was applied solubilizing portions for up to 26.5% TRR leading to final radioactive residues being below or equal to 8.9% TRR.

Table 5.1.1-15: Extractability of Combined Faeces Samples Obtained from Male Rats 1-4 after a Single Oral Dose of 10 mg/kg bw 500M106

Time Interval [h]	TRR calculated[1] [mg/kg] [%TRR] [% Dose]	Combined Acetonitrile Extract [mg/kg] [%TRR] [% Dose]	Combined Water Extract [mg/kg] [%TRR] [% Dose]	Sum of Combined Extracts [mg/kg] [%TRR] [% Dose]	Residue after Acetonitrile and Water Extraction [mg/kg] [%TRR] [% Dose]	Acetonitrile / Acetone [mg/kg] [%TRR] [% Dose]	ERR[2] [mg/kg] [%TRR] [% Dose]	RRR[3] [mg/kg] [%TRR] [% Dose]
0-24	321.718 100.0 65.22	222.636 69.2 45.13	8.367 2.6 1.70	231.003 71.8 46.83	90.715 28.2 18.39	7.712 2.4 1.56	238.715 74.2 48.39	69.832 21.7 14.16
24-48	90.343 100.0 18.11	48.695 53.9 9.76	3.486 3.9 0.70	52.181 57.8 10.46	38.162 42.2 7.65	2.383 2.6 0.48	54.564 60.4 10.94	29.977 33.2 6.01
48-72	9.163 100.0 0.80	4.113 44.9 0.97	0.475 5.2 0.11	4.589 50.1 1.09	4.575 49.9 1.08	0.181 2.0 0.04	4.769 52.0 1.13	4.015 43.8 0.95
72-96	0.507 100.0 0.18	1.041 45.6 0.36	0.149 6.5 0.05	1.189 52.1 0.42	1.093 47.9 0.38	0.047 2.1 0.02	1.236 54.2 0.43	0.943 41.3 0.33
96-120	0.265 100.0 0.09	0.229 45.2 0.08	0.044 8.8 0.02	0.273 53.9 0.10	0.234 46.1 0.08	0.010 1.9 <0.01	0.283 55.8 0.10	0.200 39.5 0.07
120-144	0.265 100.0 0.09	0.123 46.5 0.04	0.029 10.8 0.01	0.152 57.3 0.05	0.113 42.7 0.04	0.005 1.9 <0.01	0.157 59.2 0.05	0.101 38.2 0.03
144-168	0.146 100.0 0.07	0.061 42.0 0.03	0.019 12.7 0.01	0.080 54.7 0.04	0.066 45.3 0.03	0.003 2.4 <0.01	0.083 57.1 0.04	0.057 39.0 0.03

[1] Total radioactive residue (TRR) was calculated as the sum of the combined acetonitrile extract, combined water extract and the residue after acetonitrile and water extraction.
[2] Extractable Radioactive Residue (ERR) was calculated as sum of the combined acetonitrile extract, combined water extract and combined acetonitrile / acetone extract.
[3] RRR = Residual radioactive Residue after solvent extraction with acetonitrile, water and acetonitrile / acetone extraction.

Table 5.1.1-16: Investigation of the Radioactive Residues after Solvent Extraction of Combined Faeces Samples Obtained from Male Rats after a Single Oral Dose of 10 mg/kg bw 500M106

Fraction	0-24 h [mg/kg] [%TRR] [% Dose]	24-48 h [mg/kg] [%TRR] [% Dose]	48-72 h [mg/kg] [%TRR] [% Dose]	72-96 h [mg/kg] [%TRR] [% Dose]	96-120 h [mg/kg] [%TRR] [% Dose]	120-144 h [mg/kg] [%TRR] [% Dose]	144-168 h [mg/kg] [%TRR] [% Dose]
Residue after Solvent Extraction	*69.832* *21.7* *14.16*	*29.977* *33.2* *6.01*	*4.015* *43.8* *0.95*	*0.943* *41.3* *0.33*	*0.200* *39.5* *0.07*	*0.101* *38.2* *0.03*	*0.057* *39.0* *0.03*
Protease Solubilizate	5.613 1.7 1.14	3.264 3.6 0.65	0.278 3.0 0.07	0.076 3.3 0.03	0.019 3.7 0.01	0.009 3.4 <0.01	0.006 4.2 <0.01
Pepsin Solubilizate	1.492 0.5 0.30	0.871 1.0 0.17	0.103 1.1 0.02	0.021 0.9 0.01	0.004 0.9 <0.01	0.002 0.9 <0.01	0.001 1.0 <0.01
Pancreatin Solubilizate	2.669 0.8 0.54	1.355 1.5 0.27	0.343 3.7 0.08	0.035 1.5 0.01	0.008 1.6 <0.01	0.004 1.5 <0.01	0.003 1.9 <0.01
Sum of Solubilized Radioactive Residues	**9.774** **3.0** **1.98**	**5.490** **6.1** **1.10**	**0.724** **7.9** **0.17**	**0.132** **5.8** **0.05**	**0.032** **6.3** **0.01**	**0.015** **5.8** **0.01**	**0.010** **7.1** **<0.01**
Residue after Enzyme Solubilization	*59.416* *18.5* *12.05*	*24.836* *27.5* *4.98*	*3.361* *36.7* *0.80*	*0.810* *35.5* *0.28*	*0.170* *33.5* *0.06*	*0.082* *30.9* *0.03*	*0.048* *32.7* *0.02*
NaOH Solubilizate	41.055 12.8 8.32	17.983 19.9 3.60	2.334 25.5 0.55	0.606 26.5 0.21	0.122 24.1 0.04	0.058 21.9 0.02	0.037 25.7 0.02
Final Residue	13.019 4.0 2.64	4.739 5.2 0.95	0.819 8.9 0.19	0.174 7.6 0.06	0.043 8.6 0.02	0.022 8.2 0.01	0.011 7.8 0.01
Sum of Solubilized Radioactive Residues + NaOH Solubilizate + Final Residue	**63.847** **19.8** **12.94**	**28.211** **31.2** **5.66**	**3.877** **42.3** **0.92**	**0.912** **40.0** **0.32**	**0.197** **38.9** **0.07**	**0.095** **35.8** **0.03**	**0.059** **40.6** **0.03**

Values in italics were not considered for the calculation of "totals".

3. Identification, Characterisation and Quantification of Residues

3.1. Identification, Characterization and Quantification of Metabolites in Urine

Structure elucidation of metabolites in urine was based on HPLC-MS/MS analysis of the pooled urine sampled from rat 3 within 48 hours. The following metabolites were identified (order by retention time of the HPLC-MS/MS analysis):

- 500M109 (isomers) - 12.4 min 13.1 min and 15.9 min
- 500M03 - 13.9 min
- 500M05 -15.9 min
- 500M04 - 18.4 min
- 500M21 - 20.6 min

Metabolites 500M05, 500M109 and two not identified compounds were not separated by radio-HPLC and co-eluted in one peak at 20.5 min (15.9 min, RT of HPLC-MS/MS analysis).

The two unidentified compounds are most likely adducts of 500M05 and urine matrix. Further, metabolites 500M109 and 500M03 co-eluted in one peak during the analysis of the pooled and combined urine sample. The results of the quantitative analysis of urine are summarised in Table 5.1.1-17 and Table 5.1.1-18.
For all rats, 500M109 and 500M04 were identified as the main components in urine sampled within 48 hours ranging from 2.95 to 5.16% and 3.26 to 6.10% of the dose, respectively. All other metabolites were present at lower quantities ranging from 0.36-2.63% of the dose (0-48 hours). In pooled and combined urine sampled within 48-168 hours, metabolites 500M109/500M03, 500M05/500M109, 500M04 and 500M21 were present at similar low levels ranging from 0.12 to 0.49% of the dose. The administered compound 500M106 was not detected in urine sampled within 168 hours.
The excreted amount via urine within 168 hours ranged from 15.93 (rat 3) to 17.84% of the dose (rat 4), where 10.77 (rat 3) to 14.93% of the dose (rat 4) were identified in urine sampled within 48 hours. Additional 1.95 (rat 4) to 4.46% of the dose (rat 2) were characterized by HPLC, whereby each peak (up to 10 further peaks per sample) was below or equal to 0.83% of the dose. Furthermore, 1.16% of the dose recovered in urine sampled within 48-168 hours, were identified by HPLC.

Table 5.1.1-17: Summary of Radioactive Residues in Urine Obtained from Rat 1-4 (0-168 h)

Metabolite Identity	Composition of Radioactive Residues [% Dose]				
Animal No.	Rat 1	Rat 2	Rat 3	Rat 4	Rat 1-4
Time Interval	0-48 h	0-48 h	0-48 h	0-48 h	48-138 h
Identified					
500M109[1]	5.16	3.49	3.66	2.95	-
500M109 (RT 16.0-16.2)	0.90	0.90	1.00	1.69	-
500M109 (RT 17.6-18.4)	4.26	2.59	2.66	1.26	-
500M109/500M03	-	-	-	-	0.32
500M03	0.38	2.20	2.44	2.63	-
500M05/500M109	0.92	1.65	0.71	2.60	0.23
500M04	6.10	3.26	3.60	5.55	0.49
500M21	1.08	0.92	0.36	1.19	0.12
Total identified peaks	**13.65**	**11.52**	**10.77**	**14.93**	**1.16**
Characterized by HPLC					
Number of further HPLC peaks	6	10	7	4	-
% Dose of maximum peak	0.78	0.83	0.78	0.75	-
Total characterized	**2.36**	**4.46**	**3.51**	**1.95**	**-**
Total identified and characterized	**16.01**	**15.98**	**14.28**	**16.88**	**1.16**

[1] For metabolite 500M109, no unambiguous structure is assigned, but given as a generic structure. Several isomers of 500M109 elute in three different peaks (RT 16.0-16.2 min, RT 17.6-18.4 min and RT 20.2-20.5 min), and is even co-eluting with metabolite 500M05 and two matrix adducts of metabolite 500M05 (RT 20.2-20.5 min), where the metabolite ratio could not be determined. Hence, the sum of peaks RT 16.0-16.2 min and RT 17.6-18.4 min is given additionally. Values in italics were not considered for the calculation of "totals".

Table 5.1.1-18: Identified Metabolites and Components in Urine and Faeces Sampled within 168 hours from Male Rats after Single Oral Treatment with 10 mg/kg bw ^{14}C-500M106

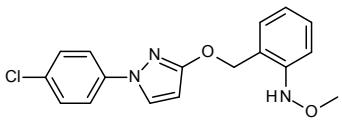
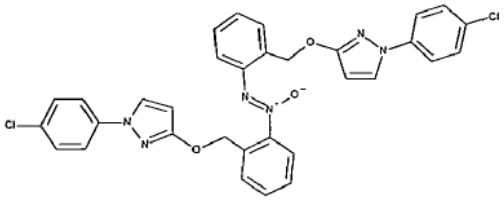
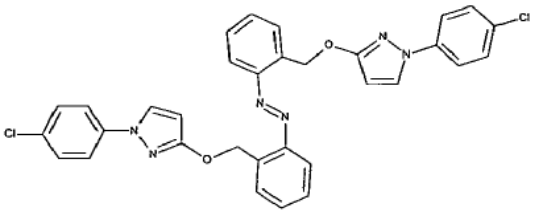
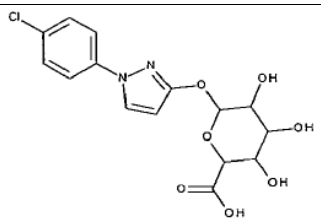
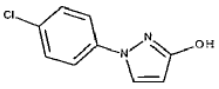
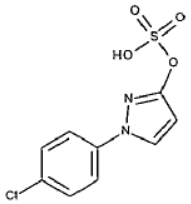
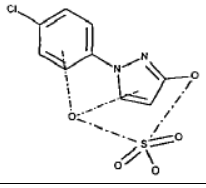
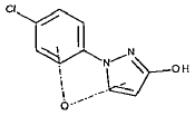
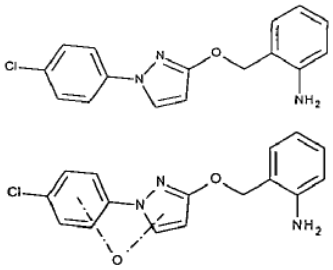
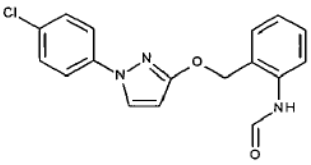
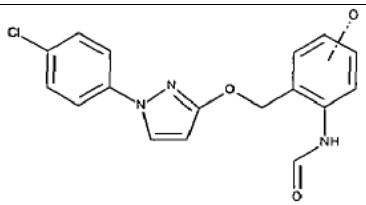
Metabolite	Structure	Urine[1]	Faeces
		Composition of Radioactive Residues [% Dose]	
Test item 500M106		not detected	not detected
500M01		not detected	8.02
500M01 / 500M02	See above and below, respectively	not detected	1.63
500M02		not detected	11.47
500M03		1.91	not detected
500M04		5.11	6.18
500M05 / 500M109	 for structure of 500M109 see below	1.70	not detected
500M109)[2]		3.82	not detected
500M109 / 500M03	see above	0.32	not detected

Table 5.1.1-18: Identified Metabolites and Components in Urine and Faeces Sampled within 168 hours from Male Rats after Single Oral Treatment with 10 mg/kg bw ^{14}C-500M106

Metabolite	Structure	Urine[1]	Faeces
		Composition of Radioactive Residues [% Dose]	
500M21		1.01	not detected
500M73 / 500M112[3]		not detected	31.90
500M105		not detected	0.39
500M117		not detected	0.33

[1] The sum of the mean values of 4 animals (0-48 h) plus the concentration determined in the pooled urine combined from 4 animals (48-168 h) is given.

[2] For metabolite 500M109, no unambiguous structure is assigned, but given as a generic structure. Several isomers of 500M109 elute in three different peaks (RT 16.0-16.2 min, RT 17.6-18.4 min and RT 20.2-20.5 min), and is even co-eluting with metabolite 500M05 and two matrix adducts of metabolite 500M05 (RT 20.2-20.5 min), where the metabolite ratio could not be determined. Hence, the sum of peaks RT 16.0-16.2 min and RT 17.6-18.4 min is given additionally.

[3] According to HPLC-MS/MS analysis, metabolite 500M073 accounted for the main portion of radioactive residues in the peak, where metabolites 500M073 and 500M112 co-eluted.

3.2. Identification, Characterization and Quantification of Metabolites in Faeces

Structure elucidation of metabolites in faeces was based on HPLC-MS/MS analysis of the pooled acetonitrile extract of combined faeces (rat 1-4) sampled within 24-48 hours and two fractions obtained from the pooled acetonitrile /acetone extract of combined faeces (rat 1-4) sampled within 0-24 hours. The following metabolites were identified (order by retention time of the HPLC-MS/MS analysis):

- 500M04 - 19.2 min
- 500M117 - 24.1 min
- 500M105 - 30.8 min
- 500M112 - 31.0 min
- 500M73 - 31.6 min
- 500M01 - 38.5 min
- Isomer of 500M01 - 37.5 min
- 500M02 - 40.0 min
- Isomer of 500M02 - 37.5 min

Metabolites 500M73 and 500M112 were not readily separated by radio-HPLC and co-eluted in one peak at 35.0 min. According to HPLC-MS/MS analysis, metabolite 500M73 accounted for the main portion of radioactive residues in the peak. In one quantitative analysis (acetonitrile extract of combined faeces 0-24 h) isomers of metabolite 500M01 and 500M02 were detected, which were not separated by radio-HPLC and co-eluted in one peak at 41.4 min.
Extracts and solubilizates of combined faeces samples, containing sufficient amounts of radioactive residues, were analyzed by HPLC. The results of the investigation of faeces extracts and solubilizates are summarised in Table 5.1.1-19 to Table 5.1.1-22.
The peak comprising metabolites 500M73/500M112 was the main peak for the time interval 0-24 hours (27.06% of the dose) and was approximately 4-fold higher than the next abundant metabolites 500M01 and 500M02 accounting for 6.31% and 7.64% of the dose, respectively. Further identified metabolites in the pooled acetonitrile extracts were metabolite 500M04 (2.50% of the dose) and isomers of 500M01/500M02. After 24 hours the excreted concentration of all identified metabolites decreased and ranged from 1.13-3.77% of the dose within 24-48 hours and from 0.08-0.24% of the dose within 48-72 hours. Metabolite 500M04 was detected at trace amounts (up to 0.07% of the dose per sampling interval or 0.16% of the dose in total) within 72-168 h. Further, metabolites 500M73/500M112 and 500M02 were detected at low levels within 72-120 h (0.01-0.06% of the dose and 0.01-0.10% of the dose, respectively). The administered test item 500M106 was not detected at any sampled time interval.
Residues in the pooled acetonitrile / acetone extracts of combined faeces (time intervals 0-24 h, 24-48 h and 48-72 h) were quantified by HPLC.
Metabolites 500M04, 500M117, 500M105, 500M73/500M112 and 500M02 were detected at low levels (up to 0.72% of the dose) in all investigated samples. The administered test item 500M106 was not detected at any sampled time interval.

Residues in the protease solubilizates from the RRR of combined faeces (time intervals 0-24 h, 24-48 h and 48-72 h) were also quantified by HPLC. The metabolite 500M04 was determined in all investigated samples with the highest concentration (1.08% of the dose) for the time interval 0-24 h. In addition, metabolite 500M117 was detected at trace amounts (up to 0.05% of the dose) in samples of the time intervals 0-24 h and 24-48 h. The administered test item 500M106 was not detected at any sampled time interval.
Taken together, all identified metabolites in combined faeces reached their highest concentration within the time interval 0-24 hours and decreased continuously afterwards (in concordance with the TRR). Furthermore, the applied test item 500M106 was not detected in any sample, indicating a rapid and sufficient biotransformation.
Metabolites 500M73/500M112 accounted for the main portion of radioactive residues (27.81% of the dose) in combined faeces sampled within 0-24 h. Metabolites 500M01 and 500M02 were present at levels of 6.31 and 7.79% of the dose, respectively, whereby additional 1.63% of the dose were detected representing isomers of metabolites 500M01 and 500M02. Metabolite 500M04 was quantified at considerable levels in the extracts as well as in the investigated protease solubilizate, accounting in sum for 4.33% of the dose.
Further identified metabolites M500117 and M500105 were present at low levels accounting for 0.19 and 0.27% of the dose in combined faeces 0-24 h.
In combined faeces 24-48 h, metabolites 500M073/500M112 and 500M02 were the main components (3.83 and 3.33% of the dose, respectively). Furthermore, metabolites 500M04 and 500M01 were present at considerable levels (1.55 and 1.62% of the dose, respectively) and metabolites 500M117 and 500M105 at low levels (0.13 and 0.11% of the dose, respectively).
Within 48-72 hours all identified metabolites were present only at low levels ranging from 0.1-0.2% of the dose, except for metabolites 500M117 and 500M105 being identified at trace levels (both 0.01% of the dose).
Metabolite 500M04 was further detected in all samples until the end of the experiment (72-168 h: in sum 0.16% of the dose) and metabolites 500M73/500M112 and 500M02 were quantified at low concentration in faeces sampled within 72-120 h (0.07 and 0.10% of the dose, respectively).
Out of 81.05% of the dose recovered in faeces sampled within 0-168 hours, 59.91% of the dose were identified in extracts and solubilizates (Table 5.1.1-22). Moreover, 2.11% of the dose were characterized by HPLC, whereby each peak was below or equal to 0.26% of the dose. Assuming the case that the maximum not identified peak of each investigated sample represents the same component, these components would not exceed in sum 0.6% of the dose in the investigation period of 0-168 h. In addition, 0.92% of the dose were characterized by their extraction behaviour. The sum of radioactive residues after solvent extraction for all time intervals accounted for 21.58% of the dose. The RRR was further investigated by applying different enzyme incubations and alkaline treatment. In sum, additional 14.58% of the dose were characterized by their solubilization behaviour. The main portion of radioactive residues was released by exhaustive hot alkaline treatment (up to 8.32% of the dose / time interval). The final residue accounted for 3.87% of the dose (in sum; 0-168 h). The highest final residue was observed within the first 24 hours accounting for 2.64% of the dose.

Table 5.1.1-19: Summary of Radioactive Residues in Combined Faeces Samples (Rat 1-4, 0-48h)

Metabolite Identity (Time interval)	Composition of Radioactive Residues in Combined Faeces [% Dose]							
	Aceto-nitrile Extract (0-24 h)	Water Extract (0-24 h)	Aceto-nitrile / Acetone Extract (0-24 h)	Sum (0-24 h)	Aceto-nitrile Extract (24-48 h)	Aceto-nitrile / Acetone Extract (24-48 h)	Sum (24-48 h)	Sum (0-48 h)
Identified from ERR								
500M04	2.50	0.47	0.28	3.25	1.13	0.10	1.23	4.47
500M117	n.d.	n.d.	0.14	0.14	n.d.	0.10	0.10	0.23
500M105	n.d.	n.d.	0.27	0.27	n.d.	0.11	0.11	0.38
500M73/500M112[1]	27.06	0.04	0.72	27.81	3.77	0.06	3.83	31.64
Isomers of 500M01/ 500M02	1.63	n.d.	n.d.	1.63	n.d.	n.d.	n.d.	1.63
500M01	6.31	n.d.	n.d.	6.31	1.62	n.d.	1.62	7.93
500M02	7.64	n.d.	0.16	7.79	3.24	0.09	3.33	11.12
Total Identified from ERR	**45.13**	**0.50**	**1.56**	**47.20**	**9.76**	**0.44**	**10.20**	**57.41**
Characterized from ERR by HPLC								
Number of further HPLC peaks	*0*	*17*	*0*	-	*0*	*2*	-	-
% Dose of maximum peak	-	*0.26*	-	-	-	*0.02*	-	-
Sum of Characterized HPLC Peaks	-	**1.19**	-	**1.19**	-	**0.03**	**0.03**	**1.23**
Water extract	**see above**				**0.70**			**0.70**
Identified and/or Characterized from ERR	**45.13**	**1.70**	**1.56**	**48.39**	**9.76**	**0.48**	**10.94[2]**	**59.33**
Residual Radioactive Residue (RRR)	14.16				6.01			20.17
Characterized and Identified from RRR								
Metabolite Identity (Time Interval)	Protease Solubilizate (0-24 h)				Protease Solubilizate (24-48 h)			Sum (0-48 h)
Identified from RRR[1]								
500M04	1.08				0.32			1.41
500M117	0.05				0.03			0.08
Total identified	**1.14**				**0.35**			**1.49**
Characterized from RRR by HPLC								
Number of further HPLC peaks	*0*				*12*			-
% Dose of maximum peak	-				*0.05*			-
Sum of Characterized HPLC Peaks	-				**0.30**			**0.30**

Table 5.1.1-19: Summary of Radioactive Residues in Combined Faeces Samples (Rat 1-4, 0-48h)

Metabolite Identity	Composition of Radioactive Residues in Combined Faeces [% Dose]							
	Aceto-nitrile Extract	Water Extract	Aceto-nitrile / Acetone Extract	Sum	Aceto-nitrile Extract	Aceto-nitrile / Acetone Extract	Sum	Sum
(Time interval)	**(0-24 h)**	**(0-24 h)**	**(0-24 h)**	**(0-24 h)**	**(24-48 h)**	**(24-48 h)**	**(24-48 h)**	**(0-48 h)**
Characterized from RRR by Solubilization								
Pepsin Solubilizate		0.30				0.17		0.48
Pancreatin Solubilizate		0.54				0.27		0.81
NaOH Solubilizate		8.32				3.60		11.93
Sum of Characterized from RRR		**9.17**				**4.36**		**13.52**
Identified and/or Characterized from RRR		**10.30**				**4.71**		**15.01**
Final Residue		2.64				0.95		3.59
Total Identified and/or Characterized from ERR and RRR and Final Residue		**61.34**				**16.59**		**77.93**

1 According to HPLC-MS/MS analysis of the acetonitrile extract of combined faeces sampled within 24-48 h, metabolite 500M73 accounted for the main portion of radioactive residues in the peak.
2 Sum including water extract (24-48 h)
n.d. not detected, *Values given in italics were not considered for calculations of "totals".*

Table 5.1.1-20: Summary of Radioactive Residues in Combined Faeces Samples (Rat 1-4, 48-72 h)

Metabolite Identity **(Time interval)**	Composition of Radioactive Residues in Combined Faeces [% Dose] Acetonitrile Extract **(48-72 h)**	Acetonitrile / Acetone Extract **(48-72 h)**	**Sum (48-72 h)**
Identified from ERR			
500M04	0.10	0.01	0.11
500M117	n.d.	0.01	0.01
500M105	n.d.	0.01	0.01
500M073/500M112[1]	0.19	0.01	0.19
500M01	0.08	n.d.	0.08
500M02	0.24	<0.01	0.25
Total Identified	**0.62**	**0.04**	**0.66**
Characterized from ERR by HPLC			
Number of further HPLC peaks	*5*	*1*	-
% Dose of maximum peak	*0.11*	*<0.01*	-
Sum of Characterized HPLC Peaks	**0.36**	**<0.01**	**0.36**
Water extract	**0.11**		
Identified and/or Characterized from ERR	**0.97**	**0.04**	**1.13[2]**
Residual Radioactive Residue (RRR)	0.95		
Characterized and Identified from RRR			
Metabolite Identity **(Time Interval)**	Protease Solubilizate **(48-72 h)**		
Identified from RRR[1]			
500M04	0.03		
Total identified	**0.03**		
Characterized from RRR by HPLC			
Number of further HPLC peaks	*4*		
% Dose of maximum peak	*0.02*		
Sum of Characterized HPLC Peaks	**0.04**		
Characterized from RRR by Solubilization			
Pepsin Solubilizate	0.02		
Pancreatin Solubilizate	0.08		
NaOH Solubilizate	0.55		
Sum of Characterized from RRR	**0.70**		
Identified and/or Characterized from RRR	**0.76**		
Final Residue	0.19		
Total Identified and/or Characterized from ERR and RRR and Final Residue	**2.09**		

1 According to HPLC-MS/MS analysis, metabolite 500M073 accounted for the main portion of radioactive residues in the peak, where metabolites 500M073 and 500M112 co-eluted.
2 Sum including water extract
n.d. not detected, *Values given in italics were not considered for calculations of "totals"*

Table 5.1.1-21: Summary of Radioactive Residues in Combined Faeces Samples (Rat 1-4, 72-168 h)

Metabolite Identity (Time interval)	Composition of Radioactive Residues in Combined Faeces [% Dose] Acetonitrile Extract (72-96 h)	Acetonitrile Extract (96-120 h)	Acetonitrile Extract (120-144 h)	Acetonitrile Extract (144-168 h)	Sum (72-168 h)
Identified from ERR					
500M04	0.07	0.04	0.02	0.03	0.16
500M117	n.d.	n.d.	n.d.	n.d.	n.d.
500M105	n.d.	n.d.	n.d.	n.d.	n.d.
500M073/500M112[1]	0.06	0.01	n.d.	n.d.	0.07
500M01	n.d.	n.d.	n.d.	n.d.	n.d.
500M02	0.10	0.01	n.d.	n.d.	0.10
Total Identified	**0.23**	**0.06**	**0.02**	**0.03**	**0.34**
Characterized from ERR by HPLC					
Number of further HPLC peaks	*2*	*1*	*3*	*0*	-
% Dose of maximum peak	*0.07*	*0.02*	*0.01*	*0.00*	-
Sum of Characterized HPLC Peaks	**0.14**	**0.02**	**0.02**	**0.00**	**0.18**
Acetonitrile/Acetone Extract	**0.02**	**<0.01**	**<0.01**	**<0.01**	**0.02**
Water extract	**0.05**	**0.02**	**0.01**	**0.01**	**0.09**
Identified and/or Characterized from ERR	**0.43**	**0.10**	**0.05**	**0.04**	**0.63**
Residual Radioactive Residue (RRR)	0.33	0.07	0.03	0.03	0.46
Characterized from RRR by Solubilization					
Protease Solubilizate	0.03	0.01	<0.01	<0.01	0.04
Pepsin Solubilizate	0.01	<0.01	<0.01	<0.01	0.01
Pancreatin Solubilizate	0.01	<0.01	<0.01	<0.01	0.02
NaOH Solubilizate	0.21	0.04	0.02	0.02	0.29
Characterized from RRR	**0.26**	**0.05**	**0.02**	**0.02**	**0.36**
Final Residue (after Solubilization)	0.06	0.02	0.01	0.01	0.09
Total Identified and/or Characterized from ERR and RRR and Final Residue	**0.75**	**0.17**	**0.09**	**0.07**	**1.08**

[1] According to HPLC-MS/MS analysis, metabolite 500M073 accounted for the main portion of radioactive residues in the peak, where metabolites 500M073 and 500M112 co-eluted.
n.d. not detected

Table 5.1.1-22: Summary of Radioactive Residues in Combined Faeces Samples (Rat 1-4, 0-168 h)

Metabolite Identity (Time interval)	Composition of Radioactive Residues in Combined Faeces [% Dose] Sum (0-48 h)	Sum (48-72 h)	Sum (72-168 h)	Total Sum (0-168 h)
Identified				
500M04	4.47	0.11	0.16	4.75
500M117	0.23	0.01	n.d.	0.25
500M105	0.38	0.01	n.d.	0.39
500M73/500M105[1]	31.64	0.19	0.07	31.90
500M01/500M02	1.63	n.d.	n.d.	1.63
500M01	7.93	0.08	n.d.	8.02
500M02	11.12	0.25	0.10	11.47
Total Identified	**57.41**	**0.66**	**0.34**	**58.40**
Characterized by HPLC				
Sum of Characterized HPLC Peaks	**1.23**	**0.36**	**0.18**	**1.76[2]**
Water extract	0.70[3]	0.11	0.09	0.90
Acetonitrile/Acetone Extract	-	-	0.02	0.02
Identified and/or Characterized from ERR	**59.33**	**1.13**	**0.63**	**61.09**
Residual Radioactive Residue (RRR)	*20.17*	*0.95*	*0.46*	*21.58*
Metabolite Identity	Protease Solubilizates (0-48 h)	Protease Solubilizates (48-72 h)	Protease Solubilizates (72-168 h)	Sum (0-168 h)
Identified from RRR				
500M04	1.41	0.03	n.a.	1.43
500M117	0.08	n.d.	n.a.	0.08
Total Identified	**1.49**	**0.03**	n.a.	**1.51**
Characterized from RRR by HPLC				
Sum of Characterized HPLC Peaks	**0.30**	**0.04**	-	**0.35[2]**
Characterized from RRR by HPLC				
Protease Solubilizate	-	-	0.04	0.04
Pepsin Solubilizate	0.48	0.02	0.01	0.51
Pancreatin Solubilizate	0.81	0.08	0.02	0.91
NaOH Solubilizate	11.93	0.55	0.29	12.77
Characterized from RRR	**13.52**	**0.70**	**0.36**	**14.58**
Identified and / or Characterized from RRR	**15.01**	**0.72**	**0.36**	**16.09**
Final Residue	3.59	0.19	0.09	3.87
Total Identified and/or Characterized from ERR and RRR and Final Residue	**77.93**	**2.05**	**1.08**	**81.05**

[1] According to HPLC-MS/MS analysis, metabolite 500M073 accounted for the main portion of radioactive residues in the peak, where metabolites 500M073 and 500M112 co-eluted.

[2] Assuming the worst case that the maximum characterized peak in each investigated extract / solubilizate contains the same unidentified component, leads to a not identified compound accounting for in sum approximately 0.6% of the dose.

[3] Only the water extract from combined faeces 24-48 h is given, as the water extract from combined faeces 0-24 h was analysed by HPLC and hence being already considered in the "total identified" and "sum of characterized HPLC Peaks".

n.a. not analyzed
n.d. not detected

4. Metabolic Pathway

The proposed metabolic pathway of the test item 500M106 is illustrated in Figure 5.1.1-10 and Figure 5.1.1-11. The major transformation steps in the metabolic pathway are:

- Cleavage of the N-O bond of the phenylamine moiety followed by (a) hydroxylation, (b) dimerization with subsequent N-oxidation or (c) formation of formaldehyde adducts
- Cleavage of the ether bridge between the pyrazole and phenylamine moiety followed by conjugation (sulphation / glucuronidation) or hydroxylation and sulphation

Cleavage of N-O bond of the phenylamine moiety of the administered compound 500M106 leads to metabolite 500M73, which might be subsequently hydroxylated (500M112) or dimerized forming an azo-group linking the aniline moieties (metabolite 500M02 or an isomer). Further, N-oxidation of the recently formed azo-group ends up in metabolite 500M01 (or an isomer). Besides dimerization and hydroxylation, formation of formaldehyde adducts was observed (500M105 and 500M117). It is not clear whether 500M105 and 500M117 are “real metabolites” or formed during workup.
Furthermore, cleavage of the C-O bridge between the pyrazole and phenylamine moiety of 500M106 leads to metabolite 500M04 being subsequently conjugated with glucuronic acid (500M03) or sulphate (500M05). Further, metabolite 500M04 can be hydroxylated at any position of the chlorophenyl or pyrazole ring, which is indicated as a generic bond in the structure of 500M021, followed by sulphation and resulting in metabolite 500M109 (or an isomer).

Figure 5.1.1-10: Proposed Metabolic Pathway of 500M106 in Male Rats (Part 1)

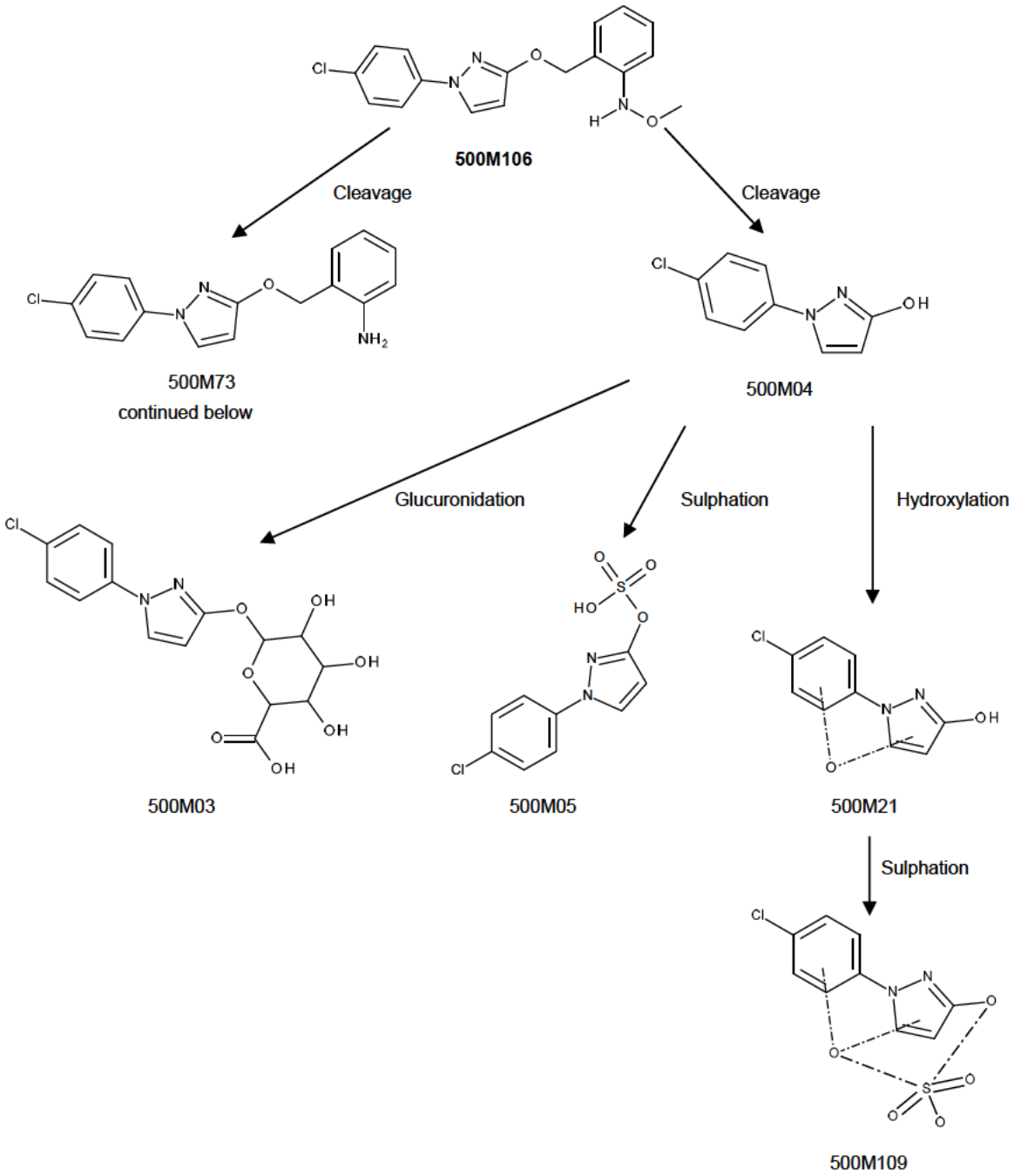
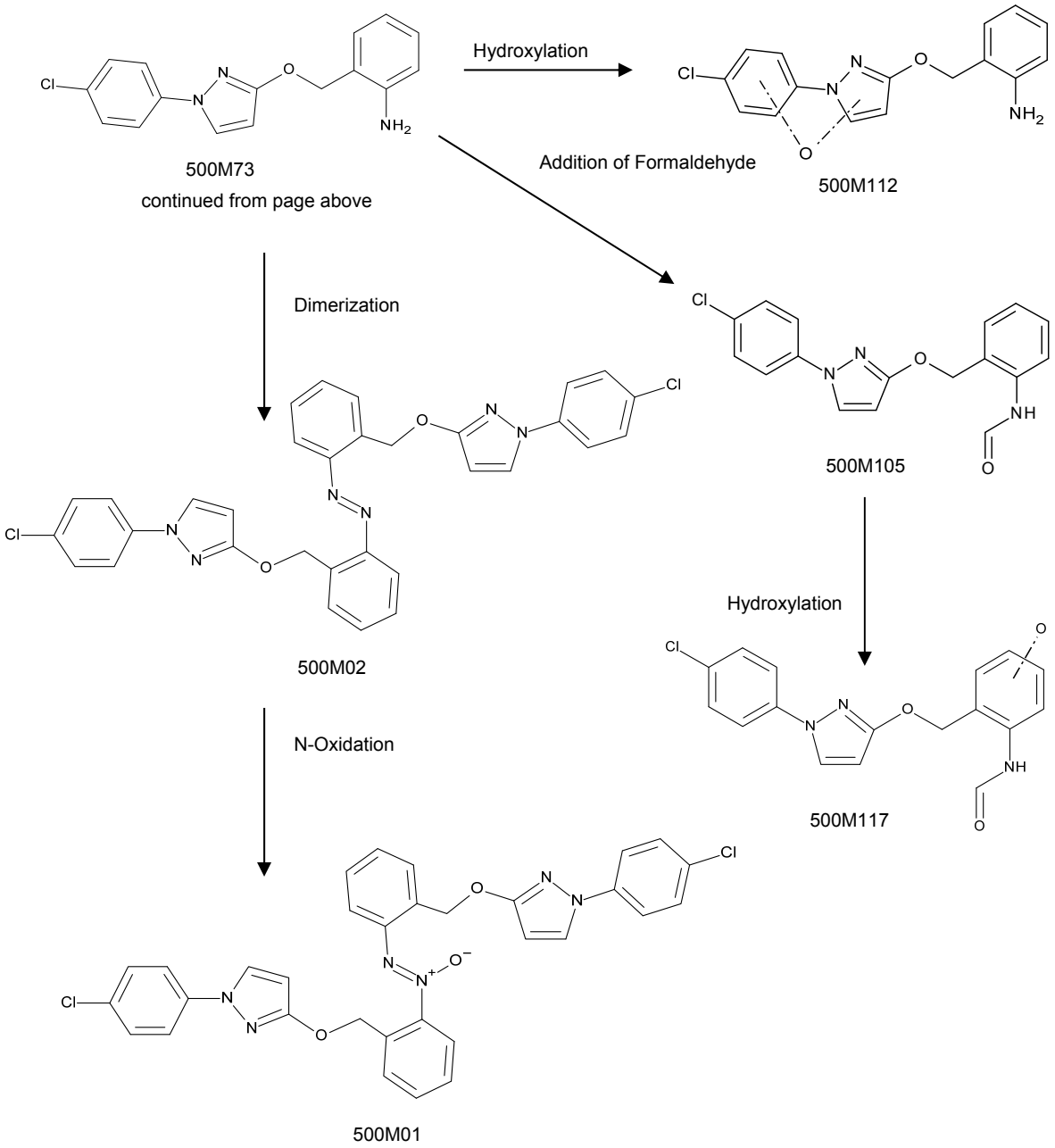


Figure 5.1.1-11: Proposed Metabolic Pathway of 500M106 in Male Rats (Part 2)



III. CONCLUSION

The metabolism of metabolite 500M106 was investigated in male rats after single oral administration of a nominal dose of 10 mg/kg bw. Metabolites were identified by HPLC-MS/MS and quantified by radio-HPLC. Urine and faeces were sampled within different time intervals up to 168 hours after treatment of male Wistar rats with 500M106 within a kinetics study.

The excretion was complete within the observation period, whereby the main excretion route was via faeces and detected portions of radioactive residues ranged from 84.61 to 89.89% of the dose within 168 hours. The excretion via urine ranged from 15.93 to 17.84% of the dose.

The combined faeces samples were extracted three times with acetonitrile and twice with water. The residue after acetonitrile and water extraction was further extracted three times with a mixture of acetonitrile and acetone.

The extractability of radioactive residues from faeces was moderate and the main portion of the radioactive residues was extracted with acetonitrile ranging from 42.0 to 69.2% TRR. Only low amounts were subsequently extracted with water and with the mixture of acetonitrile and acetone leading to radioactive residues after solvent extraction above or equal to 21.7% TRR. The residual radioactive residues after solvent extraction were subjected to a sequential solubilization procedure. Amounts up to 4.2% TRR were released by enzyme incubation (up to 8% TRR in total) and high portions were solubilized by applying hot alkaline treatment accounting for up to 26.5% TRR.

Reasonable efforts were made to identify successfully all metabolites being present for at least 1% of the administered dose.

Metabolites 500M109, 500M03, 500M05, 500M04 and 500M21 were identified in urine sampled within 48 h, whereby metabolites 500M109 and 500M04 were quantified as the main components accounting for up to 3.82 and 4.63% of the dose, respectively (average of 4 animals). Further detected metabolites were present at levels in a range from 0.89-1.91% of the dose (average of 4 animals). In pooled and combined urine sampled within 48-168 hours, metabolites 500M109/500M03, 500M05/500M109, 500M04 and 500M21 were present at similar low levels ranging from 0.12 to 0.49% of the dose. The administered compound 500M106 was not detected in urine sampled within 168 h.

Metabolites 500M04, 500M117, 500M105, 500M73/500M112, 500M01 and 500M02 as well as isomers of 500M01 and 500M02 were identified in faeces, thereby metabolites 500M73/500M112 accounted for the main fraction (in total 31.90% of the dose). Metabolites 500M01 and 500M02 were the next most abundant metabolites accounting for 8.02 and 11 .47% of the dose. Metabolite 500M04 was present at 6.18% of the dose and the remaining metabolites 500M117 and 500M105 accounted for up to 0.39% of the dose.

The applied compound 500M106 was not detected in faeces sampled within 168 h.

The major biotransformation steps in the metabolic pathway of 500M106 in male rats are:

- **Cleavage of the N-O bond of the phenylamine moiety followed by (a) hydroxylation, (b) dimerization with subsequent N-oxidation or (c) formation of formaldehyde adducts**
- **Cleavage of the ether bridge between the pyrazole and phenylamine moiety followed by conjugation (sulphation / glucuronidation) or hydroxylation and sulphation**

CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

New study

In accordance with the requirements of Commission Regulation No. 283/2013 and No. 1107/2009 and in alignment with RMS Germany a **comparative *in-vitro* metabolism study** was performed and is summarized below.

The aim of this study was

- to compare the *in-vitro* metabolism in hepatocytes (and microsomes) between animal species used for toxicological testing and humans
- to determine whether metabolic profiles are similar and
- to investigate whether a unique human metabolite occurs.

Since no guidelines exist for this type of study, the investigations were performed using a study design that has been aligned with the RMS Germany.

Report: CA 5.1.2/1
Funk D. et al., 2014a
Comparative in-vitro metabolism with 14C-BAS 500 F
2014/1001562
Guidelines: none
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** BAS 500 F (pyraclostrobin, Reg No. 304428)
Description: see table below
Batch # / purity: see table below (for structures of ^{13}C-pyrazole and ^{14}C-tolyl label: see Figure 5.1.1-3 and Figure 5.1.1-4).

Pyraclostrobin (^{14}C tolyl label)	566-5103	95%	6.8 MBq/mg
Pyraclostrobin (^{14}C chlorophenyl label)	579-6301	99.2%	6.15 MBq/mg
Pyraclostrobin (^{13}C pyrazole label)	1026-1018	99.8% (chem.)	n.a.
Pyraclostrobin	01815-65	99.9% (chem.)	n.a.

Stability of test compound: Stable during testing

2. Vehicle and/or positive control:

Hepatocytes
Positive control: Hepatocytes were incubated with ethoxy coumarin and testosterone instead of the active substance to validate the metabolic activity of the hepatocytes.
Microsomes
Positive control: Microsomes were incubated with testosterone instead of the active substance to prove the metabolic activity of the microsomes.

3. Test animals: Mammals
Species: Dog, rabbit, rat and human (all male and female)
Strain: Beagle dog (only for microsomes), rabbit (NZ), Wistar rats, human (sources: Xenotech or Gibco, Germany)

B. STUDY DESIGN AND METHODS

The study was carried out at the Agricultural Research Centre of BASF SE in Limburgerhof, Germany.

1. Dates of work: February 25, 2014 – July 11, 2014

2. Test system preparation

The radiolabelled and non-radiolabelled test materials were prepared with the following specifications. The radiolabeled test item (solution in toluene) was evaporated under nitrogen and taken up in an appropriate volume of DMSO. For the radiolabelled stock solutions, a concentration of approximately 5mg/mL was determined. For the preparation of the stock solution of the unlabelled test items, pyraclostrobin was dissolved in methanol.

For the preparation of the application solution for experiments with 10 µM pyraclostrobin, specific amounts of unlabelled and ^{13}C-labelled were combined, concentrated to dryness in a stream of nitrogen, mixed with the desired amount of radiolabelled test item and diluted with DMSO. For the preparation of the different application solutions for experiments with 1 µM, 3 µM and 5 µM pyraclostrobin, calculated amounts of ^{14}C-labelled test item were diluted with DMSO. The actual concentrations of radiolabelled pyraclostrobin in the application solutions were determined by LSC of aliquots of diluted subsamples. The purity of each application solution was confirmed by HPLC analysis.

Hepatocytes
Cryopreserved hepatocytes from dog, rabbit, rat and human were stored in liquid nitrogen. On each incubation day, the cells were thawed according to a protocol provided by the supplier using appropriate kits. Aliquots of the resulting cell suspensions in hepatocyte incubation medium were diluted with phosphate-buffered saline (PBS), and the number of viable cells was measured using an automated cell counter. The cell suspensions were then adjusted to the desired cell density of 2 x 10^6 viable cells per mL with incubation medium. In the case of dog, rabbit and rat hepatocytes, male and female cells were combined in this final step in a ratio of 1:1. The human hepatocytes were purchased as a mixture of male and female cells.

Microsomes
All preparations contained 20 mg microsomal protein per mL. For the tests, the liver microsomes of male and female animals of one species were pooled in a ratio of 1:1, relative to the protein content. The human liver microsomes were already pooled by the supplier.

1. *In-vitro* assays
The application media were incubated at a final concentration of approximately 3 and 10 µM with hepatocytes and 10 µM with microsomes.

Hepatocytes
As the previously conducted *in-vivo* rat metabolism study with pyraclostrobin showed extensive phase II-metabolism, hepatocytes were chosen as main test system for the present study. On each incubation day, the application solutions in DMSO (mix of ^{14}C-labelled, ^{13}C-labelled and unlabelled test item) were diluted with the corresponding incubation media by a factor of 100 to prepare the respective application media for hepatocyte incubations. Each sample comprised of equal amounts of the application medium and of the hepatocyte cell suspension in one of the wells of a 24-well cell culture plate (maximum concentration of DMSO: 0.5%).

For human hepatocytes, the reactions were performed for 30 min, 60 min and 180 min at approximately 37°C. In case of rabbits and rats, experiments with shorter incubation intervals were additionally performed (10 min and 30 min). Incubation was terminated by pipetting the incubation mixture into a weighed tube containing cold ethanol (final concentration of 70%) and cell lysis was assisted by ultrasonication. In this stage, the samples were stored frozen prior to concentration.

In addition, two negative controls, two positive controls and a blank control (application medium with DMSO instead of test item) were performed for each species. Under these conditions no metabolisation should occur. For the **"stability control"**, the application medium was mixed only with incubation medium instead of cell suspension. For the **"zero incubation control"** (t = 0 min), the reaction was stopped immediately after addition of the cell suspension. The stability control was performed for all test systems, but also covers all test concentrations.
In the **positive controls**, 10 µM testosterone or ethoxycoumarin instead of the active substance was incubated with hepatocytes from the different species to prove the metabolic activity of the different hepatocytes.

Microsomes
On each incubation day, appropriate amounts of the application solutions in DMSO (mix of ^{14}C-chlorophenly labelled, ^{13}C-labelled and unlabeled test item) were directly dosed resulting in a final dilution of 1:200. Each sample (2 mL total volume) comprised 1 mg of microsomal protein, a NADPH generating system and potassium phosphate buffer (approximately 0.1 M, pH 7.4). The reactions were performed at 37 °C for 90 min in a shaking water bath and stopped by adding 4.7 mL cold ethanol and sonicating for 5 minutes. Additionally, for each species negative and positive controls were performed.

The negative controls were the "heat denatured control" and the "t=0 control". In addition, the "buffer control" was performed. Under these conditions no or only very limited metabolisation or degradation should occur. For the heat denatured control, the liver microsomes were inactivated by incubating in hot water (> 95 °C) for 10 minutes before pipetting them into the mixture. For the buffer control, the application solution was mixed only with buffer. For the "t=0" control, the enzymatic reaction was stopped immediately after adding the NADPH generating system by adding 4.7 mL of cold ethanol. In the **positive control**, the different liver microsomes were incubated with testosterone instead of the active substance to prove the metabolic activity of the different liver microsomes.

In each experimental setup, the incubation of the substrates as well as all control assays were performed in triplicate.

2. Work-up of samples
The stopped incubation mixture was centrifuged and concentrated to volumes of approx.0.5 (hepatocytes) or 2 mL (microsomes) by a centrifugal evaporator. The supernatant and the pellet were frozen separately. All samples were stored in a freezer at -18°C or below.

Analysis of the supernatants
All supernatants were analysed by reversed phase HPLC. The metabolites detected in the supernatants of human hepatocytes or human liver microsomes at relative concentrations above 5% of the applied radioactivity (5% AR) were identified by HPLC MS/MS analysis of selected samples. For all other samples, the radio HPLC peaks were assigned to the identified metabolites by comparison of retention times and metabolite patterns, as well as by HPLC MS analysis of at least one of three replicate supernatant samples. The metabolite patterns formed by incubation with the animal hepatocytes were compared to the metabolite pattern formed with human hepatocytes. In addition, the metabolite patterns arising after incubation with liver microsomes (focusing on phase I metabolism) were also investigated.

Work-up of the residual pellet
If the radioactivity measurement of the supernatants after concentration and centrifugation of the terminated incubation mixtures yielded less than 90% of the applied radioactivity, the pellets after centrifugal evaporation were resuspended in 50 µL water each and mixed with 90 µL acetone and 360 µL acetonitrile (assisted by ultrasonication). After centrifugation, the radioactive residues in the extract supernatants were determined by LSC of aliquots. The remaining second pellets were resuspended in 300 µL water, and the radioactive residues in the suspended samples were measured by LSC.

II. RESULTS AND DISCUSSION

1. Control Experiments

The blank controls performed for each species without test item showed no significant amounts of radioactivity (LSC measurements), and no radioactive peaks were detected by HPLC analysis.

The triplicates of each negative control (stability control without cells and zero incubation control) were comparable and showed nearly identical HPLC profiles, which contained only one peak corresponding to the unchanged active substance pyraclostrobin. Hence, no or very little metabolisation or degradation of pyraclostrobin occurred without influence of hepatocytes and microsomes.
The positive controls showed that the metabolic activity of the hepatocytes with respect to Phase I and Phase II metabolic reactions was sufficiently high. The portions of metabolized testosterone and ethoxycoumarin reached values above 80% of the radioactive residues recovered.
The positive controls showed that the metabolic activity of the microsomes with respect to Phase I metabolic reactions was also sufficiently high; the defined threshold values (greater than 50% metabolism) were reached in all cases.

2. Viability of the Hepatocytes

The hepatocyte suspensions were adjusted to a cell density of 2 x 10^6 viable cells per mL to achieve a final cell density of approximately 10^6 cells per mL in the incubation assays. After incubation for 180 min, the viability of the cells was determined using a luminescent cell viability assay. The viability of the human hepatocytes incubated with 10 µM pyraclostrobin (measured in Relative Luminescence Units, RLU) was 55 and 46% of the viability of the cells incubated without test item for the chlorophenyl label and the tolyl label, respectively. In order to select an appropriate second concentration of the test item with a higher viability of the cells, concentrations of 1 µM, 3 µM and 5 µM pyraclostrobin were tested with human hepatocytes. Since the viability of human hepatocytes was at or above 90% of the viability of the cells incubated without test item for both labels, a concentration of 3 µM pyraclostrobin was chosen. The viability of the animal cells incubated with 10 µM pyraclostrobin was in the range of 79 to 105% for rat and in the range of 87 to 116% for rabbit. The viability of the cells incubated with 3 µM pyraclostrobin (chlorophenyl label and tolyl label, respectively) was 91 and 94% of the viability of the cells incubated without test item for human hepatocytes, 100 and 106% for rat hepatocytes and 102 and 96% for rabbit hepatocytes.

3. Determination of radioactivity in supernatants and pellets

Hepatocytes: In most of the samples, the radioactive residues recovered in the concentrated supernatants after terminating the incubations were below 90% of the applied radioactivity. For this reason, the respective incubation vials with the residual pellets were extracted with acetone / acetonitrile. The radioactive residues in the acetone / acetonitrile extracts were below 12.3% AR in all experiments. The extracts obtained were analyzed by HPLC; if sufficiently resolved, the metabolite amounts being present were considered for final quantitation (see Table 5.1.2-1 and Table 5.1.2-2). The radioactive residues in the final pellets of all experiments with pyraclostrobin were below or equal to 7.6% AR. Therefore, no further workup was performed.

Microsomes: After incubation of pyraclostrobin with liver microsomes; the radioactive residues recovered in the concentrated supernatants were often also below 90% of the applied radioactivity. Therefore, the residual pellets were extracted with acetone / acetonitrile in the same manner as the hepatocyte samples. The extracts obtained were analyzed by HPLC; if sufficiently resolved, the metabolite amounts being present were considered for final quantitation (see Table 5.1.2-3). The radioactive residues in the acetone / acetonitrile extracts (with one exception) were below 9% AR. The radioactive residues in the final pellets of the experiments with pyraclostrobin were below 11% AR. Therefore, no further workup was performed.

4. Kinetics of biodegradation of pyraclostrobin after incubation with hepatocytes

Pyraclostrobin is present in most of the samples after incubation with human hepatocytes. The mean % AR decreased in chlorophenyl and tolyl label from 73.20 and 67.31% AR at 0 minutes to 64.57 and 55.35 % AR after 30 minutes to 40.07 and 37.50% AR after 60 minutes and to 2.55 and 3.84% AR after 180 minutes of incubation in the experiments with 10 µM pyraclostrobin. At the concentration of 3 µM pyraclostrobin, the portion of the active substance showed a faster decrease of both labels from 81.33 and 79.38% AR (chlorophenyl and tolyl label) at 0 minutes to 52.98 and 42.31% AR after 30 minutes and 16.12 and 14.48% AR after 60 minutes and after 180 minutes no pyraclostrobin was detected any more.

After incubation with rat hepatocytes, pyraclostrobin was detected in all control samples with 0 minutes incubation (between 65.52 to 77.87% AR for both labels and concentrations). In the chlorophenyl labelled samples of 10 µM pyraclostrobin with rat hepatocytes the mean % AR decreased to 36.95% AR after 10 minutes and after 30 and 180 minutes, no pyraclostrobin was detected. Similar results were obtained for the tolyl labelled samples with 10 µM pyraclostrobin with rat hepatocytes; here the mean % AR decreased from 30.64% AR after 10 minutes to 0.63% AR after 30 minutes and after 180 minutes no pyraclostrobin was detected. Samples with 3 µM pyraclostrobin were incubated for 0 and 180 minutes. After 180 minutes no pyraclostrobin was detected any more.

After incubation with rabbit hepatocytes, pyraclostrobin was detected in all control samples with 0 minutes incubation (between 63.30 to 84.88% AR for both labels and concentrations). The mean % AR decreased in chlorophenyl and tolyl label from 8.86 and 7.07% AR after 10 minutes to 2.45 and 1.94% AR after 30 minutes of incubation in the experiments with 10 µM pyraclostrobin. After 180 minutes no pyraclostrobin was detected in any label or applied concentration. Detailed results are shown in Table 5.1.2-1 and Table 5.1.2-2 for chlorophenyl and tolyl labelled samples, respectively. The order of the metabolites is in accordance with the original report.

From the results is can be concluded that the transformation of pyraclostrobin proceeded faster in animal hepatocytes, whereas rabbit hepatocytes transformed pyraclostrobin faster than rat hepatocytes.

5. Metabolites formed after incubation of pyraclostrobin with hepatocytes Chlorophenyl label

HPLC-MS analysis of samples after incubation with human hepatocytes with chlorophenyl labelled pyraclostrobin allowed identification of nine metabolites being present in amounts greater than 5% AR (500M03, 500M108, 500M04, 500M103, 500M104, 500M107, 500M73, 500M106 and 500M02) in both concentrations and one additional metabolite 500M88 at 10 µM pyraclostrobin.

Each of these ten metabolites was also present in at least one sample from the incubation with animal hepatocytes.

In rat hepatocyte samples, the metabolites 500M108, 500M04, 500M104 and 500M88 were identified. In rat samples incubated with 10 µM pyraclostrobin, the detected metabolites 500M108, 500M04 and 500M88 represent a mean portion below 5% AR and metabolite 500M104 represents a mean % AR above 5% AR. Rat samples incubated with 3 µM pyraclostrobin showed the formation of metabolite 500M104, which represent a mean portion below 5% AR. Additionally, the regioisomers of metabolite 500M108 were formed.

After incubation of rabbit hepatocytes with 10 µM pyraclostrobin each metabolite identified in human hepatocyte samples was formed and identified. Metabolites 500M03, 500M103, 500M104, 500M107 and 500M88 were detected below a mean portion of 5% AR and metabolites 500M108, 500M04, 500M73, 500M106 and 500M02 represent values below as well as above 5% AR. In rabbit samples incubated with 3 µM pyraclostrobin (chlorophenyl label), the detected metabolites 500M103, 500M104, 500M73 and 500M106 represent a mean portion below 5% AR and metabolites 500M03 and 500M108 represent a mean % AR above 5% AR.
The amounts of metabolites being identified in different test species and after designated incubation times are summarized in Table 5.1.2-1. The structural formula of the metabolites are depicted in Table 5.1-1**:**

Table 5.1.2-1: Comparison of metabolites of pyraclostrobin (chlorophenyl label) formed after incubation with human, rat and rabbit hepatocytes

Metabolite code	Human Mean % AR		Rat Mean % AR		Rabbit Mean % AR	
	10 µM	3 µM	10 µM	3 µM	10 µM	3 µM
Incubation time: 10 min						
500M108 (regioisomers)			-		2.20	
			-		2.12	
500M04			-		8.73	
500M103			-		1.65	
500M104			2.32		-	
500M88			2.85		2.71	
500M73			-		9.03	
BAS 500 F			36.95		8.86	
500M106			-		11.75	
500M02			-		11.75	
Incubation time: 30 min						
500M03	-	-	-		1.95	
500M108 (regioisomers)	-	-	-		11.72	
	-	-	3.99		6.44	
500M04	-	-	2.78		5.24	
500M103	-	-	-		3.10	
500M104	-	6.34	21.19		1.51	
500M107	-	-	-		1.99	
500M88	-	-	-		1.55	
500M73	-	4.83	-		13.86	
BAS 500 F	64.57	52.98	-		2.45	
500M106	11.38	23.69	-		4.29	
500M02	-	3.20	-		4.27	
Incubation time: 60 min						
500M108 (regioisomers)	-	7.10				
	-	-				
500M04	3.24	5.29				
500M103	-	2.86				
500M104	3.13	8.46				
500M88	1.49	-				
500M73	2.68	5.62				
BAS 500 F	40.07	16.12				
500M106	17.72	27.81				
500M02	4.39	5.26				

Metabolite code	Human Mean % AR		Rat Mean % AR		Rabbit Mean % AR	
	10 µM	3 µM	10 µM	3 µM	10 µM	3 µM
Incubation time: 180 min						
500M03	4.22	6.73	-	-	-	5.68
500M108 (regioisomers)	6.62	10.43	2.29	1.83	12.28	6.45
		8.80	-	6.34	4.16	-
500M04	4.04	5.15	-	-	2.82	-
500M103	3.30	4.50	-	-	4.32	4.56
500M104	6.89	7.54	9.08	4.81	-	3.87
500M107	2.90	7.19	-	-	-	-
500M73	4.59	3.70	-	-	2.34	2.30
BAS 500 F	2.55	-	-	-	-	-
500M106	16.01	5.37	-	-	-	3.71
500M02	11.03	6.13	-	-	-	-

- = not detected

Tolyl label

HPLC-MS analysis of samples after incubation with human hepatocytes with tolyl labelled pyraclostrobin allowed identification of eight metabolites (500M108, 500M103, 500M104, 500M107, 500M88, 500M73, 500M106 and 500M02), whereas metabolite 500M88 was only observed in incubation samples with 10 µM pyraclostrobin and metabolite 500M107 was only detected in incubation samples with 3 µM pyraclostrobin.

Each of these eight metabolites was also present in at least one sample from the incubation with animal hepatocytes.

In rat hepatocyte samples incubated with 10 µM pyraclostrobin metabolites 500M88, 500M108 and 500M103 and 500M88 represent a mean portion below 5% AR and metabolite 500M104 represents a mean % AR above as well as below 5% AR. Rat samples incubated with 3 µM tolyl labelled pyraclostrobin showed only the formation of metabolite 500M104 representing a mean portion below 5% AR.

After incubation of rabbit hepatocytes with 10 µM pyraclostrobin each metabolite identified in human hepatocyte samples was formed and identified. Metabolites 500M103, 500M104, 500M107 and 500M88 were detected below a mean portion of 5% AR and metabolites 500M108, 500M73, 500M106 and 500M02 represent values below as well as above 5% AR. In rabbit samples incubated with 3 µM pyraclostrobin (tolyl label), the mean % AR of the detected metabolites 500M103 and the regioisomers of metabolite 500M108 was above 5% AR.

The amounts of metabolites being identified in different test species and after designated incubation times are summarized in Table 5.1.2-2. The structural formula of the metabolites are depicted in Table 5.1-1**:** .

Table 5.1.2-2: Comparison of metabolites of pyraclostrobin (tolyl label) formed after incubation with human, rat and rabbit hepatocytes

Metabolite code	Human Mean % AR		Rat Mean % AR		Rabbit Mean % AR	
	10 µM	3 µM	10 µM	3 µM	10 µM	3 µM
Incubation time: 10 min						
500M108 (regioisomers)			-		2.00	
			-		2.41	
500M103			-		1.31	
500M104			2.28		-	
500M88			2.36		2.75	
500M73			-		8.12	
BAS 500 F			30.64		7.07	
500M106			-		9.50	
500M02			-		7.27	
Incubation time: 30 min						
500M108 (regioisomers)	-	-	-		9.03	
	-	-	4.19		8.12	
500M103	-	2.71	1.72		3.32	
500M104	1.43	5.16	18.74		1.62	
500M107	-	-	-		1.44	
500M88	1.74	-	-		1.79	
500M73	1.14	5.60	-		9.65	
BAS 500 F	55.35	42.31	0.63		1.94	
500M106	10.06	20.69	-		-	
500M02	1.73	4.00	-		3.10	
Incubation time: 60 min						
500M108 (regioisomers)	-	5.66				
	-	3.22				
500M103	-	4.58				
500M104	3.44	8.47				
500M88	1.87	-				
500M73	2.93	4.97				
BAS 500 F	37.50	14.48				
500M106	18.56	23.60				
500M02	5.13	4.89				
Incubation time: 180 min						
500M108 (regioisomers)	5.03	11.94	-	-	-	7.57
	-	15.80	-	-	3.07	-
500M103	3.30	6.61	-	-	-	5.05
500M104	5.76	12.87	8.64	4.37	-	-
500M107	-	8.99	-	-	-	-
500M88	1.46	-	-	-	-	-
500M73	5.19	7.15	-	-	2.30	-
BAS 500 F	3.84	-	-	-	-	-
500M106	18.10	6.63	-	-	-	-
500M02	11.91	8.48	-	-	-	-

- = not detected

In summary, the *in-vitro* comparative experiments with hepatocytes showed that the metabolites, which were identified in human hepatocyte samples, are also seen in animal hepatocyte samples.

6. Kinetics of biodegradation of pyraclostrobin after incubation with microsomes

The *in-vitro* metabolism with focus on the phase I metabolic reactions was investigated with 10 µM chlorophenyl labelled pyraclostrobin incubated with human liver microsomes and with liver microsomes from rat, rabbit and dog for 0 minutes (control) and 90 minutes. An additional control after 90 minutes was performed with inactivated liver microsomes (heat denatured control).

After incubation with human and animal liver microsomes, pyraclostrobin was detected in all heat denatured control samples at 0 and 90 min with a mean % AR above 87, except after incubation of rabbit liver microsomes for 0 minutes. Besides 29.49% AR pyraclostrobin, further substances were detected indicating that metabolism of the active substance has already started.

After 90 minutes incubation with human liver microsomes, the active substance decreased to 44.70% AR. The incubation of rat and rabbit liver microsomes delivered a higher decrease of pyraclostrobin with 15.37 and 2.99% AR, respectively. This higher conversion rate indicates a faster metabolic capacity of rat and rabbit microsomes. After the incubation of pyraclostrobin with dog liver microsomes the active substance decreased to 64.42% AR.

7. Metabolites formed after incubation of pyraclostrobin with microsomes

HPLC-MS analysis of the samples incubated with human liver microsomes allowed identification of five metabolites. Three of these metabolites represent a mean portion above 5% AR with metabolite 500M04 (14.00% AR), 500M88 (9.96% AR) and 500M02 (7.25% AR). The metabolites 500M73 (2.94 %AR) and 500M106 (4.16 % AR) are present in amounts below 5 % AR. After incubation with rat liver microsomes, the three metabolites 500M04, 500M88 and 500M73 were detected. After incubation of rabbit liver microsomes, each of the 5 metabolites was identified, which was seen in human liver microsome samples. After incubation of dog liver microsomes four metabolites were observed with metabolites 500M04, 500M88, 500M73 and 500M02. The quantitative results are summarized in Table 5.1.2-3.

In summary, the *in-vitro* comparative experiments with microsomes showed that the metabolites, which were identified in human microsome samples, are also seen in animal microsome samples.

Table 5.1.2-3: Comparison of metabolites of pyraclostrobin (chlorophenyl label) formed after incubation with human, rat, rabbit and dog microsomes

Metabolite code	Human Mean % AR	Rat Mean % AR	Rabbit Mean % AR	Dog Mean % AR
Incubation time: 90 min				
500M04	14.00	9.30	17.02	18.42
500M88	9.96	7.94	1.52	7.10
500M73	2.94	2.54	8.73	1.90
BAS 500 F	44.70	15.37	2.99	64.42
500M106	4.16	-	6.35	-
500M02	7.25	-	7.91	0.82

III. CONCLUSION

In the *in-vitro* comparison study, some differences were noted between the different test species. The metabolic degradation of pyraclostrobin is considerably faster in rat and rabbit hepatocytes compared to human cells. In deviation to human, no or very low amounts of parent were detected after incubation periods of 10 or 30 min. Metabolites as e.g. 500M04, 500M108, 500M103, 500M104 and 500M88 are common to all test species.
In human and rabbit, cleavage of the amide bond resulting in the formation of the metabolite 500M106 is major degradation pathway. Subsequently, 500M106 is further metabolized by conjugation with glucuronic acid to 500M107. In human and rabbit, the metabolite 500M02 which is formed by dimerization has been also identified.
In rat hepatocytes, hydroxylation and conjugation to 500M104 seems to be more pronounced compared to human and rabbits.

In order to investigate the impact of conjugation reactions (Phase II) on kinetics, further investigations on liver microsomes were carried out. As additional test species, dogs were included. The investigations confirm the picture obtained with hepatocytes. In dogs, trace amounts of 500M02 were found.

The study did not show any human metabolite, which was not found either in rabbit or rat.

The table below provides an overview on all investigated test systems and incubation periods including indications on the amounts of detected metabolites (+ representing a detected mean portion of < 5 % AR for all time intervals, ++ representing a mean portion above 5 % AR from at least one time interval).

Table 5.1.2-4: Occurrence of metabolites of pyraclostrobin after incubation with hepatocytes or liver microsomes

Metabolite code	Human		Rat		Rabbit		Dog
	10 µM	3 µM	10 µM	3 µM*	10 µM	3 µM*	10 µM
Hepatocytes (chlorophenyl label)							
500M03	+	++	n.d.	n.d.	n.d.	++	not applied
500M108 (regioisomers)	++	++	+	++	++	++	
500M04	+	++	+**	n.d.	++	n.d.	
500M103	+	+	n.d.	n.d.	+	+	
500M104	++	++	++	+	+**	+	
500M107	+	++	n.d.	n.d.	+**	n.d.	
500M88	+	n.d.	+**	n.d.	+**	n.d.	
500M73	+	++	n.d.	n.d.	++	+	
BAS 500 F	++	++	++**	n.d.	++**	n.d.	
500M106	++	++	n.d.	n.d.	++**	+	
500M02	++	++	n.d.	n.d.	++**	n.d.	
Hepatocytes (tolyl label)							
500M108 (regioisomers)	++	++	+**	n.d.	++**	++	not applied
500M103	+	++	+**	n.d.	+**	++	
500M104	++	++	++	+	+**	n.d.	
500M107	n.d.	++	n.d.	n.d.	+**	n.d.	
500M88	+	n.d.	+**	n.d.	+**	n.d.	
500M73	++	++	n.d.	n.d.	++	n.d.	
BAS 500 F	++	++	++**	n.d.	++**	n.d.	
500M106	++	++	n.d.	n.d.	++**	n.d.	
500M02	++	++	n.d.	n.d.	++	n.d.	
Liver microsomes (chlorophenyl label, 90 min)							
500M04	++	not applied	++	not applied	++	not applied	++
500M88	++		++		+		++
500M73	+		+		++		+
BAS 500 F	++		++		+		++
500M106	+		n.d.		++		n.d.
500M02	++		n.d.		++		+

* investigations performed at one incubation period (180 min)
** detected at short incubation periods of 10 and 30 min
n.d. = not detected

In order to get a better understanding of the findings of the *in-vitro* comparison study, *in-vitro* mechanistic investigations were performed. Goal of the experiments was to identify the enzyme involved in the formation of metabolite 500M106 in human cell cultures, but also to investigate whether this enzyme is also present in rats.

Report:	CA 5.1.2/2 Funk D., Bellwon P., 2016 a Influence of WWL229 on the *in-vitro*-metabolism of 14C-BAS 500 F with human hepatocytes and human liver cytosol 2016/1225031
Guidelines:	OECD Principles of Good Laboratory Practice
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	^{14}C-BAS 500 F (pyraclostrobin, Reg. No. 304428), see Figure 1.1
	WWL229 (inhibitor of human carboxylesterase 1), see Figure 1.2
Description:	BAS 500 F: tolyl-ring-U-C14 label WWL229: unlabeled
Chemical name:	BAS 500 F: methyl-N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)-(N-methoxy)carbamate (IUPAC-Name) WWL229: not applicable
Test substance No:	not reported
Lot/Batch #:	BAS 500 F: 566-5501; WWL229: 104M4753V
CAS#:	BAS 500 F : 175013-18-0 ; WWL229: 1338575-28-2
Development code:	Pyraclostrobin: BAS 500 F (Reg.No. 304428)
Purity:	BAS 500 F: Radiochemical purity: 99.5% (RHPLC); chemical purity: 98.3% WWL229: 98.8% (HPLC)
Specific activity:	BAS 500 F: 6.31 MBq/mg (specific activity a.i.); 30 MBq/g (specific activity) WWL229: not applicable
Stability of test compound:	The test item was stable over the test period. Stability controls (test item without hepatocytes and liver cytosol) were performed as negative controls. No degradation of pyraclostrobin, neither without nor with WWL229, was observed within 180 min under these experimental conditions without cells.

2. Vehicle and/or positive control: Human hepatocytes were incubated with 7-ethoxycoumarin and testosterone instead of the active substance to validate the metabolic activity of the hepatocytes.

Figure 1.1: Structural formula of ^{14}C-pyraclostrobin labelled at the tolyl ring

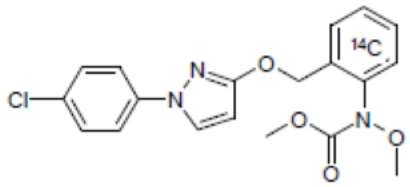
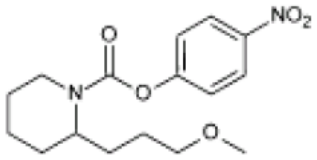


Figure 1.2: Structural formula of unlabelled WWL229



B. STUDY DESIGN AND METHODS

The study was carried out at the Agricultural Research Centre of BASF SE in Limburgerhof, Germany.

1. Dates of experimental work (analytical phase): September 17, 2015 to August 30, 2016

2. Study design

Stock solution

The stock solution of pyraclostrobin (BAS 500 F) was prepared as follows: a weighed aliquot was evaporated to dryness with nitrogen and dissolved in acetonitrile. Aliquots were diluted and analyzed by liquid scintillation counting (LSC) in order to determine the actual concentration (0.978 mg/mL).

The stock solutions of radiolabeled ^{14}C-testosterone and 7-ethoxycoumarin were also prepared within the *in-vitro* metabolism study (BASF DocID 2014/1001562), and the concentrations were determined by LSC analysis (0.591 mg/mL and 0.464 mg/mL, respectively).

WWL229 was purchased as oil. A defined weight was mixed with dimethylsulphoxide (DMSO) and application medium (hepatocytes) or application buffer (cytosol).

Application solution

Aliquots of the application solutions containing pyraclostrobin without inhibitor as well as aliquots of the application solutions containing testosterone and 7-ethoxycoumarin were obtained from the *in-vitro* metabolism study (BASF DocID 2016/1061381).

The application solutions used for incubations with hepatocytes and incubation with cytosol were prepared as follows:
The solvent from a defined volume of the stock solution was evaporated with nitrogen, and the test item was dissolved in 2 mL DMSO. For incubation with hepatocytes a nominal concentration of 3 µM pyraclostrobin was used. For incubation with liver cytosol a nominal concentration of 10 µM pyraclostrobin was intended. The specific radioactivity of the test item accounted for 378,600 dpm/µg. The actual concentration of radiolabeled pyraclostrobin in this application solution was determined by LSC of aliquots of diluted subsamples (0.226 mg/mL (3 µM) and 0.756 mg/mL (10 µM)).
For the preparation of the application solution for experiments with 10 µM testosterone a specific amount of the stock solution of the radiolabeled testosterone was evaporated with nitrogen and dissolved in DMSO (0.4 mL). The actual concentration of testosterone in the application solution was determined by LSC of aliquots of diluted subsamples (0.591 mg/mL). The specific radioactivity accounted for 402,345 dpm/µg.
For the preparation of the application solution for experiments with 10 µM 7-ethoxycoumarin a specific amount of the solid radiolabeled 7-ethoxycoumarin obtained from the supplier was dissolved in DMSO (0.325 mL). The actual concentration of 7-ethoxycoumarin in the application solution was determined by LSC of aliquots of diluted subsamples (0.464 mg/mL). The specific radioactivity accounted for 898,800 dpm/µg.
As application solution for blank experiments (negative controls), 1.0 mL DMSO was used.

Test system

Preparation of human hepatocytes

Cryopreserved human hepatocytes (pool of 50 male and 50 female donors) were purchased from XenoTech. The hepatocytes were stored in liquid nitrogen. On the incubation day, cells were thawed according to the protocol provided by the supplier using appropriate kits.

Preparation of human liver cytosol

Cryopreserved human liver cytosol was purchased from XenoTech (as a mixture from males and females). The cytosol was stored in an ultra-freezer at approximately -80°C. On the incubation day, the cytosol was thawed on ice. Aliquots of the cytosol were heat-denatured by keeping at 95°C or above for 10 minutes.

In-vitro assays

In-vitro assay for incubation with human hepatocytes

On the incubation day, the application solution was diluted with hepatocyte incubation medium to prepare the respective application medium. Human hepatocytes were incubated with application media containing 3 µM pyraclostrobin without and with 20 µM WWL229 (all final concentrations in the incubation mixture). The final concentration of DMSO was 0.5% for all experiments. Aliquots of the application media were analyzed by LSC to calculate the amounts of applied radioactivity per well and of total applied pyraclostrobin per assay (representing 100% AR in 1200 µL per well: 1.31 µg or 1.32 µg for incubation without or with WWL229, respectively). The purity of the application media for the *in-vitro* assays was determined by HPLC analyses using HPLC methods LC01 and LC02.

In the case of testosterone and 7-ethoxycoumarin, the incubations were performed at final concentrations of 10 µM each. The purity of the application medium and the retention time of testosterone were determined by HPLC analysis using HPLC methods LC01 and LC04 (optimized for testosterone), respectively. The purity of the application medium and the retention time of 7-ethoxycoumarin were determined by HPLC analysis using HPLC methods LC01 and LC03 (optimized for 7-ethoxycoumarin, radio- and UV-Vis detection), respectively. Additionally, the possible conversion products of 7-ethoxycoumarin were analyzed using HPLC method LC03 to determine the respective retention times.

The reactions were performed for 60 min and 180 min at 37°C and at 5% CO_2 in an incubator.

Two negative controls (stability and zero incubation control), two positive controls (with testosterone and 7-ethoxycoumarin) and a blank control (cells in incubation medium without test item) were performed.

In the negative controls no metabolism should occur. For the "stability control", the application medium was mixed with incubation medium instead of the cell suspension. The stability and the blank controls were incubated for 180 min. For the "zero incubation control" (t = 0 min), the reaction was stopped immediately after addition of the cell suspension.

In the positive controls, testosterone or 7-ethoxycoumarin instead of the test item were incubated with hepatocytes for 180 min, in order to determine their metabolic activity. To prove the metabolic activity, at least 50% of testosterone and 30% of 7-ethoxycoumarin should be metabolized, respectively.

Each experimental setup was performed in triplicates.

In-vitro assay for incubation with human liver cytosol

On the incubation day, the application solution was diluted with 10 mM phosphate buffer pH 7.4 to obtain the application buffer for incubation with human liver cytosol. Human liver cytosol was incubated with application buffer containing 6 µM pyraclostrobin without and with 20 µM WWL229 (all final concentrations in the incubation mixture). Aliquots of the application buffer were analyzed by LSC to calculate the amounts of applied radioactivity per vial and of total applied pyraclostrobin per assay (representing 100% AR in 500 µL per vial: 1.29 µg or 1.32 µg for incubation without or with WWL229, respectively). The final concentration of DMSO was 0.5%.

The purity of the test item in the application buffers for the *in-vitro* assays was determined by HPLC analysis using HPLC methods LC01 and LC02. The application buffers were equilibrated for five minutes in a water bath prior to addition of the human liver cytosol. The incubations were performed for 60 min and 180 min in a water bath at 37°C (± 2°C) under gentle shaking at 200 rpm.

Three negative controls (stability control, zero incubation control and control with heat-denatured liver cytosol) were performed. In the negative controls no metabolism should occur. For the "stability control", the application buffer was mixed with phosphate buffer instead of liver cytosol. The stability controls and the controls with heat-denatured liver cytosol were incubated for 180 min. For the "zero incubation control" (t = 0 min), the reaction was stopped immediately after addition of the cytosol.

Each experimental setup was performed in triplicates.

Sampling and sample storage

After incubation for 0 min, 60 min or 180 min, the reaction was terminated by mixing with ice-cold ethanol (final concentration of ethanol: 70%). The terminated incubation mixtures were concentrated at room temperature using a centrifugal evaporator. The tubes were weighed after concentration and the supernatants were pipetted into separate vials. The remaining pellet ("pellet 1") was weighed again. The volume of the supernatant collected from each tube was calculated from the difference of the two weights prior to and after removal of the supernatant (using a density of 1 g/mL) and recorded in the raw data. The weight of each pellet 1 was calculated by subtraction of the weight of the tube.

The radioactive residues in the supernatants were determined by LSC analysis of aliquots. Additionally, aliquots of the supernatants were analyzed using HPLC method LC02 and selected replicates of supernatants were subjected to HPLC-MS. For positive controls of hepatocytes with testosterone and 7-ethoxycoumarin HPLC methods LC04 and LC03, respectively, were used. The supernatants and pellets (pellet 1) were stored separately in a freezer at -18°C.

Workup procedure of the residual pellet
If the supernatant contained less than 90% of the applied radioactivity (90% AR), the corresponding pellet was extracted with a mixture of acetonitrile / acetone (80/20, v/v). The radioactive residues in the resulting extract and in the final pellet were determined by LSC. An aliquot of the pellet extract was also analyzed by HPLC method LC02 and one selected replicate from human liver cytosol (without WWL229, 180 min) was subjected to HPLC-MS analysis. In the case of the first replicate of human liver cytosol incubated with pyraclostrobin and WWL229 for 0 min, pellet 1 was again extracted with acetonitrile / acetone as described resulting in extract 2 of pellet 1. The radioactive residues in the suspended samples were measured by LSC.

Instrumental methods
HPLC method LC01 (used for analysis of application medium)
The column used was an Accucore aQ (ThermoScientific) 2.6 µm, the eluent system consisted of 2 mobile phases (A: water + 0.1% formic acid & B: acetonitrile + 0.1% formic acid), which were used applying gradient elution.

HPLC method LC02 (used for analysis of pyraclostrobin and its biotransformation products)
The column used was an Accucore aQ (ThermoScientific) 2.6 µm, the eluent system consisted of 2 mobile phases (A: water + 0.1% formic acid & B: acetonitrile + 0.1% formic acid), which were used applying gradient elution.

HPLC method LC03 (used for analysis of 7-ethoxycoumarin and its biotransformation products)
The column used was a TCI ODS-AX10 (TCI Chemicals) 5 µm, the eluent system consisted of 2 mobile phases (A: 50 mM Ammonium Formate (pH 3.5; adjusted with formic acid) + acetonitrile (950/50, v/v) & B: acetonitrile + 50 mM Ammonium Formate (pH 3.5; adjusted with formic acid) (950/50, v/v)), which were used applying gradient elution.

HPLC method LC04 (used for analysis of testosterone)
The column used was a Kinetex C18 (Phenomenex) 2.6 µm, the eluent system consisted of 2 mobile phases (A: water + 0.1% formic acid & B: methanol + acetonitrile + 0.1% formic acid (500/500, v/v)), which were used applying gradient elution.

The HPLC, recovery values observed using HPLC method LC02 ranged from 94.08 to 109.22% of the radioactive residues in the supernatants. The recovery values of the acetonitrile / acetone extracts from pellet 1 ranged from 98.8 to 109.3% of the extracted radioactive residues.

Mass Spectrometry (MS)
HPLC-MS analysis was performed on a Thermo Fisher Scientific LTQ (Linear Trap Quadrupole) FT (Fourier Transform) Ultra hybrid mass spectrometer hyphenated to an HP 1200 chromatography system with electrospray ionisation (ESI) in positive-ion mode. The column used was an Accucore aQ (ThermoScientific) 2.6 µm, the eluent system consisted of 2 mobile phases (A: water + 0.1% formic acid & B: acetonitrile + 0.1% formic acid), which were used applying gradient elution. From the triplicates of each experimental setup, selected replicates of the supernatants and of one pellet extract were analysed by HPLC-MS using the LTQ FT Ultra mass spectrometer. Chromatographic separation was performed under the same conditions as used in the analytical laboratory for quantitative evaluation (HPLC method LC02).

II. RESULTS AND DISCUSSION

Control experiments

In order to determine the metabolic activity of the human hepatocytes, positive controls were performed incubating cells with testosterone or 7-ethoxycoumarin instead of the test item. All triplicates of the controls showed similar HPLC profiles. The positive controls with testosterone showed that the metabolic activity of the hepatocytes with respect to phase I metabolic reactions was sufficiently high. The mean portion of metabolized testosterone accounted for 89% (desired value: 50%).

HPLC analyses of the positive controls with 7-ethoxycoumarin revealed portions of approximately 25-27% AR of unchanged 7-ethoxycoumarin. The phase I metabolite 7-hydroxycoumarin was not detected. The phase II metabolites 7-hydroxycoumarin glucuronide and 7-hydroxycoumarin sulphate were recovered accounting for approximately 9 and 2% AR, respectively. The mean portions of metabolised 7-ethoxycoumarin amounted to 65% (desired value: 30%).

Stability controls (test item without hepatocytes), zero incubation controls (incubation stopped immediately) and blank controls (cells in incubation medium without test item) were performed as negative controls. No degradation of pyraclostrobin, neither without nor with WWL229, was observed within 180 min under these experimental conditions without cells.

All triplicates of the zero incubation controls showed similar HPLC profiles. In general, they all consisted only of the characteristic peak of the active substance pyraclostrobin.

The blank controls performed without the test item showed no significant amounts of radioactivity (LSC measurements of the supernatants after addition of the incubation mixtures to ice-cold ethanol and subsequent concentration). Furthermore, no radioactive peaks were detected by HPLC analysis.

Negative controls, stability controls (test item without liver cytosol), heat-denatured controls (test item with heat-denatured human liver cytosol) and zero incubation controls (incubation stopped immediately) were performed in triplicates. The stability controls and heat-denatured controls were performed with an incubation time of 180 minutes. No degradation of pyraclostrobin, neither without nor with WWL229, was observed within 180 min under the experimental conditions.

All triplicates of the zero incubation control showed similar HPLC profiles. In general, they all consisted only of the characteristic peak of the active substance pyraclostrobin.

Viability of the hepatocytes

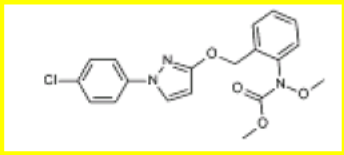
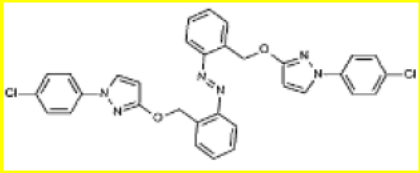
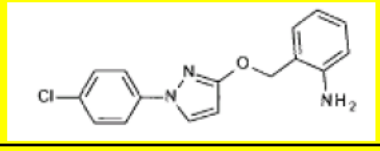
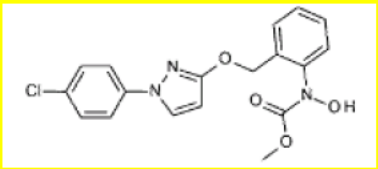
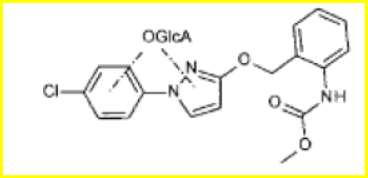
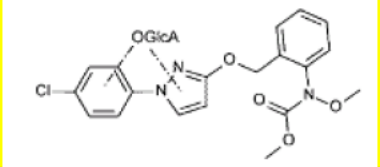
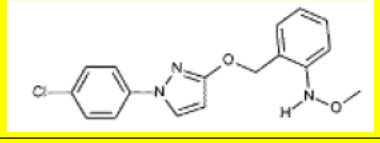
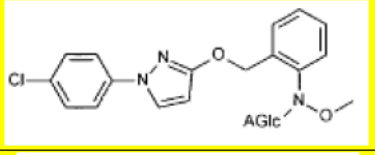
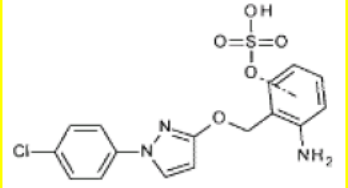
After incubation for 180 min, the viability of the cells was determined using the luminescent cell viability assay. The viability of the human hepatocytes incubated with 3 µM pyraclostrobin was 102% in comparison to untreated cells. The viability of the human hepatocytes incubated with 3 µM pyraclostrobin and 20 µM WWL229 accounted for 30% in comparison to untreated cells.

Identified metabolites
The supernatants after incubation of human hepatocytes and human liver cytosol with pyraclostrobin without and with WWL229 were analyzed using HPLC method LC02. The residual pellets after centrifugal evaporation were extracted with a mixture of acetonitrile and acetone whenever radioactive residues were below 90% AR in the supernatants. Obtained extracts were also analyzed by HPLC using method LC02. Already identified metabolites within a parallel *in-vitro* metabolism study (BASF DocID 2014/1001562) were in focus of quantitative evaluation.

In samples of human hepatocytes, the unchanged parent compound BAS 500 F and its biotransformation products 500M103, 500M107, 500M73, 500M106, 500M02 as well as an isomer of 500M02 were designated. Moreover, metabolite 500M104 and metabolite 500M88 were identified. As the signal-to-noise ratio was low for metabolite 500M106 in samples of human hepatocytes, its presence and identity were confirmed by additional co-chromatography experiments. No evidence was found for the presence of the metabolite 500M108 (analyzed by HPLC-MS) in the XIC-chromatograms and mass spectra of the samples of human hepatocytes incubated within the present study.
In samples of human liver cytosol, the unchanged parent compound BAS 500 F and its biotransformation products 500M73, 500M106, 500M02 as well as an isomer of 500M02 were designated. As expected no evidence was found for the presence of metabolite 500M107 (analyzed by HPLC-MS) in the XIC-chromatograms and mass spectra of the samples of human liver cytosol incubated within the present study.

A summary of metabolites identified in human hepatocytes and liver cytosol is shown in Table 5.1.2-5.

Table 5.1.2-5: Summary of metabolites identified in human hepatocytes and liver cytosol

Component	Structure	Metabolite identified in	
		Human Hepatocytes	Human Liver Cytosol
BAS 500 F Pyraclostrobin		x	x
500M02		x	x
Isomer of 500M02		x	x
500M73		x	x
500M88		x	-
500M103		x	-
500M104		x	-
500M106		x	x
500M107		x	-
500M108		-	-

Radioactive residues in human hepatocytes

After termination of the incubation of human hepatocytes with 3 µM pyraclostrobin without or with 20 µM WWL229 for up to 180 min, the radioactive residues in the supernatants ranged from 78.32 to 88.19% AR (without WWL229) and from 72.75 to 88.46% AR (with WWL229), except for one replicate of the incubation with WWL229 accounting for 90.27% AR. The residual pellets (pellet 1) of the replicates with radioactive residues below 90% AR in the supernatants were suspended in water and extracted with a mixture of acetonitrile and acetone (80/20, v/v). The radioactive residues in extracts ranged from 4.46 to 8.28% AR (without WWL229) and from 2.96 to 5.43% AR (with WWL229), except for one replicate of the incubation with WWL229 (11.59% AR). The radioactive residues in the final pellets were below 1% AR for zero incubation controls without and with WWL229 and were below or equal to 6.52% AR (without WWL229) and 4.35% AR (with WWL229) after longer incubation. The sum of recovery of radioactive residues in the majority of the assays accounted for more than 90% AR. Although for individual replicates the sum of radioactive residues was lower than 90% AR, this has no significant influence on the objective of this study.
Besides, the recovery of radioactive residues in the supernatants might be underestimated due to assuming the density of 100% water (1 g/mL) for calculation of the supernatant volume, while still ethanol might be present. The recovery of radioactive residues in the supernatants of positive controls amounted to at least 92.8 and 90.0% AR for testosterone and 7-ethoxycoumarin, respectively. Hence, no further workup was necessary.

An overview of the recoveries of radioactive residues after incubation of human hepatocytes with pyraclostrobin (3 µM) with and without WWL229 (20 µM) is shown Table 5.1.2-6.

Table 5.1.2-6: Overview of the recoveries of radioactive residues after incubation of human hepatocytes with pyraclostrobin (3 µM) with and without WWL229 (20 µM)

Incubation time [min]	Sample description	Recovery[1] [% AR]		Sum [% AR]	
		with WWL229	without WWL229	with WWL229	without WWL229
0	Supernatant	81.76	86.58	87.44	94.05
	Extract pellet 1	5.43	6.86		
	Final pellet	0.25	0.61		
	Supernatant	77.14	88.19	89.26	97.17
	Extract pellet 1	11.59	8.28		
	Final pellet	0.53	0.69		
	Supernatant	83.76	82.86	89.10	87.85
	Extract pellet 1	5.04	4.46		
	Final pellet	0.30	0.53		
60	Supernatant	82.54	83.21	86.74	94.53
	Extract pellet 1	3.33	5.55		
	Final pellet	0.87	5.78		
	Supernatant	88.46	84.27	93.98	94.84
	Extract pellet 1	4.40	5.02		
	Final pellet	1.12	5.55		
	Supernatant	90.27	86.82	94.62	98.24
	Extract pellet 1	4.35	5.32		
	Final pellet		6.10		
180	Supernatant	72.75	79.59	78.02	91.40
	Extract pellet 1	2.96	5.29		
	Final pellet	2.31	6.52		
	Supernatant	78.11	79.67	83.94	90.69
	Extract pellet 1	3.64	4.61		
	Final pellet	2.19	6.41		
	Supernatant	76.23	78.32	81.93	89.32
	Extract pellet 1	3.53	4.77		
	Final pellet	2.17	6.24		

1 The recovery of radioactive residues in the supernatants might be underestimated due to assuming the density of 100% water (1 g/ml) for calculation of the supernatant volume, while still ethanol might be present

Radioactive residues in human liver cytosol

After termination of the incubation of human liver cytosol with pyraclostrobin without and with WWL229 for up to 180 min, the radioactive residues in the supernatants ranged from 94.66 to 103.80% AR, except for samples obtained after incubation without WWL229 for 180 min. The residual pellets (pellet 1) of the replicates with radioactive residues below 90% AR in the supernatants were suspended in water and extracted with a mixture of acetonitrile and acetone (80/20, v/v). The recovery of radioactive residues in the pellet extracts and in the first or final pellets ranged from 15.36% AR to 18.29% AR for the extracts and from 5.40% AR to 11.10% AR for the final pellets or pellet 1. For one replicate from zero incubation controls of experiments performed with WWL229, 58.47% AR were recovered in the supernatant. Albeit the pellet 1 was extracted two times (approximately 7.5% AR in sum), the total recovery accounted for 66.02% AR. This result has no impact on the outcome of the present study, as all remaining samples revealed reliable recoveries.

An overview of the recoveries of radioactive residues after incubation of human liver cytosol with pyraclostrobin (6 µM) with and without WWL229 (20 µM) is shown Table 5.1.2-7.

Table 5.1.2-7: Overview of the recoveries of radioactive residues after incubation of human liver cytosol with pyraclostrobin (6 µM) with and without WWL229 (20 µM)

Incubation time [min]	Sample description	Recovery [% AR]		Sum [% AR]	
		with WWL229	without WWL229	with WWL229	without WWL229
0	Supernatant	58.47	101.63	66.02*	107.20
	Pellet 1		5.56		
	Extract 1 Pellet 1	7.04			
	Extract 2 Pellet 1	0.48			
	Final pellet	0.03			
	Supernatant	103.80	100.91	108.73	106.30
	Pellet 1	4.93	5.40		
	Supernatant	100.44	102.87	106.01	108.37
	Pellet 1	5.56	5.50		
60	Supernatant	101.34	94.66	106.79	105.48
	Pellet 1	5.45	10.82		
	Supernatant	102.40	95.00	107.85	106.10
	Pellet 1	5.45	11.10		
	Supernatant	97.26	95.97	103.23	106.56
	Pellet 1	5.98	10.58		
180	Supernatant	98.39	85.95	104.18	108.61
	Extract pellet 1		15.36		
	Final pellet		7.30		
	Pellet 1	5.79			
	Supernatant	97.94	79.01	103.60	105.00
	Extract pellet 1		18.29		
	Final pellet		7.71		
	Pellet 1	5.66			
	Supernatant	99.68**	84.24	99.68	107.05
	Extract pellet 1		15.38		
	Final pellet		7.43		

* This result has no impact on the outcome of the present study, as it is one replicate of the zero incubation control and all remaining samples revealed reliable recoveries.

** The vial containing pellet 1 broke not being measured. However, almost 100% AR were already recovered in the supernatant.

Metabolites formed after incubation of human hepatocytes with pyraclostrobin without WWL229

Radio-HPLC analyses of the samples after incubation of human hepatocytes with 3 µM pyraclostrobin without 20 µM WWL229 allowed the assignment of the parent compound and seven relevant metabolites known from the previous comparative in-vitro metabolism study performed on human hepatocytes or human liver microsomes with pyraclostrobin. In the following, the sum of the mean % AR recovered in supernatants and extracts of pellets is discussed, if applicable.

All metabolites were already detected after incubation for 60 min. The concentration of pyraclostrobin continuously decreased from 92.06% AR at 0 min to 15.85% AR after 60 min and was no more detected after 180 min. After 60 min, 500M02 was the most abundant metabolite accounting for 23.29% AR, but decreased to 8.99% AR after 180 min. Similarly, metabolite 500M73 and the isomer of 500M02 were detected at concentrations amounting to 15.60% AR and 5.89% AR, respectively, after 60 min decreasing to 6.17% AR and 2.14% AR, respectively, after 180 min. Further detected metabolites after 60 min were 500M103 (3.89% AR), 500M104 (8.52% AR), 500M107 (16.19% AR) and 500M106 (5.23% AR). While metabolites 500M103, 500M107 and 500M106 remained stable between 60 min and 180 min, the concentration of metabolite 500M104 increased to 18.16% AR after 180 min.

Metabolites formed after incubation of human hepatocytes with pyraclostrobin with WWL229

Three of the eight compounds detected in samples from hepatocytes incubated with pyraclostrobin without WWL229 were also recovered in samples of hepatocytes incubated with pyraclostrobin with WWL229, namely 500M103, 500M104 and the parent compound pyraclostrobin. Additionally, metabolite 500M88 was identified in samples after incubation of hepatocytes with pyraclostrobin and WWL229. In the following, the sum of the mean % AR recovered in supernatants and extracts of pellets is discussed, if applicable.

Similar to the incubation without WWL229, the concentration of the parent compound decreased constantly, but to a lesser extent (from 88.24% AR after 0 min to 48.20% AR after 180 min). Metabolites 500M104 and 500M88 were detected after 60 min accounting for 4.99% AR and 1.90% AR, respectively. The concentration of metabolite 500M104 significantly increased to 19.93% AR after 180 min, while the concentration of metabolite 500M88 remained almost stable. Besides, metabolite 500M103 was identified at levels accounting for 5.64% AR after 180 min.

In contrast, metabolites 500M73, 500M107, 500M106, 500M02 and the isomer of 500M02, resulting from an initial cleavage of the ether and / or amide bond of parent pyraclostrobin, were not detected in samples obtained after simultaneous incubation of hepatocytes with pyraclostrobin and WWL229. Instead metabolite 500M88 was formed at low levels resulting from desmethoxylation of the parent compound. These results indicate an inhibition of the biotransformation of pyraclostrobin in the presence of WWL229. This shows that especially the formation of the metabolites 500M73, 500M107, 500M106, 500M02 and the isomer of 500M02 depends on the activity of the enzyme human carboxylesterase 1.

A summary of the relevant components in human hepatocytes incubated with pyraclostrobin with and without WWL229 is shown Table 5.1.2-8.

Table 5.1.2-8: Comparison of relevant metabolites of pyraclostrobin after incubation of human hepatocytes with and without WWL229

Component	Supernatant [Mean % AR]		Extract Pellet 1 [Mean % AR]		Sum[1] [Mean % AR]	
	with WWL229	without WWL229	with WWL229	without WWL229	with WWL229	without WWL229
Incubation time: 0 min						
BAS 500 F	80.89	85.53	7.36	6.53	88.24	92.06
Incubation time: 60 min						
BAS 500 F	78.35	15.13	3.86	0.72	82.21	15.85
500M02		19.96		3.33		23.29
Isomer of 500M02		4.47		1.42		5.89
500M73		14.81		0.80		15.60
500M88	1.90		n.d.		1.90	
500M103		3.89		n.d.		3.89
500M104	4.99	8.52	n.d.	n.d.	4.99	8.52
500M106		5.23		n.d.		5.23
500M107		16.19		n.d.		16.19
Incubation time: 180 min						
BAS 500 F	45.05		3.15		48.20	
500M02		5.79		3.20		8.99
Isomer of 500M02		2.14		n.d.		2.14
500M73		5.05		1.12		6.17
500M88	2.48		n.d.		2.48	
500M103	5.64	4.19	n.d.	n.d.	5.64	4.19
500M104	19.25	16.89	0.68	1.27	19.93	18.16
500M106		5.41		1.28		6.69
500M107		17.54		n.d.		17.54

1 Sum of supernatant and pellet extract
n.d. not detected

Metabolites formed after incubation of human liver cytosol with pyraclostrobin without WWL229

Radio-HPLC analyses of the samples after incubation of human liver cytosol with 6 µM pyraclostrobin without 20 µM WWL229 allowed the assignment of the parent compound and four relevant metabolites known from the previous comparative *in-vitro* metabolism study performed on human hepatocytes or human liver microsomes with pyraclostrobin. In the following, the sum of the mean % AR recovered in supernatants and extracts of pellets is discussed, if applicable.

The concentration of pyraclostrobin continuously decreased from 101.80% AR at 0 min to 35.74% AR after 180 min. Metabolite 500M106 was the most abundant metabolite after 60 min amounting to 15.63% AR and remained almost stable (12.67% AR after 180 min). Metabolites 500M73, 500M02 and the isomer of 500M02 were present at low levels after 60 min accounting for 4.00, 5.98 and 2.03% AR, respectively. After 180 min, the concentrations of these metabolites were significantly higher accounting for 11.21% AR for 500M73, 28.59% AR for 500M02 and 9.76% AR for the isomer of 500M02.

Metabolites formed after incubation of human liver cytosol with pyraclostrobin with WWL229
Only the parent compound pyraclostrobin was detected in samples from human liver cytosol incubated with pyraclostrobin with WWL229 ranging from 94.61 to 100.33% AR for all incubation periods. Similar to incubations performed on human hepatocytes, metabolites 500M73, 500M106 and 500M02, formed after an initial cleavage of the ether and / or amide bond of the parent pyraclostrobin, were not detected in samples obtained after simultaneous incubation of human liver cytosol with pyraclostrobin and WWL229. These results indicate an inhibition of the biotransformation of pyraclostrobin to these metabolites in the presence of WWL229 and hence, the involvement of the enzyme human carboxylesterase 1.

A summary of the relevant components in human liver cytosol incubated with pyraclostrobin with and without WWL229 is shown in Table 5.1.2-9.

Table 5.1.2-9: Comparison of relevant metabolites of pyraclostrobin after incubation of human liver cytosol with and without WWL229

Component	Supernatant [Mean % AR]		Extract Pellet 1 [Mean % AR]		Sum[1] [Mean % AR]	
	with WWL229	without WWL229	with WWL229	without WWL229	with WWL229	without WWL229
Incubation time: 0 min						
BAS 500 F	87.57	101.80	7.04	n.a.	94.61	101.80
Incubation time: 60 min						
BAS 500 F	100.33	67.57	n.a.	n.a.	100.33	67.57
500M02		5.98		n.a.		5.98
Isomer of 500M02		2.03		n.a.		2.03
500M73		4.00		n.a.		4.00
500M106		15.63		n.a.		15.63
Incubation time: 180 min						
BAS 500 F	98.67	31.39	n.a.	4.36	98.67	35.74
500M02		21.57		7.02		28.59
Isomer of 500M02		7.35		2.41		9.76
500M73		10.32		0.89		11.21
500M106		12.19		0.48		12.67

1 sum of supernatant and pellet extract
n.a. not analyzed

III. CONCLUSION

In the present *in-vitro* metabolism study performed on human hepatocytes and human liver cytosol with pyraclostrobin (BAS 500 F, Reg. No. 304428), the biotransformation products after incubation without and with WWL229, an inhibitor of human carboxylesterase 1, were compared, in order to investigate a potential influence of WWL229.

For human hepatocytes incubated with pyraclostrobin without WWL229, pyraclostrobin and its biotransformation products 500M103, 500M104, 500M107, 500M73, 500M106, 500M02 as well as an isomer of 500M02 were identified. The parent molecule was time-dependently and completely metabolized after 180 min. In presence of WWL229, the biotransformation was slower, as pyraclostrobin was still present after 180 min and metabolites 500M73, 500M107, 500M106 and 500M02 as well as an isomer of 500M02, formed after an initial cleavage of the ether and / or amide bond of the parent pyraclostrobin, were not detected at all. Instead, formation of metabolite 500M88 was observed at low levels.

For human liver cytosol incubated with pyraclostrobin without WWL229, pyraclostrobin and its biotransformation products 500M73, 500M106, 500M02 as well as an isomer of 500M02 were identified. Similar to the incubations performed on human hepatocytes, metabolites 500M73, 500M106, 500M02 and an isomer of 500M02 were not detected in samples obtained after simultaneous incubation of human liver cytosol with pyraclostrobin and WWL229.

These results indicate an inhibition of the biotransformation of pyraclostrobin in the presence of WWL229. This compound inhibits especially the formation of metabolites 500M106, 500M73 and 500M02 (and isomer) as well as their conjugates showing the involvement of the enzyme human carboxylesterase 1 in the respective metabolic reactions.

Report:	CA 5.1.2/3 Funk D., Bellwon P., 2016 b Metabolisation of 14C-BAS 500 F in rat serum 2016/1225032
Guidelines:	OECD Series on Principles of GLP working group on GLP No.14: The application of GLP-Principles to in vitro studies (2004)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

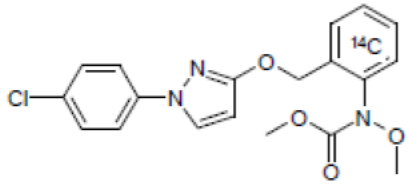
I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	^{14}C-BAS 500 F (pyraclostrobin, Reg. No. 304428)
Description:	tolyl-ring-U-C14 label, see Figure 1.1
Chemical name:	methyl-N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)-(N-methoxy)carbamate (IUPAC-Name)
Test substance No:	not reported
Lot/Batch #:	566-5501
CAS#:	175013-18-0
Development code:	Pyraclostrobin: BAS 500 F (Reg. No. 304428)
Purity:	Radiochemical purity: 99.5 % (RHPLC)
Specific activity:	6.31 MBq/mg (specific activity a.i.); 30 MBq/g (specific activity)
Stability of test compound:	The test item was stable over the test period. No degradation of pyraclostrobin, was observed within 20 h under the experimental conditions neither without serum nor in the presence of heat-denatured rat serum.
	For stability controls the application buffer was mixed with aqueous buffer (Tris/HCl buffer) instead of rat serum.

2. Test animals:	Rat
Species:	Wistar rats
Strain:	Crl: WI (Han)
Sex:	male and female
Age:	27-28 weeks (male), 14/16 weeks (female) (age at sampling)
Source:	Charles River Laboratories, Sulzfeld, Germany

Figure 1.3: Structural formula of ^{14}C-pyraclostrobin labelled at the tolyl ring



B. STUDY DESIGN AND METHODS

The study was carried out at the Agricultural Research Centre of BASF SE in Limburgerhof, Germany.

1. Dates of experimental work (analytical phase) June 13, 2016 to September 08, 2016

2. Study design

Stock solution

The stock solution of pyraclostrobin was prepared as follows (within the *in-vitro* metabolism study (BASF DocID 2014/1001562): a weighed aliquot was evaporated to dryness with nitrogen and dissolved in acetonitrile. Aliquots were diluted and analyzed by a liquid scintillation counter (LSC) in order to determine the actual concentration (0.978 mg/mL).

Application solution

The application solution of the test item was prepared as follows (within the parallel in-vitro metabolism study BASF DocID 2016/1061381): The solvent from a defined volume of the stock solution was evaporated with nitrogen, and the test item was dissolved in 2 mL DMSO. The specific radioactivity of the test item accounted for 378,600 dpm/µg. The actual concentration of radiolabeled pyraclostrobin in this application solution was determined within the separate study (0.756 mg/mL) by LSC of aliquots of diluted subsamples. A subsample (0.87 mL) of this application solution was obtained from another *in-vitro* metabolism study (BASF DocID 2016/102596).

Test system

Serum was sampled from untreated male (10 mL pooled from three rats) and female (2x 3 mL pooled from five rats each) Wistar rats (Crl: Wl(Han)). The serum was shipped to the Agricultural Centre of BASF in Limburgerhof, Germany, on dry ice, where samples were stored deep frozen until pooling. One day before incubation, serum samples were thawed in a refrigerator overnight. On the day of incubation, serum samples from female rats were completely pooled, 2.5 mL were taken and mixed with 2.5 mL of male serum to generate a mixture of female and male serum (1/1, v/v) for the experiments. An aliquot of the serum was heat-denatured by incubating at 95°C or above for 10 min.

In-vitro assays

On the incubation day, the application solution was diluted with 50 mM Tris/HCl buffer pH 7.4 to obtain the application buffer for incubation with rat serum. The identity and purity of the test item in the application buffer (and the retention time of pyraclostrobin) was confirmed by HPLC analysis of aliquots. Further aliquots of the application buffer were analyzed by LSC to calculate the amounts of applied radioactivity per vial and of total applied pyraclostrobin per assay (representing 100% AR in 1000 µL per vial: 2.328 µg). The final concentration of pyraclostrobin in the incubation mixtures with rat serum (calculated from the LSC results of the application buffer) was 6.004 µM and is given as rounded value of 6 µM throughout the present report. The deviation from the intended final concentration of 10 µM pyraclostrobin has no impact on the validity of the study. The final concentration of DMSO in the assays was approximately 0.5%.

The application buffer was equilibrated at 37°C for 5 min in a Thermomixer® (Eppendorf) prior to addition of the rat serum. Each sample (1 mL total incubation volume) comprised 0.8 mL of application buffer and 0.2 mL of rat serum in a tube. Incubations were performed for 1 h, 3 h, 5 h and 20 h in a Thermomixer® (Eppendorf) at 37 °C (± 2 °C) under shaking at 700 rpm.

Three negative controls (stability controls, controls with heat-denatured rat serum and zero incubation controls) were performed.
In the negative controls no metabolism should occur. For the “stability controls”, the application buffer was mixed with Tris/HCl buffer instead of rat serum. The stability controls and the controls with heat-denatured serum were incubated for 20 h. For the “zero incubation controls” (t = 0 h), the reaction was stopped immediately after addition of the serum.
Each experimental setup was performed in triplicates.

Sampling and sample storage

The incubation was terminated by addition of 1 mL ice-cold acetonitrile followed by ultrasonication for 5 min. The terminated incubation mixtures were concentrated at room temperature using a centrifugal evaporator (Genevac EZ-2.3 plus). The tubes were weighed after concentration and the supernatants were pipetted into HPLC vials. The tube containing the remaining pellet (“pellet 1”) was weighed again. The volume of the supernatant collected from each tube was calculated from the difference of the two weights prior to and after removal of the supernatant (using a density of 1 g/mL) and recorded in the raw data. The weight of each pellet 1 was calculated by subtraction of the weight of the tube.
The radioactive residues in the supernatants were determined by LSC analysis of aliquots. In addition, aliquots of the supernatants were analyzed by HPLC using HPLC method LC02 and selected replicates of supernatants were subjected to HPLC-MS. The supernatants and pellets (pellet 1) were stored separately in a freezer at -18 °C.

Workup procedure of the residual pellet
If the radioactive residues in the supernatant represented 90% AR or more, the residual pellet was resuspended in 0.35 mL water using a pipette, followed by ultrasonication for 5 min. Three aliquots were subjected to LSC to determine the radioactive residues in pellet 1.
If the radioactive residues in the supernatant accounted for less than 90% AR, the pellet 1 was resuspended in 0.05 mL water, and 0.25 mL of a mixture of acetonitrile / acetone (80/20, v/v) were added. The pellet was extracted for 5 min in an ultrasonication bath, and the extract was separated from the final pellet by centrifugation. Aliquots of the acetonitrile / acetone extract were measured for radioactive residues (LSC). Further aliquots of the acetonitrile / acetone extracts were analyzed using HPLC method LC02. The final pellet was resuspended in 0.35 mL water, ultrasonicated for 5 min, and the radioactive residues in the suspended samples were measured by LSC.

Instrumental methods
HPLC method LC01 (used for analysis of application buffer)
The column used was an Accucore aQ (ThermoScientific) 2.6 µm, the eluent system consisted of 2 mobile phases (A: water + 0.1% formic acid & B: acetonitrile + 0.1% formic acid), which were used applying gradient elution.

HPLC method LC02 (used for analysis of pyraclostrobin and its biotransformation products)
The column used was an Accucore aQ (ThermoScientific) 2.6 µm, the eluent system consisted of 2 mobile phases (A: water + 0.1% formic acid & B: acetonitrile + 0.1% formic acid), which were used applying gradient elution.
The HPLC recovery values observed using HPLC method LC02 ranged from 94.26 to 107.56% of the radioactive residues in the supernatants. The recovery values of the acetonitrile/acetone extracts from pellet 1 ranged from 101.88 to 110.13% of the extracted radioactive residues, except for two samples accounting for 16.29% (pellet extract of a heat-denatured control) and 147.33% (pellet extract of a replicate after 3 h incubation), respectively. This has no impact on the overall conclusion drawn from the study.

Mass Spectrometry (MS)
HPLC-MS analysis was performed on a Thermo Fisher Scientific LTQ (Linear Trap Quadrupole) FT (Fourier Transform) Ultra hybrid mass spectrometer hyphenated to an HP 1200 chromatography system with electrospray ionisation (ESI) in positive-ion mode. The column used was an Accucore aQ (ThermoScientific) 2.6 µm, the eluent system consisted of 2 mobile phases (A: water + 0.1% formic acid & B: acetonitrile + 0.1% formic acid), which were used applying gradient elution. From the triplicates of each experimental setup, selected replicates of the supernatants were analyzed by HPLC-MS using the LTQ FT Ultra mass spectrometer. Chromatographic separation was performed under the same conditions as used in the analytical laboratory for quantitative evaluation (HPLC method LC02).

II. RESULTS AND DISCUSSION

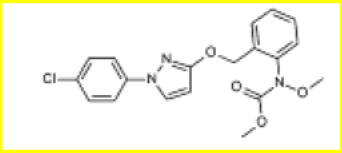
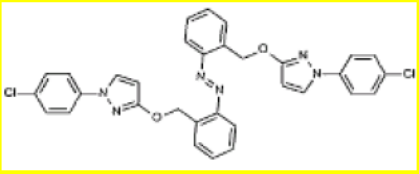
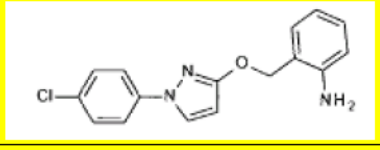
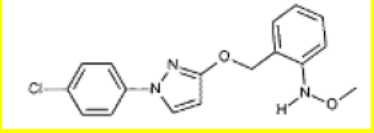
The present *in-vitro* study describes the biotransformation of ^{14}C-pyraclostrobin (BAS 500 F) during incubation with rat serum for up to 20 h. The biotransformation products were compared to the main products observed after incubation of pyraclostrobin with human hepatocytes as well as with human liver microsomes within a previous comparative *in-vitro* metabolism study (BASF DocID 2014/1001562). In addition, several negative controls were conducted.
The radioactive residues of ^{14}C-pyraclostrobin were investigated and quantified in the supernatants after incubation, and the residual pellets after centrifugal evaporation were extracted with a mixture of acetonitrile and acetone, if supernatants contained less than 90% of the applied radioactivity (90% AR). The resulting pellet extracts were also analyzed. All assays were performed in triplicates.

Control Experiments
As negative controls, stability controls (test item without rat serum), heat-denatured controls (test item with heat-denatured rat serum) and zero incubation controls (incubation stopped immediately) were performed in triplicates. The stability controls and heat-denatured controls were performed with an incubation time of 20 h. No degradation of pyraclostrobin, was observed within 20 h under the experimental conditions neither without serum nor in the presence of heat-denatured rat serum. All triplicates of the zero incubation control showed similar HPLC profiles, generally all consisting only of the characteristic peak of pyraclostrobin.

Identified metabolites
The supernatants after incubation of 6 µM pyraclostrobin with rat serum were analyzed using HPLC method LC02. The residual pellets after centrifugal evaporation were extracted with a mixture of acetonitrile and acetone, whenever radioactive residues were below 90% AR in the supernatants. Obtained extracts were also analyzed by HPLC using method LC02. Metabolites already identified within a previous in-vitro metabolism study (BASF DocID 2014/1001562) were in focus of the evaluation. The unchanged parent compound BAS 500 F and its biotransformation products 500M73, 500M106, 500M02 as well as an isomer of 500M02 were identified in rat serum. For extracts of pellets, the assignment was based on comparison of the retention times and metabolite patterns with those acquired by RHPLC analysis of the supernatants. For some supernatants, where the signal-to-noise ratio was too low for an unambiguous metabolite identification, the assignment was additionally based on comparison of the retention time and the metabolite pattern with the results obtained from the analysis of the supernatant sampled after incubation for 20 h.

A summary of metabolites identified in rat serum is shown in Table 5.1.2-10.

Table 5.1.2-10: Summary of metabolites identified in rat serum

Component	Structure
BAS 500 F Pyraclostrobin	
500M02	
Isomer of 500M02	
500M73	
500M106	

Radioactive residues in rat serum

After termination of the incubation of pyraclostrobin with rat serum for up to 20 h, the radioactive residues in the supernatants ranged from 81% AR to 104% AR (means of three replicates for each incubation period). The residual pellets (pellet 1) of the replicates with radioactive residues below 90% AR in the supernatants were suspended in water and extracted with a mixture of acetonitrile and acetone (80/20, v/v). It ranged from 44.24 to 75.81% AR for the extracts, from 13.01 to 25.67% AR for pellet 1 and from 2.53 to 7.36% AR for the final pellet.

The sum of recovery of radioactive residues ranged from 119 to 134% AR (means of three replicates for each incubation period) and accounted for more than 100% for each replicate. This overestimation can be attributed to plastic binding of pyraclostrobin during sample preparation.

An overview on the recoveries of radioactive residues after incubation of 6 µM pyraclostrobin with rat serum is shown Table 5.1.2-11.

Table 5.1.2-11: Overview on recoveries of radioactive residues after incubation of 6 µM pyraclostrobin with rat serum

Incubation time [h]	Sample description	Recovery [% AR]	Sum[1] [% AR]
0	Supernatant Extract Pellet 1 Final pellet	83.21 49.74 7.12	140.06
	Supernatant Extract Pellet 1 Final pellet	64.12 56.34 2.53	122.99
	Supernatant Pellet 1	120.08 13.79	133.87
1	Supernatant Extract Pellet 1 Final pellet	63.91 75.81 6.04	145.76
	Supernatant Extract Pellet 1 Final pellet	85.94 53.01 4.51	143.46
	Supernatant Pellet 1	93.18 21.73	114.91
3	Supernatant Pellet 1	99.46 25.52	124.98
	Supernatant Pellet 1	94.55 25.67	120.22
	Supernatant Extract Pellet 1 Final pellet	78.15 58.49 7.27	143.91
5	Supernatant Pellet 1	110.57 14.14	124.71
	Supernatant Pellet 1	101.03 13.01	114.04
	Supernatant Pellet 1	101.36 16.26	117.62
20	Supernatant Extract Pellet 1 Final pellet	82.71 44.24 7.33	134.28
	Supernatant Extract Pellet 1 Final pellet	86.25 47.42 7.36	141.04
	Supernatant Pellet 1	91.12 17.24	108.36

1 The sum of recovery of radioactive residues accounted for more than 100 % AR due to plastic binding of pyraclostrobin during sample preparation.

Metabolites formed after incubation of pyraclostrobin with rat serum

HPLC analyses of the samples after incubation of 6 µM pyraclostrobin with rat serum allowed the assignment of the parent compound and four relevant metabolites known from the previous comparative *in-vitro* metabolism study (BASF DocID 2014/1001562) performed on human hepatocytes and human liver microsomes with pyraclostrobin. In the following, the sum of the mean % AR recovered in supernatants and extracts of pellets is discussed, if applicable.

The concentration of pyraclostrobin decreased continuously from 142.04% AR or 142.52% AR at 0 h or 1 h, respectively, to 66.29% AR after 20 h. Formation of metabolites 500M73, 500M106, 500M02 and an isomer of 500M02 was observed in trace amounts already after 1 h. The concentration of metabolite 500M106, being the main biotransformation product for all investigated periods, increased time-dependently to up to 48.81% AR after 20 h. Similarly, concentrations of metabolites 500M73, 500M02 and the isomer of 500M02 increased with time amounting to 1.55, 11.03 and 4.27% AR after 20 h, respectively.

These results indicate a significant biotransformation of pyraclostrobin to several metabolites occurring in rat serum within 20 h.

A summary of the relevant components in rat serum with pyraclostrobin is shown Table 5.1.2-12.

Table 5.1.2-12: Summary of relevant metabolites of pyraclostrobin (6 µM) after incubation of rat serum

Incubation time [h]	Sample description	Component [% AR]				
		BAS 500 F	500M02	Isomer of 500M02	500M73	500M106
0	Supernatant	89.14	-	-	-	-
	Extract Pellet 1	52.90	-	-	-	-
	Sum	**142.04**	-	-	-	-
1	Supernatant	79.93	0.29*	-	0.32*	0.78*
	Extract Pellet 1	62.59	0.68*	0.27*	0.30*	0.57*
	Sum	**142.52**	**0.98***	**0.27***	**0.62***	**1.34***
3	Supernatant	84.90	0.60	-	0.58*	5.03*
	Extract Pellet 1	53.48	1.78	0.69*	0.59*	1.60*
	Sum	**138.38**	**2.38**	**0.69***	**1.17***	**6.63***
5	Supernatant	93.01	2.03	0.37*	0.95*	8.29*
	Extract Pellet 1	n.e.	n.e.	n.e.	n.e.	n.e.
	Sum	**93.01**	**2.03**	**0.37***	**0.95***	**8.29***
20	Supernatant	52.38	5.98	1.94	0.70	25.55
	Extract Pellet 1	13.91	5.05	2.32	0.84	23.26
	Sum	**66.29**	**11.03**	**4.27**	**1.55**	**48.81**

* Peak assignment was based on comparison of the retention time and the metabolite pattern with the chromatogram obtained from analysis of the supernatants collected from assays incubated for 20 h.

n.e. not extracted

III. CONCLUSION

In the present *in-vitro* metabolism study with pyraclostrobin (BAS 500 F), the biotransformation of 6 µM pyraclostrobin was investigated after incubation with rat serum for up to 20 hours. Within the first 1 h of incubation, no significant differences were observed in comparison to the zero incubation controls. After 3 h, the concentration of pyraclostrobin started to decrease continuously to 66.29% AR after 20 h. Formation of metabolites 500M73, 500M106, 500M02 and an isomer of 500M02 was observed in trace amounts already after 1 h. The concentration of all metabolites increased time-dependently with metabolite 500M106 being the main biotransformation product for all incubation periods (48.81% AR after 20 h). These results indicate a significant biotransformation of pyraclostrobin occurring in rat serum within 20 h leading to metabolites, which are also formed with human hepatocytes or human liver microsomes.

Overall conclusion

Pyraclostrobin has been extensively studied for absorption, distribution, metabolism and excretion. Endpoints set during the previous Annex I inclusion process reflect the rapid absorption and excretion as well as the extensive metabolism in mammalian systems.

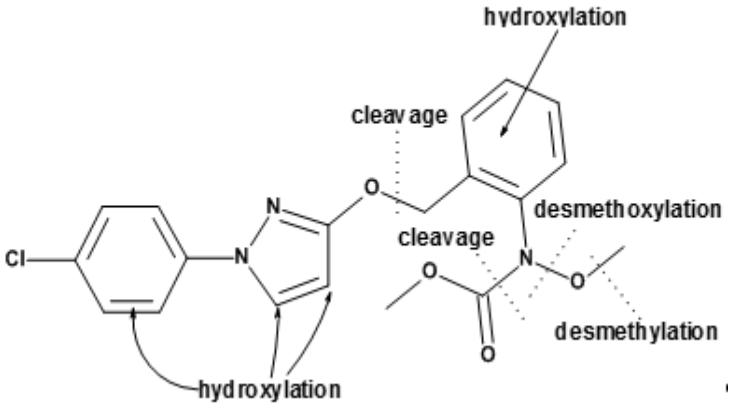
In addition, a new rat study focussing on detailed metabolism in plasma is available, which extends the knowledge obtained during previous studies. Given the degree of metabolism of pyraclostrobin, it is not to be expected to match all metabolites or percentage between the old and new studies. In the recently performed plasma study in rats, several metabolites have been reconfirmed and some new metabolites (500M104, 500M106, 500M107 and 500M108) have been identified.

Since the submission of the supplemental dossier in July 2014, further investigations have been performed; main purpose of these studies was to get a better understanding of the formation, but also the metabolism of the metabolites 500M106, 500M107 and 500M02 which were predominantly found in human and rabbit cell cultures, but only in minor amounts in rats.

When all studies (including the comparative *in-vitro* metabolism and the subsequent investigations on 500M106) are compared, the following general conclusions can be drawn:

- Pyraclostrobin is rapidly excreted via urine and feces.
- Excretion of radioactivity is similar for both sexes. The majority of the radioactivity was excreted via feces (>80% of the dose) and smaller amounts via urine (<15% of the dose). Via bile, about 35% of dose is excreted resulting in a bioavailability of approximately 50%.
- There is no evidence of any cumulative potential of pyraclostrobin. Throughout the time course of the experiments, highest radioactivity concentrations were found in the GI tract. Most organs and tissues had values similar to the ones of the plasma. Liver (factor: 4 – 5) and to a lesser extent kidneys (factor: 2) were tissues with higher values than plasma. Radioactivity concentrations were lowest in bone and brain.

- The metabolite patterns in feces, urine, bile, liver, kidney and plasma were largely comparable for both sexes and for all dose groups investigated.
- In total six transformation steps were observed in rats, which are depicted in the figure below:



  - Desmethoxylation of the side chain
  - Hydroxylation of the chlorophenyl pyrazole ring system
  - Hydroxylation of the tolyl ring system
  - Cleavage of the ether bond resulting in chlorophenyl pyrazole or anthranilic acid derivatives
  - Desmethylation of the side chain
  - Cleavage of the amide bond in the side chain

- The combination of these reactions followed by conjugation steps results in a huge number of metabolites.
- An *in-vitro* comparison of metabolic profiles produced in human hepatocyte / microsome samples and those from several mammalian species has been performed.
- In this study, the same key metabolic degradation steps were observed as under *in-vivo* conditions. Metabolites as 500M04, 500M73, 500M108, 500M103, 500M104 and 500M88 are common to all test species.
- Some quantitative differences were noted between the different test species. In human and rabbit, cleavage of the amide bond resulting in the formation of metabolite 500M106 is the major degradation pathway. Subsequently, 500M106 is further metabolized by conjugation with glucuronic acid to 500M107. In human and rabbit, metabolite 500M02 (which is formed by dimerization) has been also identified.
- However, no unique human metabolite was detected based on the study procedures for comparison at a 5% TRR level.
- Based on these results, in total three metabolites require further consideration:
  - 500M106
  - 500M107 (conjugate of 500M106)
  - 500M02

- For metabolites 500M106 and 500M02, further toxicological studies have been performed (see M-CA 5.8). The glucuronic acid conjugate 500M107 is covered by the investigations of 500M106. The relevant endpoints are considered for estimating the dietary exposure (see M-CA 6.9) as the conversion of parent into the three metabolites cannot be excluded under *in-vivo* conditions.
- For improving the understanding of the formation and metabolism of 500M106, in total four new studies are included in the updated version of the supplemental dossier.
- In a rat metabolism study where 500M106 was dosed to male rats, the same excretion pattern was found as for pyraclostrobin. Two major biotransformation steps in the metabolic pathway of 500M106 were observed:
  - Cleavage of the N-O bond of the phenylamine moiety followed by (a) hydroxylation, (b) dimerization with subsequent N-oxidation or (c) formation of formaldehyde adducts
  - Cleavage of the ether bridge between the pyrazole and phenylamine moiety followed by conjugation (sulphation / glucuronidation) or hydroxylation and sulphation
- The results of the metabolism study demonstrate that rats are an appropriate species for further toxicological testing, but they also show that metabolite 500M02 is covered by the investigations on 500M106 as it has been found in considerable amounts.
- By means of further *in-vitro* investigations, the enzyme resulting in the formation of 500M106 has been identified as human carboxylesterase 1.
- According to literature, this type of enzymes is also present in rats in high amounts, however, in serum instead of the liver. In order to confirm the publications, rat serum was incubated with ^{14}C-pyraclostrobin. The results indicate a significant biotransformation of pyraclostrobin leading to metabolites, which are also formed with human hepatocytes or human liver microsomes. These findings and the fact that in rats the key degradation steps are different (desmethoxylation and hydroxylation) can well explain the outcome of the *in-vivo* study in rats (see M-CA 5.1.1), where 500M106 / 500M107 were detected in minor amounts in plasma.

Altogether and taking the results summarized in M-CA 5.8 into account, the investigations support the use of previous studies for end point setting (ADI: rat, ARfD: rabbit) and interpretation of consumer risk.

When the information from previous and new studies is reviewed in total, the description of proposed endpoints is proposed to be slightly modified as shown below. In this context, the metabolites being considered for dietary exposure assessment are designated as “significant”. After the conduct of the assessments (see M-CA 6.7 and 6.9), none of them was identified as “relevant”.

Proposed endpoints

Rate and extent of absorption:	Rapid absorption: Tmax ~ 1 hour: 50% (based on urinary and biliary excretion within 48 hours)
Distribution:	Widely, highest concentrations in the liver
Potential for accumulation:	No potential for accumulation
Rate and extent of excretion:	Complete within 5 d; mainly via feces (80-90%, biliary excretion amounting to 35%), via urine 11-15%
Metabolism in animals	Extensive (>95%) with more than 50 metabolites Main metabolic pathways included N-desmethoxylation, hydroxylation, cleavage of ether bond and further oxidation of the resulting molecule parts, conjugation with glucuronic acid or sulphate
Toxicologically significant compounds (animals and plants)	Parent and metabolites
Toxicologically significant compounds (environment)	Parent

CA 5.2 Acute Toxicity

Studies submitted in the original Annex I Dossier (2000):
Pyraclostrobin (BAS 500 F) has been tested in various species and via different routes of administration. All studies are scientifically valid. The studies listed in Table 5.2-1 have been evaluated and peer reviewed during the previous Annex I inclusion process.

Table 5.2-1: Summary of already peer-reviewed acute toxicity studies with pyraclostrobin

Route/species/sex	Dose range	Vehicle	Result	Reference (BASF DocID)
Oral Rat, Wistar (CHBB:THOM), m/f	2000, 5000 mg/kg bw	0.5% aqueous Tylose (CMC)	LD_{50} > 5000 mg/kg bw	1998/10965
Dermal Rat, Wistar (CHBB:THOM), m/f	2000 mg/kg bw	0.5% aqueous Tylose (CMC)	LD_{50} > 2000 mg/kg bw	1998/10966
Inhalation Rat, Wistar (CHBB:THOM), m/f	0.31, 1.07, 5.27 mg/L	Acetone	0.31 mg/L < LC_{50} < 1.07 mg/L	1997/11472
Skin irritation Rabbit, (NZW), m/f	0.5 g/animal	None	Irritant	1998/10959
Eye irritation Rabbit, (NZW), m/f	0.1 g/animal	None	Non irritant	1998/10963
Skin sensitisation, Maximisation Test Guinea pig, Dunkin Hartley (Crl:(HA)BR), f	Intradermal: 5% in mixture Freund's adjuvant /Tylose Epidermal: 5% in Tylose	1% aqueous Tylose	Not sensitising	1998/10964

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):
In vivo studies characterising the acute inhalation toxicity profile were performed with pyraclostrobin, which were not submitted during the previous Annex I inclusion process.
In accordance with the requirements of Commission Regulation SANCO/11802/2010 an in vitro NRU Phototoxicity Test in Balb/c 3T3 cells has been performed and is described in detail in chapter M-CA 5.2.7. The respective studies are listed in Table 5.2-2.

Table 5.2-2: Summary of not yet peer-reviewed acute toxicity studies with pyraclostrobin

Type of study	Test substance	Result Classification	Reference (BASF DocID)
Inhalation route - rat	pyraclostrobin (solvent: Solvesso)	4.07 mg/L < LC_{50} < 7.3 mg/L CLP classification not required	2001/1010625
Inhalation route - rat	pyraclostrobin (solvent: acetone)	LC_{50} = 0.58 mg/L CLP classification H331	2002/1012053, 2003/1009200, 2003/1009427
In vitro NRU Phototoxicity Test in Balb/c 3T3 cells	pyraclostrobin	Not phototoxic	2012/1189936, 2014/1092412

Pyraclostrobin has low acute toxicity by the oral and dermal route of administration.

The toxicity by the inhalation route is to a great extent dependent on the vehicle/solvent used to generate an inhalable aerosol. Whereas for pyraclostrobin dissolved in acetone LC_{50} values of 0.31 mg/L < LC_{50} < 1.07 mg/L and 0.58 mg/L were derived (H331 - Toxic if inhaled), generation of an aerosol of pyraclostrobin dissolved in Solvesso revealed a value of 4.07 mg/L < LC_{50} < 7.3 mg/L. More detailed information on the acute inhalation toxicity can be found in M-CA 5.2.3.

Pyraclostrobin is irritant to the skin (H315 - Causes skin irritation), but not irritant to the eye.

In a Maximisation Test pyraclostrobin was not sensitising.

No indications for a phototoxic potential was observed in an in vitro NRU Phototoxicity Test in Balb/c 3T3 cells:

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the Review Report of pyraclostrobin (SANCO/1420/2001-Final, 8. September 2004):

Rat LD_{50} oral:	> 5000 mg/kg bw (mouse mortality at ≥ 300 mg/kg bw)
Rat LD_{50} dermal:	> 2000 mg/kg bw
Rat LC_{50} inhalation:	0.69 mg/L
Skin irritation:	Irritating
Eye irritation:	Not irritating
Skin sensitization (test method used and result):	Not sensitising (M & K maximisation test)

The proposed endpoints based on all available studies are shown below in Table 5.2-3.

Table 5.2-3: Proposed acute toxicity endpoints of pyraclostrobin*

Study type/species	Results	Classification	
		EU Dir. 67/548/EEC 2001/59 EC	**Reg. EC 1272/2008 (CLP)**
Acute oral toxicity, rat	LD_{50} > 5000 mg/kg bw		
Acute dermal toxicity, rat	LD_{50} > 2000 mg/kg bw		
Acute inhalation toxicity, rat	**LC_{50} = 0.58 mg/L**	R23	H331
Dermal irritation, rabbit	Irritating	R36	H315
Eye irritation, rabbit	Not irritating		
Maximisation test, guinea pig	Not sensitising (M & K maximisation test)		
In vitro NRU Phototoxicity Test, Balb/c 3T3 cells	**Not phototoxic**		

* new endpoints or values differing from the current agreed EU endpoints are marked in bold

CA 5.2.1 Oral

The acute oral toxicity study of pyraclostrobin was evaluated and peer reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Pyraclostrobin has low acute oral toxicity.

As already mentioned since 2004 in the list of endpoints, a higher acute toxicity of pyraclostrobin was observed in mice administered pyraclostrobin in the course of the in-vivo mouse micronucleus assay. While in the acute oral LD_{50} study in rats pyraclostrobin was administered as a coarsely ground suspension of pyraclostrobin in 0.5% aqueous tylose in bi-distilled water, it was administered in the mouse micronucleus assay (BASF DocID 1998/10460) as solution in olive oil. The detailed results of the range finding toxicity study for setting the dose levels in the main mutagenicity study are provided in a report amendment (see M-CA 5.4.2/1, BASF DocID 2016/1309356).
Even though not designed as a study to determine a LD_{50} in mice, the mortality data in the mouse micronucleus assay are suitable to derive an acute oral toxicity value in mice. Based on the mortality observed in the range finding and main study, acute LD_{50} values of 449, 453 and 451 mg/kg bw can be calculated for male mice, female mice and combined sexes, respectively. This calculation used the Probit analysis according to Finney (Finney, D.J. (1971): "Probit Analysis" Cambridge University Press, pp. 1 – 50) using SAS 9.3.

Besides potential differences in species susceptibility, different bioavailability of pyraclostrobin administered as aqueous suspension or as solution in olive oil is considered to be the main driver for the observed differences in the oral LD_{50} values.

CA 5.2.2 Dermal

The acute dermal toxicity study of pyraclostrobin was evaluated and peer reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Pyraclostrobin has low acute dermal toxicity.

CA 5.2.3 Inhalation

Report: CA 5.2.3/1
[redacted] et al., 2001a
BAS 500 F 40% in Solvesso (technical active ingredient) - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure
2001/1010625
Guidelines: OECD 403, EEC 92/69 B 2, EPA 870.1300
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an acute inhalation toxicity study, four groups of Wistar CrlGlxBrlHan:Wi (5/sex) were exposed to liquid aerosols of 40% pyraclostrobin in Solvesso at concentrations of 0.89, 1.96, 4.07 and 7.3 mg/L for 4 hours. The animals were observed for 14 days after exposure.

Mortality was observed in 0, 1, 0, and 5 males and 0, 0, 1, 4 females at 0.89, 1.96, 4.07 and 7.3 mg/L, respectively. The following LC_{50} values / LC_{50} ranges were estimated:

LC_{50}, male and female rats	**4.07 mg/L < LC_{50} < 7.3 mg/L**
LC_{50}, female rats	**5.47 mg/L**
LC_{50}, male rats	**4.07 mg/L < LC_{50} < 7.3 mg/L**

Clinical signs observed consisted of visually accelerated respiration, gasping, respiratory sounds, eyelid closure, apathy, squatting posture, piloerection and smeared fur. Except of accelerated respiration all clinical signs were observed after the end of the exposure only. Clinical signs resolved latest by day 7 of the observation period. Gross necropsy of animals dying as well as necropsied at the end of the observation period revealed mainly discolorations in the lung. Additionally, wet and contaminated fur was observed in dead animals exposed to the highest concentration of pyraclostrobin.

The mass mean aerodynamic diameter (MMAD) ranged from 2.7 µm (7.3 mg/L) to 4.3 µm (0.89 mg/L) with geometric standard deviations in the range of 2.5 to 2.7. Greater than 74.9% of the particles had a diameter of ≤ 5.5 µm and thus may have reached the alveolar space of the lung.

The extent of mortality as well as the clinical signs observed at 4.07 mg/L strongly indicate that the LC_{50} is above 5 mg/L. According to the EU CLP (1272/2008) no classification is necessary based on this test.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin 40% in Solvesso
(BAS 500 03 F; as given in the Certificate of Analysis)

Description:	Liquid, brown, clear
Lot/Batch #:	2001-1
Purity/content:	38.1%
Stability of test compound:	According to the Certificate of Analysis the test substance was stable until April 2003 when stored under ambient temperature.

2. Vehicle and/or positive control: Solvesso 200

3. Test animals:

Species:	Rat
Strain:	Wistar CrlGlxBrlHan:Wi
Sex:	20 males and 20 females
Age:	males: 8 to 12 weeks; females: 14 to 18 weeks
Weight at dosing:	262 ± 17.8 g (males), 224.0 ± 11.5 g (females)
Source:	Charles River Laboratories, Germany, 97633 Sulzfeld
Acclimation period:	at least one week
Diet:	Kliba rat/mouse/hamster diet, pelleted, Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum except during exposure
Water:	Tap water ad libitum except during exposure
Housing:	single housing in stainless steel wire mesh cages type DK-III (Becker & Co., Castrop-Rauxel, FRG)
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1.Dates of work: 11-Apr-2001 to 13-Jun-2001

2. Animal assignment and treatment:
For determination of the acute inhalation toxicity (single 4-hour-exposure) of pyraclostrobin dissolved in Solvesso as a liquid aerosol groups of five male and 5 female rats were exposed to aerosols at four concentrations.

3. Clinical examinations:
For each test group, the body weight of the animals was determined just prior to exposure (day 0), weekly thereafter and at the end of the observation period. A check for overt clinical signs of toxicity or mortality as well as a check for the presence of feed and drinking water was made twice a day on workdays and once daily on weekends and public holidays. Detailed clinical observations were recorded for each animal separately several times during exposure and at least once on each workday of the observation period.

4. Pathology:
At the end of the 14-day observation period the animals were sacrificed with CO_2 and were subjected to gross-pathological examination like those performed for animals which had died before the end of the exposure period.

5. Statistics/Calculations:
The statistical evaluation of the concentration-response relationship was carried out using a computer program. Depending on the data of the concentration-response relationship obtained by the experiment, this program is used to estimate the LC_{50} or to perform a Probit analysis[1]. Estimation of the LC_{50} will produce types "LC_{50} greater than", "LC_{50} approx.", or "LC_{50} smaller than". If the results are type "LC_{50} greater than" or "LC_{50} smaller than", an additional binomial test is carried out, in order to verify these Statements statistically[2].
The calculation of the particle size distribution was carried out in the inhalation laboratory on the basis of mathematical methods for evaluating particle measurements[3].

[1] FINNEY, D.J. (1971): "Probit Analysis" Cambridge University Press, pp. 1 - 50

[2] SNEDECOR, G.W. (1989): "Statistical methods" 8th ed., lowa State University Press/Ames

[3] DIN 661 41: Darstellung von Korngrößenverteilungen, DIN 661 61: Partikelgrößenanalyse (Beuth-Vertrieb GmbH, Berlin 30, FRG und Köln 1, FRG)

6. Generation of the test atmosphere / chamber description:
A head-nose inhalation system INA 20 (glass-steel construction, BASF Aktiengesellschaft, volume V $\approx$ 55 l) was used. The animals were restrained in glass tubes and their snouts projected into the inhalation system.

A liquid aerosol was generated. The unchanged test-substance (pyraclostrobin in Solvesso) was used for the test. The aerosol was produced by continuously pumping the test-substance to a two-component atomizer. Using compressed air, the aerosol was generated inside the exposure system.

The exposure system was located inside an exhaust cabin in an air-conditioned laboratory. A supply airflow (compressed air) of 0.65 m³/h for Test groups 1 to 3 and of 0.75 m³/h for Test group 4 was used for the exposure. Additionally, 1.35 and 1.25 m³/h of air was pumped into the exposure system for Test groups 1 to 3 and 4, respectively. The exhaust airflow was 1.8 m³/h. An air change of about 36 times per hour can be calculated by dividing the supply air flow by the volume of the inhalation system. The lower amount of exhaust air, which was adjusted by means of a separate exhaust air system, achieved a positive pressure inside the exposure system. This ensured that the mixture of test substance and air was not diluted with laboratory air in the breathing zones of the animals.

The animals were exposed to the inhalation atmosphere for 4 hours plus an equilibration time for the inhalation system of about 10 minutes.

7. Analytical investigation:

The flows of supply and exhaust air was adjusted and continuously measured with flow meters. Air flows, the temperature and the humidity in the exposure system were recorded four times at about 1-hour intervals.
The oxygen content in the inhalation system was not measured. The air change was judged to be sufficient to prevent oxygen depletion by the breathing of the animals, and the concentrations of the test substance used could not have a substantial influence on oxygen partial pressure.

The **nominal concentration** was calculated from the amount of substance dosed and the supply airflow.

The sampling for determination of the **actual aerosol concentration** was performed with a sampling station using a sampling probe (diameter 7 mm) with quartz wool plug and 3 impingers connected in series filled with sorption solvent.

- Sorption solvent: 2-propanole
- Sampling position: immediately adjacent to the animals' noses
- Sampling flow: 3 l/min
- Sampling velocity: 1.25 m/s
- Sampling frequency: 4 samples at about hourly intervals
- Sample volume: Test group 1: 15 l; Test group 2: 7 l; Test groups 3 and 4: 5 l
  The sampling volumes were adjusted to achieve suitable amounts of test substance for analysis

The quantification of pyraclostrobin was performed by HPLC using a Hewlett Packard HP 1050. The analytical conditions were as follows:

- Colum: metal 250 mm x 4 mm; Polygosil 60-5-C18
- Detection: UV detector at 274 nm
- Injection volume: 10 µl
- Column temperature: ambient
- Mobil phase: 70% Acetonitrile + 0.5 M H_2SO_4 (1000 ml + 5 ml)
  30% bidistilled water + 0.5 M H_2SO_4 (1000 ml + 5 ml)
- Flow rate: 1.2 ml/min

The calibration curve of pyraclostrobin in the solvent (Solvesso) had a linear range from 1.01 to 30.3 mg/50 ml. Samples were taken up in an appropriate volume of solvent, transferred to a calibrated 50 ml flask and filled up to the volume.

The particle size analysis was done using a Stack Sampler Mark III (Andersen) and a vacuum pump (Millipore). The sampling probe had an internal diameter 6.9 mm.
Before sampling, the impactor was assembled and equipped with a backup particle filter. The impactor was connected to the vacuum pump and for each test group samples were taken from the breathing zone of the animals starting not earlier than 30 minutes after the beginning of the exposure. The sample volumes were 45, 27, 12 and 6 l for Test groups 1 to 4, respectively. Two samples were taken for Test group 1 whereas one sample each was taken for Test groups 2 to 4.
After sampling the impactor was taken apart. The impactor stages and the backup particle filter were eluted individually with the solvent indicated above. The samples obtained were analyzed as described above. The amounts of material adsorbed to the walls of the impactor and in the sampling probe (wall losses) were also determined quantitatively.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality was observed in Test groups 2 to 4. Details are given in Table 5.2.3-1

Table 5.2.3-1: Mortality of rats exposed for 4 hours to a liquid aerosol of pyraclostrobin in Solvesso

Test group	Concentration [mg/L]	Cumulative mortality		Time of deaths
		Male	Female	
1	0.89	0/5	0/5	-
2	1.96	1/5	0/5	d0
3	4.07	0/5	1/5	d0
4	7.3	5/5	4/5	≤ 4h (6), d0 (2), d1 (1)

h = hour; d0 =post exposure at day 0; d1 = first day after exposure

Based on the observed mortality the following LC_{50} values respectively ranges were determined:

- LC_{50} (both sexes combined) 4.07 mg/L < LC_{50} < 7.3 mg/L
- LC_{50} (female rats) 5.47 mg/L
- LC_{50} (male rats) 4.07 mg/L < LC_{50} < 7.3 mg/L

The 95% confidence interval for the LC_{50} in females is 2.3 - 18.06 mg/L.

B. CLINICAL OBSERVATIONS

Clinical signs observed consisted of visually accelerated respiration, gasping, respiratory sounds, eyelid closure, apathy, squatting posture, piloerection and smeared fur. The maximum incidence and duration of the observations are indicated in Table 5.2.3-2.

Table 5.2.3-2: Clinical signs in rats exposed for 4 hours to a liquid aerosol of pyraclostrobin in Solvesso

Concentration [mg/L]	0.89	1.96	4.07	7.3
Males				
- Total number of animals	5	5	5	5
- Respiration, visually accelerated	5 / ≤1h - d1	5 / ≤1h - d6	5 / ≤1h - d6	5 / ≤1h - ≤ 4h
- Gasping	-	2 / d0 - d1	-	-
- Respiratory sounds	-	-	1 / d1	-
- Eyelid closure	5 / d0	4 / d0	5 / d0	-
- Apathy	-	-	-	1
- Squatting posture	5 / d0	5 / d0 - d3	5 / d0 - d6	1 / d0
- Piloerection	5 / d0 - d1	-	-	-
- Fur, smeared	-	5 / d0 - d1	5 / d0 - d1	1 / d0
Females				
- Total number of animals	5	5	5	5
- Respiration, visually accelerated	5 / ≤1h - d1	5 / ≤1h - d3	5 / ≤1h - d6	5 / ≤1h - d3
- Gasping	-	-	-	1 / d0
- Respiratory sounds	-	-	2 / d1 - d6	1 / d0
- Eyelid closure	5 / d0	2 / d0 - d2	4 / d0	-
- Apathy	-	-	-	2 /d0
- Squatting posture	5 / d0	5 / d0 - d3	4 / d0 - d6	1 / d0 - d3
- Piloerection	5 / d0 - d1	-	-	-
- Fur, smeared	5 / d1	5 / d0 - d3	4 / d0 - d1	2 / d0 - d3

Abbreviations see Table 5.2.3-1; dn = day n after exposure

C. BODY WEIGHT

Mean body weights of male and female animals at 0.89 and 1.96 mg/L as well as males at 4.07 mg/L increased throughout the study period. In females exposed to pyraclostrobin at 4.07 mg/L a body weight loss was observed during the first week. However, these animals re-gained weight during the second week. Overall body weight gain of this group was marginal (+0.3%) compared to that in females at 0.89 mg/L (+4.8%) and 1.96 mg/L (+2.9%). Overall body weight gain in males ranged from 8.5 to 13.3%.

Body weight data for animals exposed to pyraclostrobin at 7.3 mg/L are only available for one surviving female. This female gained weight throughout the study (overall +3.7%).

D. NECROPSY

Gross necropsy of animals dying as well as necropsied at the end of the observation period revealed mainly discolorations in the lung. In dead animals exposed to the highest concentration of pyraclostrobin, wet and contaminated fur was observed additionally. Incidences are given in Table 5.2.3-3.

Table 5.2.3-3: Necropsy findings in rats exposed for 4 hours to a liquid aerosol of pyraclostrobin in Solvesso

Concentration [mg/L]	0.89	1.96	4.07	7.3
Animals dying (males/females)				
Number of affected animals	-/-	1/-	-/1	5/4
Organs without particular findings	-/-	1/-	-/-	-/-
Fur:				
- head and sternal region contaminated wet	-/-	1/-	-/-	5/4
Lung:				
- dark red discoloration, all lobes	-/-	-/-	-/1	4/3
- grey-red discoloration, all lobes	-/-	-/-	-/-	1/1
Animals killed at the end of the observation period (males/females)				
Number of affected animals	5/5	4/5	5/4	-/1
Animals without particular findings	5/5	-/-	-/-	-/-
Lung:				
- grey-red discoloration, all lobes	-/-	4/5	-/-	-/-
- dark red discoloration, all lobes	-/-	-/-	5/4	-/-
- red discoloration, most lobes	-/-	-/-	-/-	-/1

- not detected

E. ATMOSPHERE ANALYSIS

The exposure conditions are summarized in Table 5.2.3-4.

Table 5.2.3-4: Exposure conditions

Test group	Supply air (compressed) [m³/h]	Supply air (conditioned) [m³/h]	Exhaust air [m³/h]	Substance flow [ml/h]	Temp. [°C]	Relative humidity [%]
1	0.65	1.35	1.8	150	23.1	46.5
2	0.65	1.35	1.8	227.5	22.7	56.8
3	0.65	1.35	1.8	550	23.0	55.9
4	0.74	1.25	1.8	400	22.1	57.8

The results of the concentration measurements (mean of 4 measurements) are presented in Table 5.2.3-5.

Table 5.2.3-5: Measurement of concentrations

Test group	Mean concentration [mg/L]	Standard deviation	Nominal concentration [mg/L]
1	0.89	0.12	81.8
2	1.96	0.15	151.2
3	4.07	0.42	299.8
4	7.30	1.09	218.0

The measurements of particle-size distribution revealed mass mean aerodynamic diameters (MMAD) in the range of 2.7 to 4.3 µm with geometric standard deviations in the range of 2.5 to 2.7 (see Table 5.2.3-6).

Table 5.2.3-6: Particle size measurements

Test group	Sample	MMAD [µm]	GSD	Mass % < 3 µm
1	1	4.0	2.5	37.7
1	2	4.3	2.6	35.5
2	1	3.5	2.6	43.3
3	1	3.8	2.7	40.6
4	1	2.7	2.7	54.7

Greater than 35.5% of the particles had a diameter of ≤ 3.0 µm and thus may have reached the alveolar space of the lung.

III. CONCLUSION

Under the conditions of this study the combined 4 hour inhalation LC_{50} was estimated to be 4.07 mg/L < LC_{50} < 7.3 mg/L after exposure to a liquid aerosol of 40% pyraclostrobin in Solvesso. This estimate was true if males or both sexes combined were considered. For females an LC_{50} of 5.47 mg/L was estimated.

Based on the results of this study no classification according EU CLP criteria would be proposed. The extent of mortality as well as the clinical signs observed at 4.07 mg/l strongly indicate that the LC_{50} is above 5 mg/L.

Report:	CA 5.2.3/2 [redacted] 2002a BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2002/1012053
Guidelines:	OECD 403, EEC 92/69 B 2, EPA 870.1300
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 5.2.3/3 [redacted] 2003a Amendment No. 1 to the report: BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2003/1009200
Guidelines:	OECD 403, EEC 92/69 B 2, EPA 870.1300
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 5.2.3/4 [redacted] 2003b Amendment No. 2 to the report: BAS 500 F - Acute inhalation toxicity study in Wistar rats - 4-hour liquid aerosol exposure 2003/1009427
Guidelines:	OECD 403, EEC 92/69 B 2, EPA 870.1300
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an acute inhalation toxicity study, four groups of Wistar CrlGlxBrlHan:Wi (5/sex) were exposed to liquid aerosols of pyraclostrobin (batch 96/0308; purity 98.2%) in acetone at concentrations of 0.52, 0.65 and 0.85 mg/L for 4 hours. The animals were observed for 14 days after exposure.

Mortality was observed in 0, 5, and 5 males and 1, 4 and 5 females at 0.52, 0.65 and 0.85 mg/L, respectively. The following LC_{50} values were estimated:

LC_{50}, male and female rats	**0.58 mg/L**
LC_{50}, female rats	**0.58 mg/L**
LC_{50}, male rats	**0.58 mg/L**

Clinical signs observed consisted of visually accelerated respiration, attempts to escape, squatting posture and piloerection. Clinical signs resolved latest by day 7 of the observation period. Gross necropsy of animals dying revealed mainly red discolorations in the lung. In most cases all lung lobes were affected. Additionally, wet and contaminated fur was observed in animals dying at the mid concentration level. One mid concentration male displayed lung edema in all lobes.

The mass mean aerodynamic diameter (MMAD) ranged from 1.2 µm (0.52 mg/L) to 1.7 µm (0.85 mg/L) with geometric standard deviations in the range of 2.5 to 2.7. Greater 94.3% of the particles had a diameter of ≤ 5.5 µm and thus may have reached the alveolar space of the lung.

Based on the results of this study pyraclostrobin has to be classified as "Toxic by inhalation" (Category 3) according to the EU CLP (1272/2008).

A. MATERIALS

1. Test Material: Pyraclostrobin (BAS 500 F)

Description:	Viscous melt, brownish-clear
Lot/Batch #:	CP026063
Purity/content:	98.2%
Stability of test compound:	The test material was repeatedly analyzed between 1996 and 2003. The purity did not change over time. The stability of the test substance was guaranteed in the last analysis until February 2007.

2. Vehicle and/or positive control: Acetone

3. Test animals:

Species:	Rat
Strain:	Wistar CrlGlxBrlHan:Wi
Sex:	15 males and 15 females
Age:	males: 8 to 12 weeks; females: 14 to 18 weeks
Weight at dosing:	247.4 ± 12.7 g (males), 223.1 ± 10.3 g (females)
Source:	Charles River Laboratories, Germany, 97633 Sulzfeld
Acclimation period:	at least one week
Diet:	Kliba rat/mouse/hamster diet, pelleted, Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum except during exposure
Water:	Tap water ad libitum except during exposure
Housing:	single housing in stainless steel wire mesh cages type DK-III (Becker & Co., Castrop-Rauxel, FRG)

Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 27-Aug-2002 to 09-Oct-2002

2. Animal assignment and treatment:
For determination of the acute inhalation toxicity (single 4-hour-exposure) of pyraclostrobin dissolved in acetone as a liquid aerosol groups of five male and 5 female rats were exposed to aerosols at three concentrations. The animals were randomly selected from a pool of animals.

3. Clinical examinations:
For each test group, the body weight of the animals was determined just prior to exposure (day 0), weekly thereafter and at the end of the observation period. A check for overt clinical signs of toxicity or mortality as well as a check for the presence of feed and drinking water was made twice a day on workdays and once daily on weekends and public holidays. Detailed clinical observations were recorded for each animal separately several times during exposure and at least once on each workday of the observation period.

4. Pathology:
At the end of the 14-day observation period the surviving animals were sacrificed with CO_2 and were subjected to gross-pathological examination like those performed for animals which had died prematurely.

5. Statistics/Calculations:
The statistical evaluation of the concentration-response relationship was carried out using a computer program. Depending on the data of the concentration-response relationship obtained by the experiment, this program is used to estimate the LC_{50} or to perform a Probit analysis[1]. Estimation of the LC_{50} will produce types "LC_{50} greater than", "LC_{50} approx.", or "LC_{50} smaller than". If the results are of the type "LC_{50} greater than" or "LC_{50} smaller than", an additional binomial test is carried out, in order to verify these Statements statistically[2].

The calculation of the particle size distribution was carried out in the inhalation laboratory on the basis of mathematical methods for evaluating particle measurements[3].

[1] FINNEY, D.J. (1971): "Probit Analysis" Cambridge University Press, pp. 1 - 50

[2] SNEDECOR, G.W. (1989): "Statistical methods" 8th ed., lowa State University Press/Ames

[3] DIN 661 41: Darstellung von Korngrößenverteilungen, DIN 661 61: Partikelgrößenanalyse (Beuth-Vertrieb GmbH, Berlin 30, FRG und Köln 1, FRG)

6. Generation of the test atmosphere / chamber description:
A head-nose inhalation system INA 20 (glass-steel construction, BASF Aktiengesellschaft, volume V ≈ 55 l) was used. The animals were restrained in glass tubes and their snouts projected into the inhalation system.

A liquid aerosol was generated. For test the unchanged test-substance (pyraclostrobin in Solvesso) was used. The aerosol was produced by continuously pumping the test-substance preparation (2:1 (w/w) acetone : pyraclostrobin) to a two-component atomizer. The 2:1 acetone pyraclostrobin mixture was the maximum concentration suitable for pumping and spraying. Using compressed air, the aerosol was generated which was produced inside the exposure system.

The exposure system was located inside an exhaust cabin in an air-conditioned laboratory. A supply airflow (compressed air) of 1.5 m³/h were used for the exposure. The exhaust airflow was set at 1.35 m³/h. An air change of about 27 times per hour can be calculated by dividing the supply air flow by the volume of the inhalation system. The lower amount of exhaust air, which was adjusted by means of a separate exhaust air system, achieved a positive pressure inside the exposure system. This ensured that the mixture of test substance and air was not diluted with laboratory air in the breathing zones of the animals.

The animals were exposed to the inhalation atmosphere for 4 hours plus an equilibration time for the inhalation system of about 10 minutes.

7. Analytical investigation:

The flows of supply and exhaust air was adjusted and continuously measured with flow meters. Air flows, the temperature and the humidity in the exposure system were recorded four times at about 1-hour intervals.
The oxygen content in the inhalation system was not measured. The air change was judged to be sufficient to prevent oxygen depletion by the breathing of the animals, and the concentrations of the test substance used could not have a substantial influence on oxygen partial pressure.

The **nominal concentration** was calculated from the amount of substance dosed and the supply airflow.

The sampling for determination of the **actual aerosol concentration** was performed with a filtration equipment using a sampling probe (diameter 7 mm). The quantification of pyraclostrobin was performed by gravimetric determination. For this pre-weighed filters were placed into the filtration equipment. By means of the vacuum pump metered volumes of the liquid aerosol were drawn through the filter.

- Filter: MN 85/90 BF (d = 47 mm)
- Sampling position: immediately adjacent to the animals' noses
- Sampling flow: 3 l/min
- Sampling velocity: 1.25 m/s
- Sampling frequency: 4 samples at about hourly intervals
- Sample volume: Test group 1: 9 l; Test group 2 and 3: 6 l;
  The sampling volumes were adjusted to achieve suitable amounts of test substance for analysis

The gravimetric method was validated by HPLC analysis in the previously submitted study [BASF DocID 1997/11472]. For each sample the liquid aerosol concentration in mg/L was calculated from the difference between the pre-weighed of the filter and the weight of the dried filter after sampling and, with reference to the sample volume of the inhalation atmospheres.

The particle size analysis was done using a Stack Sampler Mark III (Andersen) and a vacuum pump (Millipore). The sampling probe had an internal diameter 6.9 mm.
Before sampling, the impactor was assembled with pre-weighed glass-fiber collecting disks and equipped with a backup particle filter. The impactor was connected to the vacuum pump and for each test group samples were taken from the breathing zone of the animals starting not earlier than 30 minutes after the beginning of the exposure. The sample volumes were 45, 30 and 24 l for Test groups 1 to 3, respectively. Two samples were taken for Test group 1 whereas each one sample was taken for Test groups 2 and 3.
After sampling the impactor was taken apart. The impactor stages and the backup particle filter were freed of acetone and re-weighed.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality was observed in Test groups 2 to 4. Details are given in Table 5.2.3-7.

Table 5.2.3-7: Mortality of rats exposed for 4 hours to a liquid aerosol of pyraclostrobin dissolved in acetone

Test group	Concentration [mg/l]	Cumulative mortality		Time of deaths
		Male	Female	
1	0.52	0/5	1/5	d0
2	0.65	5/5	4/5	h2 (1), h3 (4), d0 (4)
3	0.85	5/5	5/5	h1 (3), d0 (7)

h = hour; d0 =post exposure at day 0; d1 = first day after exposure

Based on the observed mortality the following LC_{50} values respectively ranges were determined (see Report Amendment No. 1):

- LC_{50} (both sexes combined) 0.58 mg/L (0.54 - 0.63 mg/L)
- LC_{50} (female rats) 0.58 mg/L
- LC_{50} (male rats) 0.58 mg/L (0.39 - 0.78 mg/L)

The 95% confidence interval for the LC_{50} in both sexes and in females is given in above in brackets. As no LC_{50} for male rats could be derived by statistical evaluation, a point estimate of the LC_{50} value was calculated as the geometrical mean of the highest test substance concentration causing no lethality and the lowest concentration, at which 100 % lethality occurred in male rats.

B. CLINICAL OBSERVATIONS

Clinical signs observed consisted of visually accelerated respiration, attempts to escape, squatting posture and piloerection. The maximum incidence and duration of the observations are indicated in Table 5.2.3-8.

Table 5.2.3-8: Clinical signs in rats exposed for 4 hours to a liquid aerosol of pyraclostrobin in acetone

Concentration [mg/l]	0.52	0.65	0.85
Males			
- Total number of animals	5	5	5
- Respiration, visually accelerated	5 / h0 - d3	5 / h0 - d3	5 / h0 - d3
- Attempts to escape	1 / h0	-	-
- Squatting posture	5 / d0	-	-
- Piloerection	3 / d0 - d3	-	-
Females			
- Total number of animals	5	5	5
- Respiration, visually accelerated	5 / h0 - d3	5 / h0 - d6	5 / h0 - h3
- Attempts to escape	2 / h0	-	2 / h0
- Squatting posture	4 / d0	1/ d0 - d1	-
- Piloerection	1 / d0 - d1	-	-

Abbreviations see Table 5.2.3-7; dn = day n after exposure; - not detected

C. BODY WEIGHT

Mean body weights of males at 0.52 mg/L increased throughout the study period. In the 4 surviving females exposed to pyraclostrobin at 0.52 mg/L and the one surviving female at 0.65 mg/L a body weight loss was observed during the first week. However, these animals re-gained weight during the second week.

D. NECROPSY

Gross necropsy of animals dying revealed mainly diffuse red discolorations in the lung. At the mid concentration level (0.65 mg/L) wet and contaminated fur was observed. Additionally, one mid dose male displayed edema in all lung lobes. Incidences are given in Table 5.2.3-9.

Table 5.2.3-9: Necropsy findings in rats exposed for 4 hours to a liquid aerosol of pyraclostrobin in acetone

Concentration [mg/l]	0.52	0.65	0.85
Animals dying (males/females)			
Number of affected animals	-/1	5/4	5/5
Organs without particular findings	-/-	-/-	-/-
Fur:			
- head and abdominal region contaminated wet	-/-	5/4	-/-
Lung:			
- red and diffuse discolored lung lobes	-/1	-/-	-/-
- diffuse dark red discoloration, all lobes	-/-	5/4	5/5
- edema, all lobes	-/-	1/-	-/-

- not detected

E. ATMOSPHERE ANALYSIS

The exposure conditions are summarized in Table 5.2.3-10.

Table 5.2.3-10: Exposure conditions

Test group	Supply air (compressed) [m³/h]	Exhaust air [m³/h]	Substance flow[1] [ml/h]	Atomizer pressure [bar]	Temp. [°C]	Relative humidity [%]
1	1.5	1.35	3.2	1.5	23.2 ± 0.3	22.5 ± 5.8
2	1.5	1.35	4.5	1.5	22.8 ± 0.0	17.9 ± 1.8
3	1.5	1.35	6.0	1.6	23.1 ± 0.1	20.8 ± 2.3

[1] Correlated to the substance dilution the substance flows were 2.13 (test group 1), 3.00 (test group 2) and 4.00 ml/h (test group 3).

The low humidity values measured in this study may indicate an influence of the test substance atmosphere on the measuring device. However, if there was any deviation from the guideline recommendations (30% - 70% relative hum.; especially low humidity in liquid aerosols), it is considered not of influence for the test results, because of the relatively short exposure time.

The results of the concentration measurements (mean of 4 measurements) are presented in Table 5.2.3-11.

Table 5.2.3-11: Measurement of concentrations (4 hourly measurements)

Test group	Mean concentration [mg/l]	Standard deviation	Nominal concentration [mg/L]
1	0.52	0.04	0.71
2	0.65	0.06	1.00
3	0.85	0.07	1.33

The measurements of particle-size distribution revealed mass mean aerodynamic diameters (MMAD) in the range of 1.2 to 1.7 µm with geometric standard deviations in the range of 2.5 to 2.7 (see Table 5.2.3-12).

Table 5.2.3-12: Particle size measurements

Test group	Sample	MMAD [µm]	GSD	Mass % < 3 µm
1	1	1.2	2.5	84.5
1	2	1.3	2.6	82.0
2	1	1.4	2.5	79.9
3	1	1.7	2.7	72.4

Greater than 72.4% of the particles had a diameter of ≤ 3.0 µm and thus may have reached the alveolar space of the lung.

III. CONCLUSION

Under the conditions of this study the 4 hour inhalation LC_{50} was estimated to be 0.58 mg/L after exposure to a liquid aerosol of pyraclostrobin dissolved in acetone.

Based on the results of this study pyraclostrobin has to be classified as "Toxic by inhalation" (Category 3) according to the EU CLP (1272/2008).

Overall assessment of inhalation toxicity

Dissolved in acetone pyraclostrobin was toxic to rats when inhaled as an aerosol for 4 hours. A study performed 1997 revealed an acute toxicity value of 0.31 mg/L < LC_{50} < 1.07 mg/L with no and 100% mortality at 0.31 and 1.07 mg/L, respectively. The derived LC_{50} in the List of Endpoints was 0.69 mg/L. A new study performed in 2002 at concentrations of 0.52, 0.65 and 0.85 mg/L, using also acetone as solvent, revealed an LC_{50} of 0.58 mg/L. The LC_{50} values of pyraclostrobin dissolved in acetone are leading to the CLP classification “H331 - Toxic if inhaled”.

A substantially different result was obtained in a second new acute inhalation study performed in 2001, using Solvesso 200 as a solvent, revealing an acute inhalation toxicity value of 4.07 < LC_{50} < 7.3.

Thus, the solvent (used in the acute inhalation studies to generate an inhalable aerosol) is strongly influencing the acute inhalation toxicity of pyraclostrobin. In addition, the size of aerosol particles may modulate the inhalation toxicity.

CA 5.2.4 Skin irritation

The acute skin irritation study of pyraclostrobin was evaluated and peer reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Pyraclostrobin is irritant to the skin.

In the study report (see BASF DocID 1998/10959) it is stated in section 4.1 "that the stability of the test substance over the study period will be investigated by reanalysis and reported in the form of an amendment". A respective amendment was generated (BASF DocID 2000/1018990) and is now included in the updated dossier. Details are shown below.

Report:	CA 5.2.4/1 [redacted] 2000 a Amendment No. 1: BAS 500 .. F - Acute dermal irritation/corrosion in rabbits 2000/1018990
Guidelines:	OECD 404, EEC 92/69 B 4, EPA 81-5
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive summary

The test substance was stable over the study period.

CA 5.2.5 Eye irritation

The acute eye irritation study of pyraclostrobin was evaluated and peer reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Pyraclostrobin is not irritant to the eye.

In the study report (see BASF DocID 1998/10963) it is stated in section 4.1 "that the stability of the test substance over the study period will be investigated by reanalysis and reported in the form of an amendment". A respective amendment was generated (BASF DocID 2000/1018989) and is now included in the updated dossier. Details are shown below.

Report:	CA 5.2.5/1 [redacted] 2000 b Amendment No. 1: BAS 500 .. F - Acute eye irritation in rabbits 2000/1018989
Guidelines:	OECD 405, EPA 81-4, EEC 92/69 B 5
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive summary

The test substance was stable over the study period.

CA 5.2.6 Skin sensitisation

The skin sensitisation study (Maximisation Test) of pyraclostrobin was evaluated and peer reviewed during the previous Annex I inclusion process (for details please see Table 5.2-1). Pyraclostrobin is not sensitising.

CA 5.2.7 Phototoxicity

Report:	CA 5.2.7/1 Cetto V., Landsiedel R., 2012a BAS 500 F (Pyraclostrobin) - In vitro 3T3 NRU Phototoxicity Test 2012/1189936
Guidelines:	OECD 432 (2004) In vitro 3T3 NRU Phototoxicity Test, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.41 No. L 142
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 5.2.7/2 Cetto V., Landsiedel R., 2014a Amenment No. 1 - BAS 500 F (Pyraclostrobin) - In vitro 3T3 NRU phototoxicity test 2014/1092412
Guidelines:	OECD 432 (2004) In vitro 3T3 NRU Phototoxicity test, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.41 No. L 142
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: The report amendment (CA 5.2.7/2) corrects some clerical errors, which do not affect the outcome or interpretation of the study.

Executive Summary

Pyraclostrobin (batch COD-001236; purity 99.02%) was tested for its ability to induce phototoxic effects in Balb/c 3T3 cells in vitro. The photo-cytotoxicity was estimated by the means of the Neutral Red Uptake (NRU) method. Three experiments were carried out with and without irradiation with an UVA source. Vehicle and positive controls were included into each experiment. The 1st experiment failed to fulfil the acceptance criteria and the results of the 2nd experiment were confirmed in a 3rd experiment. The latter two experiments fulfilled the acceptance criteria.

Based on an initial range-finding phototoxicity test for the determination of the experimental concentrations, the following concentrations were tested in this study with and without UVA irradiation: 0, 0.5, 1.0, 2.2, 4.6, 10.0, 21.5, 46.4 and 100.0 µg/mL.

Precipitation was seen at the top dose of 100 µg/mL with and without irradiation. In the absence and the presence of UVA irradiation cytotoxicity was noted and EC_{50} values were calculated. Based on the results of the present study, the test substance was predicted to have no phototoxic potential as indicated by Photo-Irritancy-Factor (PIF) values of 1.6 and 0.9 for the 2nd and 3rd experiment, respectively. The threshold for a negative response is ≤ 2. The positive control chlorpromazine led to the expected increased cytotoxicity with UVA irradiation as indicated by PIF values of 29.8 and 40.8.
Thus, under the experimental conditions of this study, pyraclostrobin is not considered to be a phototoxic substance in the in vitro 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	BAS 500 F (pyraclostrobin)
Description:	Solid, melt, red to brown
Lot/Batch #:	COD-001236
Purity:	99.02% (tolerance ± 1.0%)
Stability of test compound:	Expiry date: 01.10.2015
Solvent used:	Dimethylsulfoxide (DMSO) Even though the report indicated that the stability in DMSO was not determined analytically in the course of this study, the stability of pyraclostrobin in DMSO was demonstrated in earlier studies (e.g. study 08B0308/966022).
2. Control Materials:	
Vehicle control:	DMSO 1% (v/v) in PBS
Positive control compounds:	Chlorpromazine (CPU) was dissolved in DMSO; 8 concentrations tested - 1.9 to 180 µg/mL without radiation, 0.03 to 3.2 µg/mL with radiation
3. Test organisms:	BaIb/c 3T3, clone A31: fibroblast cell line isolated from the muscle tissue of a mouse embryo. The Balb/c 3T3 cell line which was used in this experiment was obtained from the "European Collection of Cell Cultures" Salisbury, Wiltshire SP4 OJG, UK on 09 Aug 2006 and is stored at -196°C (liquid nitrogen).

4. Culture media and reagents:

Culture medium:	Dulbecco's Modified Eagle's Medium (DMEM) supplemented with - 10% (v/v) newborn calf serum (NCBS) - 4 mM L-glutamine - 100 IU penicillin - 100 µg/mL streptomycin
Neutral Red solution:	- 0.4 g Neutral Red powder (NR; Sigma N4638) - 100 mL deionized water
Neutral Red medium:	- 1 mL Neutral Red solution - 79 mL culture medium (DMEM incl. supplements) (incubated overnight at 37° C with 5% CO_2 and filtered with a 0.22 µm filter prior to use)
Other solutions and reagents:	- phosphate buffered saline (PBS) without Ca/Mg - trypsin/EDTA solution (0.05%; 0.02%) - Neutral Red desorb solution (1 mL acetic acid, 50 mL ethanol, 49 mL deionized water)

5. Irradiation source: The Sol 500 solar simulator (Dr. Hönle AG, 82166 Gräfelfing, Germany) used with filter H1 produced wavelength > 320 nm. The exposure rates were determined with UV-meter RM-21 (Dr. Gröbel GmbH, 76275 Ettlingen, Germany).

6. Test concentrations:

Pretest:	Up to 1000 µg/mL with and without irradiation. The EC_{50} values determined were 56.7 µg/mL without and 26.3 µg/mL with UVA irradiation (no detailed data provided in the report).
Main NRU test:	Based on the results of the pretest the following concentrations were used in the main study: Without UVA: 0.5, 1.0, 2.2, 4.6, 10.0, 21.5, 46.4, 100.0 µg/mL With UVA: 0.5, 1.0, 2.2, 4.6, 10.0, 21.5, 46.4, 100.0 µg/mL

B. TEST PERFORMANCE:

1. Dates of experimental work: 04-Jun-2013 to 04-Jul-2013

2. Treatment and NRU Phototoxicity Test:

Two 96 well-plates per substance (test substance or positive control) were used for cultivation of cells (1.5 x 10^5 cells/well). After an attachment period of about 24 hours the cells were washed once with 100 µL PBS and subsequently treated with the respective substance (8 concentrations each with 6 replicates of the test substance or the positive control) and the vehicle control. After pre-incubation for 1 hour in the dark (5% (v/v) CO_2, ≥90% humidity; 37°C) one 96 well-plate per substance was irradiated for 50 minutes with UVA (UV intensity underneath the lid 1.5 - 2.1 mW/cm^2 = 5 J/cm^2) whereas the respective reference plate was kept in the dark for the same period. Thereafter the test- respectively control-substance was removed and the cells washed at least once with 100 µL PBS. After replenishing the wells with culture medium the cells were incubated overnight under the conditions indicated above. The medium was removed 24 hours after the start of treatment and after washing with 100 µL PBS the wells were filled with 100 µL medium containing 50 µg/mL Neutral Red. Subsequently the plates were incubated for another 3 hours. Each step was performed under light protected conditions in the lab to prevent uncontrolled photo activation. Finally, the cells were washed again with 100 µL and the dye was extracted by 100 µL Neutral Red desorb solution. Cytotoxicity was determined by measuring the Neutral Red Uptake by means of a microplate reader (Perkin Elmer, Waltham, Massachusetts, US; Wallac 1420 multilabel counter) equipped with a 550 nm filter to read the absorption of the extracted dye. The absorption shows a linear relationship with the number of surviving cells.

In this study 3 main experiments were conducted. The 1st revealed no indication of phototoxicity but the standard deviation of both vehicle controls of the non-irradiated plate treated with the test substance exceeded the acceptance criteria and therefore the 1st experiment was considered invalid. The data of this experiment were not included in the report. The 2nd and 3rd experiment likewise did not reveal indications for cytotoxicity. The 3rd experiment was performed to confirm the results of the 2nd assay.

3. Evaluation/Assessment

3.1 Cytotoxicity

The mean absorbance values obtained for each test group of every plate were used to calculate the percentage of cell viability relative to the respective vehicle control, which is arbitrarily set at 100 %.

$$Viability^{\S}\ [\%] = \frac{\text{Absorbance}_{\text{mean of the test group}}}{\text{Absorbance}_{\text{mean of the vehicle control}}} \times 100$$

§ The authors of the study denominate the above quotient as 'cytotoxicity', which is strictly speaking not correct. Thus, in this summary the appropriate term 'viability' is used. This applies also to Table 5.2.7-1 to Table 5.2.7-4 .

In case of cytotoxicity, an EC_{50} value (concentration at which the viability is reduced by 50% relative to the respective vehicle control) was calculated by a linear interpolation method (linear dose-response curve). Therefore, two viability values were needed: one between 100% and 50% and one between 50% and 0%. From these two points the concentration that inhibits the Neutral Red uptake by 50% of the respective control was calculated.

For the assessment of the phototoxic potential of a compound two prediction models are currently available:

- The Photo-Irritancy-Factor (PIF) prediction model for substances which allow the comparison of two equi-effective concentrations (EC_{50}) in the concurrently performed experiments in the presence and absence of light. This model includes two special cases: Case 1 accounts for situations in which an EC_{50} can only be calculated in the presence of UVA irradiation. Case 2 accounts for situations where an EC_{50} cannot be calculated in absence and presence of irradiation. These special cases do not apply to this study. Even though described in the report these cases are not described in this summary.
- The Mean Photo Effect prediction model which is used if no EC_{50} was obtained in the absence and presence of UV light. This is not the case in this study. Even though described in the report this prediction model is not described in this summary.

3.2 Photo-Irritancy-Factor (PIF)

For substances which induce a 50 % cytotoxicity (EC_{50}) in the presence and absence of light the Photo-Irritancy-Factor (PIF) is calculated based on comparison of the EC_{50} values in the absence (-UVA) and presence (+UVA) of UVA irradiation.

$$PIF = \frac{EC50\ (-UVA)}{EC50\ (+UVA)}$$ resulting in the following classification rules:

PIF ≥5	phototoxic potential predicted
2 < PIF < 5:	probable phototoxic potential predicted
PIF ≤ 2:	no phototoxic potential predicted

3.3 Other parameters

pH:
The pH was measured at least for the two top doses and for the vehicle controls with and without irradiation.

Osmolarity:
Osmolarity was measured at least for the two top doses and for the vehicle controls with and without irradiation.

Solubility:
Test substance precipitation was checked immediately after treatment and at the end of treatment.

Cell morphology:
Test cultures of all test groups were examined microscopically before staining with NRU, which allows conclusions to be drawn about attachment of the cells.

4. Statistics:

No special statistical tests were performed.
Mean absorbance values and standard deviations were calculated from the single values using calculation software (e.g. MS Excel). The calculations were made using the unedited values. For the report the values were rounded, therefore there may be deviations in the given relative values. If technical errors occurred in single wells (outlier) at least 4 single values per test group were sufficient for calculating reliable mean values. Outliers are defined as values that have half or double the value of the respective mean.

5. Acceptance criteria:

The assay has to be considered valid if the following criteria are met:

- The vehicle control needs to fulfill the following criteria:
  - The mean OD550 value (with and without UVA irradiation) should be > 0.3.
  - Cell viability after irradiation should be at least 80% of the concurrent non-irradiated vehicle control.
  - The standard deviation of the mean values of both vehicle control rows should not exceed ± 15%.
- The positive control chlorpromazine needs to fulfill the following criteria:
  - the EC50 value should be in the ranges:
    With irradiation (+UVA): 0.1 - 2.0 µg/mL
    Without irradiation (-UVA): 7.0 - 90.0 µg/mL
  - and the PIF ≥ 6.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Osmolarity and pH values were not influenced by test substance treatment. In this study, in the absence and the presence of UVA irradiation precipitation in culture medium was observed at test substance concentrations of ≥ 46.4 µg/mL.

B. CYTOTOXICITY OF THE TEST SUBSTANCE

After treatment with the test substance, clear cytotoxic effects indicated by Neutral Red absorbance values of below 50% of control were observed in the main experiments in the absence and the presence of UVA irradiation (see Table 5.2.7-1 (2nd experiment) and Table 5.2.7-2 (3rd experiment)).

Without UVA irradiation, there was a decrease in the cell number at concentrations ≥ 46.4 µg/mL (EC_{50}: 37.2 µg/mL) in the 2nd experiment and at ≥ 10 µg/mL (EC_{50} 8.6 µg/mL) in the 3rd experiment. With UVA irradiation, there was a decrease in the cell number at concentrations ≥ 21.5 µg/mL (EC_{50}: 23.2 µg/mL) in the 2nd experiment and at ≥ 10 µg/mL (EC_{50}: 10.0 µg/mL) in the 3rd experiment. Cell morphology changes were restricted to the highest concentration (100 µg/mL) in the 3rd experiment with and without UVA irradiation.

Based on the EC_{50} values a PIF of 1.6 and 0.9 were calculated for the 2nd and 3rd experiment, respectively, indicating no phototoxic potential for pyraclostrobin.

Table 5.2.7-1: Mean relative viability of pyraclostrobin with (+) and without (-) UVA irradiation in Balb 3T3 cells – 2nd experiment

Test group	UVA irradiation*	Precipitation**	Mean $OD_{corr.}$ ***	Relative viability [% of control]	
				Mean	SD
Vehicle control 1	-	-	0.382	-	3.9
Vehicle control 2	-	-	0.323	-	11.7
Vehicle mean	-	-	0.353	100.0	12.0
Pyraclostrobin					
0.5 µg/mL	-	-	0.342	97.1	5.2
1.0 µg/mL	-	-	0.336	95.2	8.5
2.2 µg/mL	-	-	0.288	81.7	2.8
4.6 µg/mL	-	-	0.246	69.8	3.4
10.0 µg/mL	-	-	0.209	59.3	3.0
21.5 µg/mL	-	-	0.188	53.4	3.5
46.4 µg/mL	-	+	0.170	48.1	5.6
100.0 µg/mL	-	+	0.146	41.4	4.8
Vehicle control 1			0.381	-	3.3
Vehicle control 2			0.353	-	4.6
Vehicle mean	-	-	0.367	100.0	5.5
Pyraclostrobin					
0.5 µg/mL	+	-	0.326	88.9	4.0
1.0 µg/mL	+	-	0.314	85.5	4.6
2.2 µg/mL	+	-	0.266	72.5	4.7
4.6 µg/mL	+	-	0.251	68.4	5.9
10.0 µg/mL	+	-	0.205	55.7	4.1
21.5 µg/mL	+	-	0.185	50.3	2.5
46.4 µg/mL	+	+	0.168	45.9	3.0
100.0 µg/mL	+	+	0.151	41.1	2.6

*: Irradiation with Sol 500 solar simulator for 50 minutes (approx. 5 J/cm²)
**: Precipitation in PBS at the end of exposure period
***: Mean OD corrected: mean absorbance (test group) minus mean absorbance (blank)

Table 5.2.7-2: Mean relative viability of pyraclostrobin with (+) and without (-) UVA irradiation in Balb 3T3 cells – 3rd experiment

Test group	UVA irradiation*	Precipitation**	Mean $OD_{corr.}$ ***	Relative viability [% of control]	
				Mean	SD
Vehicle control 1			0.294	-	10.9
Vehicle control 2			0.278	-	10.7
Vehicle mean	-	-	0.286	100.0	10.7
Pyraclostrobin					
0.5 µg/mL	-	-	0.263	92.0	7.7
1.0 µg/mL	-	-	0.229	80.2	4.7
2.2 µg/mL	-	-	0.191	67.0	2.6
4.6 µg/mL	-	-	0.151	52.8	6.5
10.0 µg/mL	-	-	0.140	49.0	4.5
21.5 µg/mL	-	-	0.102	35.6	2.2
46.4 µg/mL	-	+	0.070	24.3	5.0
100.0 µg/mL	-	+	0.062	21.6	4.0
Vehicle control 1			0.245	-	6.5
Vehicle control 2			0.212	-	9.0
Vehicle mean	-	-	0.229	100.0	10.6
Pyraclostrobin					
0.5 µg/mL	+	-	0.274	119.7	12.0
1.0 µg/mL	+	-	0.206	89.9	8.8
2.2 µg/mL	+	-	0.186	81.5	8.5
4.6 µg/mL	+	-	0.152	66.3	11.8
10.0 µg/mL	+	-	0.114	49.9	6.2
21.5 µg/mL	+	-	0.092	40.4	10.7
46.4 µg/mL	+	+	0.081	35.3	9.4
100.0 µg/mL	+	+	0.044	19.2	10.0

*: Irradiation with Sol 500 solar simulator for 50 minutes (approx. 5 J/cm²)
**: Precipitation in PBS at the end of exposure period
***: Mean OD corrected: mean absorbance (test group) minus mean absorbance (blank)

C. CYTOTOXICITY OF THE POSITIVE CONTROL

After treatment with the positive control chlorpromazine clear cytotoxic effects indicated by Neutral Red absorbance values of below 50% of control were observed in the absence and the presence of UVA irradiation (see Table 5.2.7-3 (2nd experiment) and Table 5.2.7-4 (3rd experiment)).
In the 2nd and 3rd experiment without UVA irradiation, there was a decrease in viability at ≥ 30.0 µg/mL (EC_{50}: 23.2 µg/mL and 24.0 µg/mL). With UVA irradiation, there was a decrease in viability at ≥ 0.8 µg/mL (EC_{50}: 0.7 µg/mL and 0.6 µg/mL). Cell morphology was distinctively changed in both experiments at ≥ 30 µg/mL and ≥ 0.8 µg/mL without and with UVA irradiation.

Based on the EC_{50} values PIF's of 29.8 and 40.8 were obtained in the 2nd and in the 3rd experiment, respectively, indicating a strong phototoxic potential, thus confirming the sensitivity of the test system.

Table 5.2.7-3: Mean relative viability of Chlorpromazine with (+) and without (-) UVA irradiation in Balb/c 3T3 cells – 2nd experiment

Test group	UVA irradiation	Mean OD *	Mean $OD_{corr.}$ **	Relative viability [% of control]	
				Mean	SD
Blank	-	0.036	-	-	-
Vehicle control 1	-	0.425	0.389	-	6.8
Vehicle control 2	-	0.493	0.456	-	6.1
Vehicle mean	-	0.459	0.423	100.0	10.4
Chlorpromazine					
1.9 µg/mL	-	0.483	0.447	105.7	4.8
3.8 µg/mL	-	0.483	0.446	105.6	3.8
7.5 µg/mL	-	0.471	0.434	102.8	5.0
15.0 µg/mL	-	0.432	0.396	93.7	4.7
30.0 µg/mL	-	0.093	0.056	13.3	0.8
60.0 µg/mL	-	0.037	0.001	0.2	1.1
90.0 µg/mL	-	0.035	-0.001	-0.2	0.1
180.0 µg/mL	-	0.036	0.000	-0.1	0.1
Blank	+	0.037	-	-	-
Vehicle control 1	+	0.423	0.386	-	6.0
Vehicle control 2	+	0.450	0.413	-	3.0
Vehicle mean	+	0.436	0.399	100.0	5.7
Chlorpromazine					
0.03 µg/mL	+	0.408	0.371	93.0	1.4
0.05 µg/mL	+	0.430	0.393	98.3	8.2
0.10 µg/mL	+	0.428	0.391	97.9	6.4
0.20 µg/mL	+	0.422	0.385	96.4	6.2
0.40 µg/mL	+	0.378	0.341	85.3	4.9
0.80 µg/mL	+	0.154	0.117	29.2	4.7
1.60 µg/mL	+	0.036	-0.001	-0.2	0.3
3.20 µg/mL	+	0.039	0.002	0.4	0.2

*: Mean absorbance at 550 nm of 6 wells, in general
**: Mean absorbance (test group) minus mean absorbance (blank)

Table 5.2.7-4: Mean relative viability of Chlorpromazine with (+) and without (-) UVA irradiation in Balb/c 3T3 cells – 3rd experiment

Test group	UVA irradiation	Mean OD *	Mean $OD_{corr.}$ **	Relative viability [% of control]	
				Mean	SD
Blank	-	0.035	-	-	-
Vehicle control 1	-	0.375	0.340	-	9.7
Vehicle control 2	-	0.431	0.396	-	4.0
Vehicle mean	-	0.403	0.368	100.0	10.6
Chlorpromazine					
1.9 µg/mL	-	0.431	0.396	107.6	4.1
3.8 µg/mL	-	0.414	0.378	102.9	8.7
7.5 µg/mL	-	0.388	0.353	95.9	8.9
15.0 µg/mL	-	0.381	0.346	94.1	9.5
30.0 µg/mL	-	0.110	0.075	20.4	2.9
60.0 µg/mL	-	0.035	0.000	0.0	0.2
90.0 µg/mL	-	0.036	0.001	0.4	0.7
180.0 µg/mL	-	0.037	0.002	0.5	1.0
Blank	+	0.037	-	-	-
Vehicle control 1	+	0.345	0.308	-	4.3
Vehicle control 2	+	0.375	0.338	-	6.2
Vehicle mean	+	0.360	0.323	100.0	7.0
Chlorpromazine					
0.03 µg/mL	+	0.347	0.310	96.0	11.0
0.05 µg/mL	+	0.318	0.281	86.9	12.5
0.10 µg/mL	+	0.314	0.277	85.8	8.4
0.20 µg/mL	+	0.320	0.283	87.7	16.2
0.40 µg/mL	+	0.286	0.249	77.0	16.6
0.80 µg/mL	+	0.099	0.062	19.2	9.1
1.60 µg/mL	+	0.036	-0.001	-0.2	0.5
3.20 µg/mL	+	0.038	0.002	0.5	1.2

*: Mean absorbance at 550 nm of 6 wells, in general
**: Mean absorbance (test group) minus mean absorbance (blank)

III. CONCLUSION

According to the results of the present study, pyraclostrobin is not considered to be a phototoxic substance in the in vitro 3T3 NRU Phototoxicity Test.

CA 5.3 Short-Term Toxicity

Studies evaluated in the pyraclostrobin draft monograph of Rapporteur Member State Germany (August 1, 2001) consisted of: short-term toxicity studies (28 - 90 days) with oral administration in three different species (rats, mice, dogs). In addition, a 1-year dog study and a 28-day dermal toxicity study in rats were evaluated. These studies have been evaluated by European authorities and Germany as RMS and were considered to be acceptable. For the convenience of the reviewer, these studies are summarized below in tabular form as extracted from the monograph (see Table 5.3-1).

"The signs of toxicity, as observed in the three species tested, were comparable. The critical clinical effects were reduction of body weight and body weight gain in all three species. In dogs, vomitus and diarrhoea occurred additionally. The target organ in all three species was the duodenum, showing mucosal hypertrophy, which was characterised by an increased ratio of cytoplasm to the nuclei in the villi, and by hyperplastic changes in the epithelial cells.
These substance-related effects were associated with changes of several clinical-chemical parameters. The typical findings included a decrease of protein, glucose and triglycerides. These effects might be associated with diminished vacuolization of hepatocytes as observed histologically.
The increase in serum urea values in mice might be indicative of increased protein catabolism or a slightly impaired renal function. The histopathological examination of the kidneys has shown diminished vacuolisation of proximal tubular epithelial cells.
In all three species investigated, hematological changes with correlating compensatory reactions were observed which were considered indicative of toxic effects on red blood cells. Platelet counts were increased in mice and dogs. Concerning white blood cell parameters, rats demonstrated an increase in white blood cells. In mice, adverse effects on white blood cells as well on lymphatic organs (thymus, mesenteric lymph modes) and the adrenals (decreased vacuolisation of cortical cells) were observed.
Only in the rat liver, an increase of liver cell hypertrophy occurred at high doses. The reduction in liver enzyme activity (alanine aminotransferase, alkaline phosphatase) in both sexes is considered to be of equivocal toxicological significance.
In a 4-week dermal toxicity study in rats, no substance related systemic toxicity was detected up to the highest dose tested (250 mg/kg bw/d).

For rats, a short-term NOAEL of 150 ppm (10.7 mg/kg bw/d) has been established. For dogs, the short-term NOAEL is 200 ppm, equivalent to about 6 mg/kg bw/d, based on the 3-month and 1-year feeding study in this species.
For male mice, the NOAEL in the 3-month study was lower than 50 ppm (9.2 mg/kg bw/d). Taking into consideration all available data for this species, a NOAEL of 30 ppm (ca 4 mg/kg bw/d) can be established for male mice based on the body weight data after 91 days from the carcinogenicity study. For females, the no observed adverse effect level was 50 ppm (12.9 mg/kg bw/d).

Overall, the lowest relevant NOAEL for short-term oral toxicity is 4 mg/kg bw/d, based on the body weight data after 91 days from the carcinogenicity study in mice."

Table 5.3-1: Summary of reviewed pyraclostrobin short-term toxicity studies

Study	Dosages (mg/kg bw/ day)	NOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
4-week feeding Wistar rat 0, 20, 100, 500, 1500 ppm	M: 1.8, 9.0, 42.3 and 120; F: 2.0, 9.6, 46.6 and 123.2	100 ppm 9.0 (M); 9.6 (F)	Effects on body weight, red blood cells, duodenum and liver.	1999/22970
13-week feeding Wistar rat 0, 50, 150, 500, 1000, 1500 ppm	M: 3.5, 10.7, 35, 69 and 106; F: 4.2, 12.6, 41, 80 and 119	150 ppm 10.7 (M); 12.6 (F)	Reduced body weight and food consumption, effects on clinical-pathology parameters, liver hypertrophy, and mucosal hypertrophy of the duodenum.	(1999/10195 1999/11899 *2000/1012360* # *2003/1013399* *2004/1027673*
13-week feeding B6C3F1 mice 0, 50, 150, 500, 1000, 1500 ppm	M: 9.2, 30.4, 119, 274 and 476; F: 12.9, 40.4, 162, 374 and 635	< 50/50 ppm <9.2/12.9 m/f (NOAEL ~ 4 mg/kg (30 ppm); based on mouse carcinogenicity study)	50 ppm (males): Reduced body weight (gain) and increased urea values in males. At higher dose levels (≥ 150 ppm), adverse effects in the gastrointestinal tract, on red blood cells, on white blood cells and lymphatic organs, as well as on adrenals, liver and kidney.	1998/11345 1999/11900 *2003/1013400* #
13-week feeding Beagle dog 0, 100, 200, 450 ppm	M: 2.8, 5.8 and 12.9; F: 3.1, 6.2 and 13.6	200 ppm 5.8 (M) 6.2 (F)	Body weight loss in females, vomitus, diarrhoea, clinical-chemical and hematological changes in females, hypertrophy of the duodenal mucosa in both sexes	1999/11678
1-year feeding Beagle dog 0, 100, 200, 400	M: 2.7, 5.4 and 10.8; F: 2.7, 5.4 and 11.2	200 ppm 2.7 (M&F)	Body weight loss in females, vomitus, diarrhoea, clinical-signs and hematological changes in both sexes	1999/11677
28-day dermal Wistar rat 40, 100, 250 mg/kg bw/day	M&F: 40, 100, 250	250 (systemic effects)	No systemic effects observed. Local signs of irritation at all dose levels	1999/11458

References (BASF DocIDs) in italic are submitted the first time in this supplemental dossier. As these report amendment do not affect the outcome and interpretation of the studies they are listed in this table.

Studies submitted in this AIR 3 dossier (not yet peer-reviewed):
Upon request of US-EPA the applicant conducted a 28-day inhalation toxicity study. This study employed atmosphere concentrations of 0, 1, 30 and 300 mg/m³ (0, 001, 0.03 and 0.3 mg/L) and identified a NOAEC of 1 mg/m³. The wide dose spacing between the mid and low concentration by a factor of 30 resulted in a NOAEC, which was not sufficient to allow to pass all US as well as EU exposure scenarios. Therefore, and to demonstrate the reversibility of the observed local effects in the upper respiratory tract, a second 28-day inhalation study was performed at lower concentrations (0, 3, 10 and 30 mg/m³). The results of these studies are listed in Table 5.3-2; comprehensive summaries are provided further below (see CA 5.3.3/1 and CA 5.3.3/2).

Table 5.3-2: Summary of not yet reviewed pyraclostrobin short-term toxicity studies

Study	Dosages (mg/m³)	NOAEC (mg/m³)	Main adverse effect	Reference (BASF DocID)
4-week inhalation (5 day/week) Wistar rat 0, 1, 30, 300 mg/m³	M&F: 1.17 ± 0.3, 30.4 ± 1.2 299 ± 15	1	Mortality and body weight effects at 300 mg/m³, hyperplasia of the duodenal mucosa, local effects in the upper respiratory tract at ≥ 30 mg/m³	2005/1013950
4-week inhalation (5 day/week) Wistar rat 0, 3, 10, 30 mg/m³	M&F: 3.01 ± 0.8 10.1 ± 1.5 29.1 ± 2.9	10	Local irritation in the upper respiratory tract (atrophy/ necrosis of olfactory epithelium)	2014/1003946

For convenience of the reviewer brief summaries of the respective studies were extracted from the monograph of are provided under the respective chapters.

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the Review Report of pyraclostrobin (SANCO/1420/2001-Final, 8. September 2004):

Short term toxicity	
Target / critical effect:	Reduced body weight, gastrointestinal tract, red blood cells, diarrhoea (dog), hepatocellular hypertrophy (rat), white blood cells and lymphatic organs (mouse)
Lowest relevant oral NOAEL / NOEL:	90-day mouse[#]: 30 ppm (4 mg/kg bw/d)
Lowest relevant dermal NOAEL / NOEL:	28-day rat: > 250 mg/kg bw/d (systemic)
Lowest relevant inhalation NOAEL/NOEL	No data - not required (because of physical and chemical properties)

[#] based on effects on body weight after 90 days in the carcinogenicity study in male mice

The proposed endpoints based on all available studies are shown below.

Target / critical effect	Reduced body weight, gastrointestinal tract, red blood cells, diarrhoea (dog), hepatocellular hypertrophy (rat), white blood cells and lymphatic organs (mouse), local irritation of the upper respiratory tract (rat inhalation)
Lowest relevant oral NOAEL / NOEL	90-day mouse[#]: 30 ppm (4 mg/kg bw/d)
Lowest relevant dermal NOAEL / NOEL	28-day rat: > 250 mg/kg bw/d (systemic)
Lowest relevant inhalation NOAEL / NOEL	0.01 mg/L (local) 0.3 mg/L (systemic)

[#] based on effects on body weight after 90 days in the carcinogenicity study in male mice

CA 5.3.1 Oral 28-day study

28-day study in rats
Groups of 5 male and 5 female Wistar rats (Chbb: THOM (SPF)) per dose group received pyraclostrobin of two different batches (purity about 94 and 99%) at dietary doses of 0, 20, 100, 500 and 1500 ppm for 28 consecutive days.

Clinical signs of toxicity consisted of a dose-dependent reduction of food consumption and impaired body weight development at ≥ 500 ppm.

Hematology investigations revealed reduced red blood cell counts and hemoglobin concentrations in females at ≥ 500 ppm. Additionally, increased MCV and decreased MCHC values were noted. Clotting analysis revealed a prolonged clotting time in males and females at 500 and/or 1500 ppm.
Changes of clinical chemistry parameters were mainly seen in top dose (1500 ppm) females and consisted of decreased serum cholinesterase activity (in absence of effects on erythrocyte and brain cholinesterase) as well as reduced glucose levels. Minor, but statistically significant changes in males consisted of decreased phosphate and increased total bilirubin levels.

Significantly reduced terminal body weights were observed in both sexes at the top dose level. Secondary to this a number of absolute and relative organ weights displayed statistically significant deviations from the respective control values. Only the increased relative liver and spleen weights were accompanied by treatment-related histopathological findings. These consisted of hepatocellular hypertrophy and decreased fat storage in the liver and increased extramedulary hematopoiesis in the spleen of males and females at 500 and or 1500 ppm. Additionally, mucosal hyperplasia of the duodenum was noted in both sexes at ≥ 1500 ppm.

Conclusion
Based on the described findings at 500 ppm and above, the NOAEL in this study was identified at 100 ppm, which is equivalent to mean daily intakes of 9.0 and 9.6 mg/kg bw/day in male and females rats, respectively.

CA 5.3.2 Oral 90-day study

Note: The main rat 90-day study report (CA 5.3.2/1) together with the 1st report amendment (CA 5.3.2/2) was already submitted and reviewed in the course of the initial registration of pyraclostrobin. This study is submitted for completeness as the basis for the assessment of report amendments submitted under CA 5.3.2/3 to CA 5.3.2/5. Whereas report amendments 1 and 2 correct clerical errors in the original report, amendment 3 provides the result of an additional histopathological evaluation of the spleen for intermediate dose levels. Amendment 4, which was submitted to Japanese authorities, provides a reference for the BASF trimming procedure used in this and other repeated dose studies. To take into account that the majority of the data were already reviewed, the following study summary will be more comprehensive than for other studies already reviewed, but will be less detailed than the summary of new studies.

Report: CA 5.3.2/1
[redacted], 1999a
BAS 500 F - Subchronic oral toxicity study in Wistar rats. Administration in the diet for 3 months
1999/10195
Guidelines: EEC 87/302, OECD 408, JMAFF, EPA 82-1
GLP: yes
(certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 3180, 6500 Mainz)

Report: CA 5.3.2/2
[redacted] 1999b
Amendment No. 1 to the report: BAS 500 F - Subchronic oral toxicity study in Wistar rats. Administration in the diet for 3 months
1999/11899
Guidelines: EEC 87/302, OECD 408, JMAFF, EPA 82-1
GLP: yes
(certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Report: CA 5.3.2/3
[redacted] 2000a
Amendment No. 2 to the report: BAS 500 F - Subchronic oral toxicity study in Wistar rats administration in the diet for 3 months
2000/1012360
Guidelines: EEC 87/302, OECD 408, JMAFF, EPA 82-1
GLP: yes
(certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Report: CA 5.3.2/4
[redacted], 2003a
Amendment No. 3 to the report: BAS 500 F - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months
2003/1013399
Guidelines: EEC 87/302, OECD 408, JMAFF, EPA 82-1
GLP: yes
(certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 3180, 6500 Mainz)

Report:	CA 5.3.2/5 [redacted] 2004a Amendment to the report for Japanese registration: BAS 500 F - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months 2004/1027673
Guidelines:	EEC 87/302, OECD 408, JMAFF, EPA 82-1
GLP:	yes (certified by Ministerium fuer Arbeit, Soziales, Familie und Gesundheit, Postfach 3180, 6500 Mainz)

Material and Methods:
Pyraclostrobin was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 50, 150, 500, 1000 and 1500 ppm for 3 months. Food consumption and body weight were determined each week. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week.

Ophthalmological examinations were carried out prior to the start and towards the end of dosing. Urinalysis, clinical-chemical and hematological examinations were carried out at the end of the administration period. All animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Findings:
The stability and homogeneous distribution of the test substance in the diet were confirmed by analysis. The correctness of the concentrations was analytically demonstrated.

Based on food consumption and body weight data average daily compound intakes of 3.5, 10.7, 35, 69 and 106 mg/kg bw/d in male and of 4.2, 12.6, 41, 80 and 119 mg/kg bw/d in female rats were calculated at dietary dose levels of 50, 150, 500, 1000 and 1500 ppm, respectively.

Figure 5.3.2-1: Body weight development of rats administered pyraclostrobin for at least 91 days

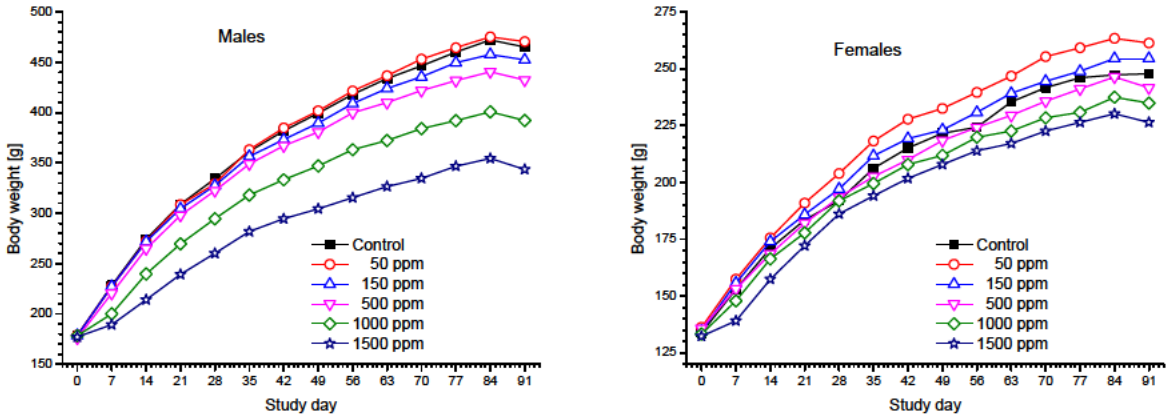


Table 5.3.2-1: Mean body weight and food consumption of rats administered pyraclostrobin for at least 91 days

Dose level [ppm]	0	50	150	500	1000	1500
	Males					
Body weight [g]						
- Day 0	178.7	178.8	178.5	176.1	178.2	177.1
- Day 91	465.1	470.5	452.4	**432.4***	**392.2****	**343.6****
Δ% (compared to control) #		1.2	-2.7	-7.0	-15.7	-26.1
Overall body weight gain [g]	286.4	291.7	273.9	**256.3***	**214.0***	**166.5****
Δ% (compared to control) #		1.9	-4.4	-10.5	-25.3	-41.9
Food consumption [g/animal/day]						
Average daily food consumption§ (weeks 1 to 13)	26.1	26.0	25.5	24.3	22.2	20.2
Number of weeks with stat. sign. difference to control		0/13	0/13	6/13	13/13	13/13
	Females					
Body weight [g]						
- Day 0	133.8	136.2	134.6	135.7	133.3	132.3
- Day 91	247.8	261.3	254.5	241.6	234.9	**226.4***
Δ% (compared to control) #		5.4	2.7	-2.5	-5.2	-8.6
Overall body weight gain [g]	114	125.1	119.9	105.9	101.6	**94.1***
Δ% (compared to control) #		9.7	5.2	-7.1	-10.9	-17.5
Food consumption [g/animal/day]						
Average daily food consumption§ (weeks 1 to 13)	17.1	18.5	18.0	16.9	16.2	15.7
Number of weeks with stat. sign. difference to control		0/13	0/13	2/13	2/13	4/13

Values may not calculate exactly due to rounding of mean values

§ Calculated from the weekly means. The values given in the DAR are the mean values for week 13

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's test, two sided)

Like in the 28-day study changes of haematological parameters were mainly observed in females at ≥ 1000 ppm (see Table 5.3.2-2) . In males only some calculated red blood cell parameters (MCV, MCHC) were changed at 500 ppm or above in addition to the changes of reticulocyte counts and prothrombin time.

Table 5.3.2-2: Treatment-related hematology findings in rats administered pyraclostrobin for at least 91 days

Parameter		Sex	Dietary dose level [ppm]					
			0	50	150	500	1000	1500
White blood cells	[G/L]	m	8.41	8.97	8.05	8.92	8.93	9.59
		f	3.90	4.22	4.85	4.69	**6.65*** **	**6.55** **
Red blood cells	[T/L]	m	8.53	8.53	8.79	8.59	8.36	8.22
		f	7.95	7.91	7.95	7.70	**7.36*** **	**7.10*** **
Hemoglobin	[mmol/L]	m	9.7	9.5	9.8	9.7	9.5	9.4
		f	9.2	9.3	9.3	9.3	**8.7** **	**8.6*** **
Reticulocytes	[‰]	m	17	17	16	19	**24** **	**33*** **
		f	14	17	14	13	15	**23*** **
Prothrombin time	[s]	m	26.0	26.5	26.4	27.1	**28.9*** **	**29.4*** **
		f	25.6	24.7	25.5	26.1	**27.5*** **	26.3

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02; ***p<0.002

A number of treatment-related changes clinical chemistry parameters were observed in both sexes at 500 ppm and above (see Table 5.3.2-3). These were either associated with the impaired nutritional condition at higher dose levels (e.g. ALT, ALP, triglycerides, cholesterol or glucose) or related to the anemia (total bilirubin). Like in the 28-day study a decrease of serum cholinesterase levels was noted in females at ≥ 1000 ppm. As before, these changes were not accompanied by changes of erythrocyte or brain cholinesterase and thus of uncertain toxicological relevance

Table 5.3.2-3: Treatment-related clinical chemistry findings in rats administered pyraclostrobin forat least 91 days

Parameter		Sex	Dietary dose level [ppm]					
			0	50	150	500	1000	1500
Total bilirubin	[µmol/L]	m	1.69	1.70	1.76	2.20	**2.67*** **	**3.29*** **
		f	2.17	1.93	1.94	1.93	**2.69**	**2.94** **
Alanine aminotransferase	[µkat/L]	m	1.05	1.03	0.91	**0.73*** **	**0.68*** **	**0.79*** **
		f	0.98	0.77**	0.89	**0.78***	**0.65*** **	**0.71** **
Alkaline phosphatase	[µkat/L]	m	5.55	5.65	5.94	5.29	**4.28** **	**4.34** **
		f	4.45	4.06	4.38	**3.82** **	**3.83**	**3.55*** **
Triglycerides	[mmol/L]	m	3.83	3.57	4.43	2.89	**1.92** **	**1.51*** **
		f	1.61	2.61	2.34	2.16	1.53	2.13
Cholesterol	[mmol/L]	m	2.26	2.16	1.94	1.84*	**1.67*** **	**1.60*** **
		f	1.83	2.10	1.82	1.82	1.72	1.79
Glucose	[mmol/L]	m	7.94	8.07	7.55	7.63	**7.34** **	**7.16** **
		f	7.93	8.07	7.69	7.23	**6.99** **	**7.25**

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02; ***p<0.002

No treatment-related changes of urinalysis parameters were noted.

Most the statistically significant changes of organ weights were secondary to the reduced terminal body weight. Only the changes of liver and spleen weights (Table 5.3.2-4) were corroborated by histopathological findings (see below).

Table 5.3.2-4: Selected mean absolute and relative organ weights of rats administered pyraclostrobin for at least 91 days

Sex		Males				Females			
Organ weight [mg]	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ% #	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ% #
Terminal weight [g]	0	439.32				230.01			
	50	444.42	(1.2)			242.67	(5.5)		
	150	423.58	(-3.6)			234.54	(2.0)		
	500	405.71*	(-7.7)			224.08	(-2.6)		
	1000	370.37**	(-15.7)			217.80	(-5.3)		
	1500	319.24**	(-27.3)			209.57**	(-8.9)		
Liver [g]	0	15.005		3.409		6.772		2.942	
	50	13.956	(-7.0)	3.132	(-8.1)	7.266	(7.3)	3.006	(2.2)
	150	13.182*	(-12.1)	3.111	(-8.7)	7.083	(4.6)	3.015	(2.5)
	500	13.054*	(-13.0)	3.217	(-5.6)	6.931	(2.3)	3.095	(5.2)
	1000	12.322**	(-17.9)	3.331	(-2.3)	7.274	(7.4)	3.34	(13.5)
	1500	11.942**	(-20.4)	3.746	(9.9)	**8.253****	**(21.9)**	**3.935**	**(33.8)**
Spleen [mg]	0	0.874		0.199		0.555		0.241	
	50	0.900	(3.0)	0.202	(1.5)	0.625	(12.6)	0.258	(7.1)
	150	0.844	(-3.4)	0.199	(0.0)	0.601	(8.3)	0.255	(5.8)
	500	**0.848**	**(-3.0)**	0.210	(5.5)	**0.654***	**(17.8)**	**0.293***	**(21.6)**
	1000	**0.946**	**(8.2)**	**0.256****	**(28.6)**	**0.740****	**(33.3)**	**0.340****	**(41.1)**
	1500	**1.018***	**(16.5)**	**0.320****	**(60.8)**	**0.878****	**(58.2)**	**0.420****	**(74.3)**

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may not calculate exactly due to rounding of figures

Table 5.3.2-5: Treatment-related histopathological findings in rats administered pyraclostrobin for at least 91 days

Sex		Males						Females					
Dose [ppm]		0	50	150	500	1000	1500	0	50	150	500	1000	1500
Animals in group		10	10	10	10	10	10	10	10	10	10	10	10
Duodenum	# examined	10	10	10	10	10	10	10	10	10	10	10	10
- Mucosal hyperplasia		2	1	1	**4**	**5**	**10**	2	1	2	1	1	**10**
		[1.0]	*[1.0]*	*[1.0]*	***[1.3]***	***[1.2]***	***[1.9]***	*[1.0]*	*[1.0]*	*[1.5]*	*[1.0]*	*[2.0]*	***[1.4]***
Liver	# examined	10	10	10	10	10	10	10	10	10	10	10	10
- hypertrophy, centrilobular (Zone 3)		-	-	-	**3**	**6**	**10**	-	-	-	-	-	**4**
		-	-	-	***[1.0]***	***[1.2]***	***[1.8]***	-	-	-	-	-	***[1.0]***
- Fatty change, diffuse		10	8	9	**6**	**2**	-	4	7	5	**2**	**1**	-
		[2.5]	*[2.1]*	*[2.2]*	***[1.5]***	***[1.0]***	-	*[1.5]*	*[1.4]*	*[1.4]*	***[1.0]***	***[3.0]***	-
Spleen	# examined	10	10	10	10	10	10	10	10	10	10	10	10
- Extramedulary hematopoiesis		2	-	3	1	**2**	**3**	-	-	3	3	**9**	**9**
		[1.0]	-	*[1.0]*	*[1.0]*	***[1.5]***	***[1.7]***	-	-	*[1.7]*	*[1.7]*	***[1.3]***	***[1.8]***
- Hemosiderin deposition		10	10	10	10	**10**	**10**	10	10	10	10	**10**	**10**
		[2.7]	*[2.5]*	*[2.1]*	*[2.5]*	***[1.8]***	***[1.7]***	*[2.9]*	*[2.7]*	*[2.8]*	*[2.5]*	***[2.3]***	***[2.3]***
- Sinusoid distension		-	-	-	1	**10**	**8**	-	-	-	2	**8**	**10**
		-	-	-	*[1.0]*	***[1.4]***	***[2.0]***	-	-	-	*[1.0]*	***[1.1]***	***[1.7]***
- Histiocytosis		-	-	1	3	**6**	**10**	-	-	1	2	7	7
				[1.0]	*[1.7]*	***[1.5]***	***[1.8]***	-	-	*[1.0]*	*[1.0]*	***[1.3]***	***[1.7]***

$ [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

Histopathology revealed the following findings (Table 5.3.2-5)

- Increased incidence and/or severity of mucosal hyperplasia of the duodenum in males at ≥ 500 ppm and in females at 1500 ppm.
- Increased incidence and severity of centrilobular hepatocyte hypertrophy in males at ≥ 500 ppm and in females at 1500 ppm.
- Diminished indicence and/or severity of hepatocellular fat storage (fatty change, diffuse) in both sexes at 500 ppm and above.
- Increased severity of extramedulary hematopoiesis in the spleen of males at ≥ 1000 ppm. In females at ≥ 1000 ppm increased incidence of this finding was noted.
- As reported in the 3rd Amendment to the report, a slightly decreased severity of hemosiderin deposition in spleen was noted in both sexes at ≥ 1000 ppm.
- Increased incidence and severity of sinusoid distension and histiocytosis in the spleen of both sexes at ≥ 1000 ppm.

The new data provided with report amendment 3 are in line with the other previously reported effects in the spleen.

Conclusion:

The no observed adverse effect level (NOAEL) in this 3-month rat study was 150 ppm (10.7 mg/kg bw/d in males and 12.3 mg/kg bw/d in females), based on reduction of body weight and food consumption, effects on clinical-chemical parameters, liver hypertrophy, and mucosal hypertrophy in the duodenum at 500 ppm and above. At the two highest dose levels (1000 and 1500 ppm), the oral administration of pyraclostrobin to female rats resulted additionally in leucocytosis and effects on red blood cells with compensatory reactions.

Note: The main mouse 90-day study report (CA 5.3.2/6) together with the 1st report amendment (CA 5.3.2/7) was already submitted and reviewed during the previous Annex I inclusion process. This study and it's 1st amendment is submitted for completeness as the basis for the assessment of the 2nd report amendment submitted under CA 5.3.2/8. Whereas report amendment 1 mainly corrects clerical errors in the original report, amendment 2 provides the result of additional histopathological evaluation of the spleen of intermediate dose levels. To take into account that the majority of the data were already reviewed, the following study summary will be more comprehensive than for other studies already reviewed, but will less detailed than the summary of new studies.

Report:	CA 5.3.2/6 [redacted] 1998a BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl BR mice. Administration in the diet for 3 months 1998/11345
Guidelines:	EEC 87/302, OECD 408, EPA 82-1, JMAFF
GLP:	yes (certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Report:	CA 5.3.2/7 [redacted] 1999c Amendment No. 1 to the report: BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl mice. Administration in the diet for 3 months 1999/11900
Guidelines:	EEC 87/302, OECD 408, EPA 82-1, JMAFF
GLP:	yes (certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Report:	CA 5.3.2/8 [redacted] 2003b Amendment No. 2 to the report: BAS 500 F - Subchronic oral toxicity study in B6C3F1 Crl mice - Administration in the diet for 3 months 2003/1013400
Guidelines:	EEC 87/302, OECD 408, EPA 82-1, JMAFF
GLP:	yes (certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Material and Methods:
Pyraclostrobin was administered to groups of 10 male and 10 female B6C3F1 mice at dietary concentrations of 0, 50, 150, 500, 1000 and 1500 ppm for 3 months. Food consumption and body weight were determined each week. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week.

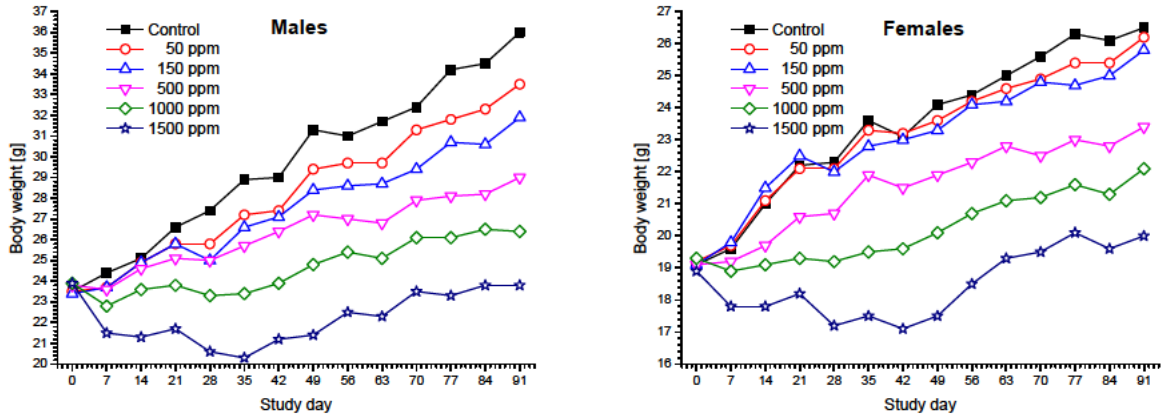
Ophthalmological examinations were carried out prior to the start and towards the end of dosing. Urinalysis, clinical-chemical and hematological examinations were carried out at the end of the administration period. All animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Findings:
The stability and homogeneous distribution of the test substance in the diet were confirmed by analysis. The correctness of the concentrations was analytically demonstrated.

No treatment-related mortality or clinical signs of toxicity were observed throughout the study. However, on top dose females (1500 ppm) died accidentially when squeezed between food hopper and wire top.

Based on food consumption and body weight data average daily compound intakes of 9.2, 30.4, 119, 274 and 476 mg/kg bw/d in male and of 12.9, 40.4, 162, 374 and 635 mg/kg bw/d in female rats were calculated at dietary dose levels of 50, 150, 500, 1000 and 1500 ppm, respectively. As food spilling was observed in all groups irrespective of dose, these values may not represent the real compound intakes.

Figure 5.3.2-2: Body weight development of mice administered pyraclostrobin for at least 91 days



Occasionally, statistically significant increases of food consumption were recorded in both sexes at ≥ 1000 ppm. Due to the above mentioned food spilling it is not clear whether this was related to treatment. At least a dose-dependent trend towards higher mean food intake was observable if the overall mean food consumption is considered (see Table 5.3.2-6).

Table 5.3.2-6: Mean body weight and food consumption of mice administered pyraclostrobin for at least 91 days

Males						
Dose level [ppm]	0	50	150	500	1000	1500
Body weight [g]						
- Day 0	23.5	23.5	23.4	23.8	23.9	23.9
- Day 91	36	**33.5**	**31.9****	**29.0****	**26.4****	**23.**8**
Δ% (compared to control) #		-6.9	-11.4	-19.4	-26.7	-33.9
Overall body weight gain [g]	12.6	**10.0**	**8.4****	**5.1****	**2.5****	**-0.1****
Δ% (compared to control) #		-20.6	-33.3	-59.5	-80.2	-100.8
Food consumption [g/animal/day]						
Average daily food consumption§ (weeks 1 to 13)	5.7	5.2	5.6	6.3	6.8	7.0
Δ% (compared to control) #l		-8.1	-1.2	10.5	18.8	23.2
Females						
Body weight [g]						
- Day 0	19.1	19.2	19.1	19.1	19.3	18.9
- Day 91	26.5	26.2	25.8	**23.4****	**22.1****	**20****
Δ% (compared to control) #		-1.1	-2.6	-11.7	-16.6	-24.5
Overall body weight gain [g]	7.5	7.0	6.7	**4.3****	**2.8****	**1.1****
Δ% (compared to control) #		-6.6	-10.7	-42.7	-62.7	-85.3
Food consumption [g/animal/day]						
Average daily food consumption§ (weeks 1 to 13)	6.1	6.0	6.2	7.0	7.6	7.8
Δ% (compared to control) #		-0.4	2.7	15.5	24.5	28.1

Values may not calculate exactly due to rounding of mean values
§ Calculated from the weekly means. The values given in the DAR are the mean values for week 13
* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's test, two sided)

A dose dependent and statistically significant decrease of body weight and body weight gain was observed in males at ≥ 150 ppm and in females ≥ 500 ppm (Figure 5.3.2-2 and Table 5.3.2-1). In the course of the European evaluation of this study, the body weight development in low dose males was likewise considered to be affected by treatment, even though no statistical significance was obtained.

Changes of haematological parameters were mainly observed in males. In addition to the parameters given in Table 5.3.2-7 calculated red blood cell parameters (MCV, MCH and/or MCHC) were altered in both sexes at 500 ppm and above.
The statistically significant decrease of white blood cell counts in males at ≥ 1000 ppm was mainly due to a decrease in the number of lymphocytes as indicated by the differential blood cell count. In females a numerical, but not statistically significant decrease of white blood cell counts was observed, which in part was also due to a decrease in lymphocyte counts.

Table 5.3.2-7: Treatment-related hematology findings in mice administered pyraclostrobin for at least 91 days

Parameter		Sex	Dietary dose level [ppm]					
			0	50	150	500	1000	1500
White blood cells	[G/L]	m	5.92	5.56	5.20	6.36	**2.72*** **	**2.67*** **
		f	6.04	5.15	4.10	3.72	3.25	3.17
Hemoglobin	[mmol/L]	m	11.8	11.6	11.6	11.4	11.4	**10.6*** **
		f	11.4	11.5	11.2	11.0	**10.9****	**10.4****
Hematocrit	[l/L]	m	0.572	0.564	**0.551***	**0.542****	**0.543****	**0.518****
		f	0.519	0.531	0.517	0.516	0.513	0.495

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02; ***p<0.002

Treatment-related changes clinical chemistry parameters were observed in both sexes at 150 ppm and above (see Table 5.3.2-8). These were associated with the impaired nutritional condition at higher dose levels (e.g. total protein, triglycerides, urea). Other clinical chemistry changes displayed no strict dose dependency and were considered of low or no toxicological relevance.

Table 5.3.2-8: Treatment-related clinical chemistry findings in rats administered pyraclostrobin forat least 91 days

Parameter		Sex	Dietary dose level [ppm]					
			0	50	150	500	1000	1500
Total protein	[g/L]	m	63.89	67.56**	66.66**	64.88	**61.42***	**56.97*** **
		f	60.00	62.16	61.69	59.67	**55.73*** **	**55.22*** **
Albumin	[µkat/L]	m	63.89	67.56**	66.66**	64.88	**61.42***	**56.97*** **
		F	37.97	39.32	39.32	39.11	36.77	36.75
Globulin	[µkat/L]	m	25.56	27.46**	27.26	26.19	**23.28*** **	**21.02*** **
		f	22.03	22.83	22.37	**20.56****	**18.68*** **	**18.47*** **
Triglycerides	[mmol/L]	m	1.70	1.64	**1.15****	**0.78*** **	**.0.59*** **	**0.47*** **
		f	1.53	1.22	**0.96***	**0.58*** **	**0.59*** **	**0.58*** **
Cholesterol	[mmol/L]	m	3.49	3.98*	3.96**	3.71	3.81	3.33
		f	2.50	2.90**	2.76	2.98**	3.20***	3.79***
Urea	[mmol/L]	m	7.25	7.93*	**8.75*** **	**10.55*** **	**12.01*** **	**11.97*** **
		f	6.09	6.73	**9.07****	**11.06*** **	**10.89*** **	**9.85*** **

Kruskal-Wallis + Mann-Whitney U-test *p<0.05; **p<0.02; ***p<0.002

The statistically significant changes of organ weights were considered secondary to the reduced terminal body weight. Except for adrenals in males for all organs (liver, kidney, ovaties/testes), spleen, brain) decreased absolute weights were recorded partially down to 150 ppm. In turn, relative weights were mostly increased with the exception of andrenal gland and ovary weights in females.

Table 5.3.2-9: Treatment-related histopathological findings in mice administered pyraclostrobin for at least 91 days

Sex		Males						Females					
Dose [ppm]		0	50	150	500	1000	1500	0	50	150	500	1000	1500
Animals in group		10	10	10	10	10	10	10	10	10	10	10	10
Adrenal cortex	# examined	10	10	10	10	10	10	10	10	10	10	10	9
- X-zone: lipid decrease		-	-	-	-	-	-	1	3	**5**	**10**	**10**	**9**
		-	-	-	-	-	-	*[2.0]*	*[3.0]*	*[3.2]*	*[3.7]*	*[5.0]*	*[5.0]*
Duodenum	# examined	10	10	10	10	10	10	10	10	10	10	10	9
- Mucosal hyperplasia		-	-	-	**10**	**10**	**10**	-	-	-	**6**	**10**	**9**
					[2.4]	*[2.8]*	*[3.0]*	-	-	-	*[2.0]*	*[2.0]*	*[2.2]*
Kidney	# examined	10	10	10	10	10	10	10	10	10	10	10	9
- Lipid vacuoles in prox. tubular		10	10	10	**2**	**1**	-	10	10	9	**7**	**7**	**7**
epithelium		*[2.0]*	*[1.7]*	*[1.7]*	*[1.0]*	*[1.0]*	-	*[1.8]*	*[1.6]*	*[1.7]*	*[1.1]*	*[1.3]*	*[1.6]*
Liver	# examined	10	10	10	10	10	10	10	10	10	10	10	9
- Fatty infiltration, diffuse		10	10	10	**10**	**10**	**4**	10	10	8	6	9	7
		[3.8]	*[3.8]*	*[3.6]*	*[3.1]*	*[3.0]*	*[1.8]*	*[3.7]*	*[3.7]*	*[3.8]*	*[3.5]*	*[3.0]*	*[3.4]*
Lymph node, mesentric	# examined	10	10	10	10	10	10	10	10	10	10	10	9
- Apoptosis, increased		-	-	-	**1**	**1**	**9**	-	-	**2**	**4**	**6**	**7**
		-	-	-	*[1.0]*	*[1.0]*	*[2.0]*	-	-	*[2.0]*	*[2.0]*	*[1.2]*	*[1.3]*
Spleen	# examined	10	10	10	10	10	10	10	10	10	10	10	9
- Hemosiderin deposition		10	1	1	6	7	10	10	10	10	10	10	9
		[1.2]	*[1.0]*	*[1.0]*	*[1.2]*	*[1.0]*	*[1.1]*	*[2.1]*	*[1.8]*	*[1.9]*	*[2.1]*	*[1.7]*	*[1.8]*
Stomach, glandular	# examined	10	10	10	10	10	10	10	10	10	10	10	9
- Erosion/ulcer		1	1	2	**4**	**5**	**8**	1	2	**5**	7	**6**	**6**
Thymus	# examined	10	10	10	10	10	10	10	10	10	10	10	9
- Atrophy		-	-	-	**3**	**6**	**8**	-	-	**6**	**7**	**8**	**4**
		-	-	-	*[1.8]*	*[2.5]*	*[3.4]*	-	-	*[2.7]*	*[2.7]*	*[2.8]*	*[3.5]*

[ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

Histopathology revealed the following findings (Table 5.3.2-9)

- Decreased lipid in the X-zone of the adrenal cortex of females at 150 ppm and above.
- Increased incidence and/or severity of mucosal hyperplasia of the duodenum in both sexes at ≥ 500 ppm.
- Decreased incidence and/or severity of lipid vacuoles in cells of the proximal epithelial cells of the kidney
- Diminished indicence and/or severity of hepatocellular fat storage (fatty change, diffuse) in males at 500 ppm and above.
- Increased incidence of apoptotic bodies in follicles of mesenteric lymph nodes in females at 150 ppm and in both sexes at 500 ppm and above.
- As reported in the 2nd Amendment to the report, the incidence of hemosiderin deposition was comparable between treated groups and controls whereas the severity was slightly decreased at ≥ 1000 ppm.
- Increased incidence of glandular stomach erosion/ulcer in females at 150 ppm and in both sexes at 500 ppm and above.
- Increased incidence and severity of thymus atrophy accompanied by an increased number of apoptotic bodies in females at 150 ppm and in both sexes at 500 ppm and above.

The new data provided with Report Amendment No. 2 do not alter the conclusions of the study.

Conclusion:
Contrary to the study director and the applicant, the previous EU-review concluded that the dose of 50 ppm (9.2 mg/kg bw/d) represents a LOEL in male mice because body weight and body weight change at this dose level were clearly decreased during the whole study period. Nevertheless, taking into consideration all available data for this species, a NOAEL of 30 ppm (ca. 4 mg/kg bw/d) was established for males, based on the body weight data after 91 days from the carcinogenicity study (dose levels: 0, 10, 30, 120 ppm).
For females, the no observed adverse effect level (NOAEL) was 50 ppm (12.9 mg/kg bw/d), based on effects on body weight, decreased hematocrit and triglyceride values and increased urea values at 150 ppm and above.
At higher dose levels, adverse effects in the gastrointestinal tract (erosions and ulcera in the glandular stomach; thickening of the duodenal mucosa), on red blood cells, on white blood cells and lymphatic organs (thymus, mesenteric lymph modes) as well as on adrenals, liver and kidney (decreased vacuolisation) have been observed.

90-Day Study in Dogs
Pyraclostrobin (Purity 97.09%) was administered to groups of five male and five female purebred Beagle dogs at dietary concentrations of 0, 100, 200 and 450 ppm for 3 months.

Clinical observation revealed repeated vomiting during the first three weeks of treatment as well as diarrhea almost throughout the entire study period was observed at the high dose level. Occasional observation of diarrhea at the mid dose level were considered to be of incidental nature due to isolated occurrence as well as the fact that this was not observed in the 1-year study at the same dose level.

Effects on food consumption and body weight development were restricted to high dose females for which a slight decrease of mean food consumption (-9%) and a slight overall body weight loss was noted (- 0.2 kg vs. + 1.2 kg in control females).

Treatment related hematology findings were restricted to increased platelet counts in high dose females (450 ppm) at the terminal blood collection. However, the total number of platelets was not changed compared to day -1 and no effects on blood clotting (PTT and PT). Likewise, the decrease of glucose and total protein levels in high dose females was considered treatment related, whereas the slight, but statistically significant decrease of glucose levels in mid dose females (200 ppm) was not considered to represent an adverse finding, especially since no comparable effects on glucose levels were observed at the same dose level in the 1-year dog study.

No treatment related effects on organ weights were noted. The only treatment related histopathological change consisted of hypertrophy of the duodenal mucosa in two males and one high dose female.

Conclusion: The no observed adverse effect level (NOAEL) in this 3-month oral dog study was 200 ppm (5.8 mg/kg bw/d in males and 6.2 mg/kg bw/d in females), based on body weight loss and clinico-chemical and hematological changes in females, and vomitus, diarrhoea and hypertrophy of the duodenal mucosa in both sexes at 450 ppm.

1-Year study in dogs
Pyraclostrobin (Purity 98.7%) was administered to groups of five male and five female purebred Beagle dogs at dietary concentrations of 0, 100, 200 and 400 ppm for 12 months.

Like in the 90-day study initially cases of vomiting were observed at the high dose (400 ppm), whereas diarrhea was noted at various occasions throughout the entire study duration. These clinical signs were considered to be treatment related.

A consistent effect on body weight was restricted to high dose females. Mean body weights increased by 0.3 kg and 1.1 kg after 6 and 12 months, respectively, compared to 2.2 and 2.7 kg in control females, respectively. This impairment of body weight development was accompanied by a 14% reduction in overall mean food consumption.
The marginal (-0.1 kg) and temporary mean body weight loss in high dose males at Day 7 was mainly due to one animal (#970183), which displayed an approx. 30% reduced food consumption and a body weight loss by 0.5 kg.

Hematological examinations revealed transiently decreased hemoglobin and hematocrit values in high dose females (day 90) and males (day 180). White blood cell counts (days 180, 362) and increased platelets (days 89, 180, 362) were noted in high dose males. However, similar increases (n.s.) were already noted at Day -9 and the increased platelet counts were not accompanied by significant effects on blood clotting (PTT and PT).

Ophthalmoscopy, clinical-chemical investigations, urinalysis and the pathological investigations did not reveal any test substance related effects.

Conclusion: The no observed adverse effect level (NOAEL) in this 12-month oral dog study was 200 ppm (5.4 mg/kg bw/d in males and females), based on reduced body weight gain and food consumption in females as well as clinical signs (vomitus, diarrhoea) and hematological changes in both sexes at 400 ppm.

CA 5.3.3 Other routes

Regarding other routes of exposure, the applicant performed 28-day studies by the dermal and inhalation route.

28-day dermal toxicity in rats
Pyraclostrobin was administered to groups of 10 male and 10 female Wistar rats by dermal route (6 hours/day; 5 days/week; semi-occlusive dressing) for 4 weeks at doses of 0 (vehicle control: 0.5% aqueous CMC solution), 40, 100 and 250 mg/kg bw/d. No signs of systemic toxicity were observed at any dose level. Dose related signs of local irritation were observed at all dose levels in the area of the treated skin. These consisted of (multifocal or diffuse) scale formation, erythema, epithelial thickening and hyperkeratosis.

28-day inhalation toxicity in rats

Report:	CA 5.3.3/1 [redacted] 2005a BAS 500 F - Subacute inhalation study in Wistar rats - 20 aerosol exposures during 4 weeks 2005/1013950
Guidelines:	OECD 413, EEC 87/302, EPA 870.3465, EPA 870.6200
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz

Executive Summary

In a 28-day inhalation toxicity study, three groups of Wistar Crl:Wi (Han) rats (10/sex) were exposed to liquid aerosols of pyraclostrobin (batch LJ 27822/199/b, purity 98.7%) dissolved in acetone at nominal concentrations of 1, 30 and 300 mg/m³ (0.001, 0.03 and 0.3 mg/L) for 6 hours daily on a 5 application per week basis. Air and acetone groups served as negative and vehicle control groups.
Mortality was observed for 4 male and 3 female rats at the high concentration of 300 mg/m³ (0.3 mg/L) between study days 7 and 24. This concentration is close to the acute 4h LC_{50} of 0.58 mg/L observed for a pyraclostrobin aerosol when dissolved in acetone. Body weight development in high concentration males was impaired. Hematology revealed increased white blood cell counts and an increase in absolute and relative neutrophil numbers. This was probably the result of the inflammatory processes in the respiratory tract. No treatment related clinical chemistry findings were noted between the acetone vehicle control and the treated groups, whereas there were some differences between the air and acetone control.
No treatment-related organ weight changes were observed. The respiratory tract (nasal cavity, larynx and lungs) and the duodenum were identified as target organs as indicated by dose dependent atrophy/necrosis and regeneration/repair of the olfactory epithelium, hyperplasia of the respiratory epithelium of nasal cavity and the larynx, perivascular inflammation of the lungs as well as hyperplasia of the duodenal mucosal. The low dose of 1 mg/m³ was identified as NOAEC.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin (BAS 500 F)

Description:	Solid melt / red-brown, clear
Lot/Batch #:	LJ 27882/199/b (ToxChargeIII/Teil2)
Purity/content:	98.7%
Stability of test compound:	According to the Certificate of Analysis the test substance was stable until March 28, 2008 when stored refridgerated.

2. Vehicle and/or positive control: Acetone

3. Test animals:

Species:	Rat
Strain:	Wistar Crl:WI(Han)
Sex:	males and females
Age at dosing:	about 9 weeks
Weight at dosing:	246.3 ± 11.2 g (males), 174.6 ± 9,1 g (females)
Source:	Charles River Laboratories, Germany, 97633 Sulzfeld
Acclimation period:	at least one week In order to accustom the animals to exposure they were treated with supply air for 6 hours under conditions comparable to exposure on 2 days before start of exposure (preflow period).
Diet:	milled mouse/rat laboratory diet "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum except during exposure and motor activity measurements
Water:	Tap water ad libitum except during exposure and motor activity measurements
Housing:	single housing in stainless steel wire mesh cages type DK-III (Becker & Co., Castrop-Rauxel, FRG), floor area approx. 800 cm². The motor activity measurements were conducted in Polycarbonate cages with wire covers from Ehret, Emmendingen, FRG (floor area about 800 cm²) and bedding.
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of work: 11-Jan-2005 to 22-Jun-2005
(in-life dates: 24-Jan-2005 to 25-Feb-2005)

2. Animal assignment and treatment

Groups of 10 male and 10 female Wistar rats per test group were exposed in a head-nose inhalation system to atmospheres containing aerosols of BAS 500 F for 6 hours per working day for about 28 days on a 5 days/week basis (20 exposures). Control animal were exposed to acetone. For generation of the aerosols the test substance (non volatile solid melt) was dissolved in acetone. The target concentrations were 0, 1, 30 and 300 mg/m³. Additionally, one group of male and female rats were exposed to the vehicle acetone.

Test group	Target concentration [mg/m³]	No. of animals per sex	Males Animal No.	Females Animal No.
0	Air control	10	1-10	51-60
1	Acetone control	10	11-20	61-70
2	1	10	21-30	71-80
3	30	10	31-40	81-90
4	300	10	41-50	91-100

The animals were allocated to the treatment groups according to weight using a suitable randomization software. The variation of individual weights of the animals used did not exceed 20% of the mean weight of each sex. As the capacity to perform Functional Observational Battery and the Motor Activity measurements (FOB/MA) was limited by the time needed to carry out these examinations, the study was carried out using a block design with a staggered start of treatment: The first 5 male animals of test groups 0 - 4 were assigned to exposure group 1. The remaining 5 male animals were assigned to exposure group 3. Likewise, the first five and the remaining five females of test groups 0 to 4 were assigned to exposure groups 2 and 4. By this it was ensured that the examinations of all test groups were performed on the same study day.

3. Aerosol generation and analysis

A head-nose inhalation system INA 60 (stinless-steel construction, BASF Aktiengesellschaft, volume V ≈ 90 l) was used. The animals were restrained in glass tubes and their snouts projected into the inhalation system.

A liquid aerosol was generated. The test substance was diluted 1:9 with acetone. The aerosol was produced by continuously pumping the test-substance solution to a two-component atomizer by means of a metering (test group 1) or an infusion pump (test groups 2 to 4). Using compressed air, the aerosol was generated which was produced inside the exposure system.

A positive pressure was maintained inside the exposure systems by adjusting the air flow of the exhaust air system. This ensured that the aerosol in the breathing zones of the animals was not diluted by laboratory air.
The concentrations of the inhalation atmospheres were analyzed by gravimetry in test group 2 - 4. This analytical method is judged to be valid, because pyraclostrobin does not possess an appreciable vapor pressure. Daily means were calculated based on two measured samples per concentration and exposure.
The sampling for determination of the **actual aerosol concentration** was performed with a filtration equipment using a sampling probe (diameter 7 mm). For this pre-weighed filters were placed into the filtration equipment. By means of the vacuum pump metered volumes of the liquid aerosol were drawn through the filter.

- Filter: MN 85/90 BF (d = 47 mm)
- Sampling position: immediately adjacent to the animals' noses
- Sampling flow: 3 l/min
- Sampling velocity: 1.25 m/s
- Sampling frequency: 4 samples at about hourly intervals
- Sample volume: Test group 2: 450 l; Test group 3: 90 l; Test goup 4: 9 l; The sampling volumes were adjusted to achieve suitable amounts of test substance for analysis
- Sampling frequency: two samples per exposure and concentration group

The volatile solvent acetone was analyzed by gas chromatography in test groups 1 and 4 based on one sample per exposure. The samples were withdrawn immediately adjacent to the animals' noses by means of a 100 µl gas tight Hamilton syringes. The entire sample was injected. The following equipment and analysis conditions were used:

- Gas comatograph: Hewlett-Packard 5840 A
- Column: glass
- Length: 3 m
- Internal diameter: 2 mm
- Separation phase: 10%Carbowax 20M
- Support: Supelcpoprt 80/100 mesh
- Carrier gas: He
- Carrier gas flow rate: 20 ml/min
- Hydrogen: 30 ml/min
- Air: 240 ml/min
- Oven temperature: 150°C/min
- Detector: FID
- Detector temperature: 220°C
- Injector temperature: 220°C/min
- Calibr. retention time: approx. 1.40 min.

The particle size analysis was done using a Stack Sampler Martple 298 (Sierra) and a vacuum pump (Millipore). The sampling probe had an internal diameter 7 mm. Before sampling, the impactor was assembled with pre-weighed glass-fiber collecting disks and equipped with a backup particle filter. The impactor was connected to the vacuum pump and for each test group samples were taken from the breathing zone of the animals. Samples were withrawn at a velocity of 1.25 m/sec. Depending on the nominal atmosphere concentrations the sample volumes were 1800, 60 and 6 L for Test groups 2 to 4, respectively. Each two samples were taken per concentration level.
After sampling the impactor was disassembled. The impactor stages and the backup particle filter were freed of acetone and re-weighed.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Parameter	Statistical test	References
Food consumption, body weight, body weight gains; food efficiency	Simultaneous comparison of all test groups with the vehicle control group using the DUNNETT-test (twosided) for the hypothesis of equal means	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096 – 1121 DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482 – 491
	A comparison of group 1 with group 0 was performed using the Welch t-test (two sided) for the hypothesis of equal means	Welch B.L. (1947): WELCH, B.L (1947).The generalization of Student´s problem when several different population valiances are involved. Biometrika, 34, 28-35
Feces, rearing, grip strength forelimbs, grip strength hindlimbs, landing foot-splay test, motor activity & Clinical pathology parameters, except reticulocytes and differential blood count	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of the dose groups with the vehiclecontrol group was performed using the WILCOXON-test (twosided) for the hypothesis of equal medians A pairwise comparison of group 1 with group 0 was performed using Wilcoxontest (two sided) for the equal medians	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York

Organ weight parameters	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each concentration group with the control group was performed using the WILCOXON test for the hypothesis of equal medians	HETTMANNSPERGER, T.P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-140. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3 MILLER, R.G. (1981): Simultaneous Statistical Inference Springer-Verlag New York Inc., 165-167 NIJENHUIS, A. and S.W. WILF (1978): Combinatorial Algorithms, Academic Press, New York, 32-33
	A pairwise comparison of group 1 with group 0 was performed using Wilcoxontest (two sided) for the equal medians	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York

C. METHODS

1. Clinical examinations:

The animals were examined for evident signs of toxicity or mortality twice daily on working days and once daily on weekends and public holidays.
The clinical condition of the test animals was recorded once on preflow and on post-exposure observation days and at least 3 times (before, during and after exposure) on exposure days. During exposure only a limited examination was possible because of the animals' location in the exposure tubes.
Detailed clinical observations of all animals were performed in a standard arena prior to the administration period and weekly thereafter. The standard arena had a size of 50 x 37.5 cm with walls of 25 cm height. If applicable the findings were ranked according to the degree of severity. The following parameters were examined:

1. abnormal behavior during handling
2. fur
3. skin
4. posture
5. salivation
6. respiration
7. activity/arousal level
8. tremors
9. convulsions
10. abnormal movements
11. impairment of gait
12. lacrimation
13. palpebral closure
14. exophthalmus
15. feces (appearance/consistency)
16. urine
17. pupil size

2. Body weight:

The body weight of the animals was determined on day –7, at the start of the preflow, at the start of the exposure period and then, as a rule, once a week as well as one day prior to gross necropsy.
As a rule, the animals were weighed at the same time of the day.

3. Food consumption, food efficiency and compound intake:
Food consumption was determined parallel to body eight and calculated as mean food consumption in grams per animal and day.
Food efficiency (group means) was calculated based upon individual values for body weight and food consumption:

$$\text{Food efficiency for day x} = \frac{BW_x - BW_Y}{FC_{y\ to\ x}} \times 100$$

- BW_x and BW_y body weight [g] at day x and day y (last weighing date before day x)
- $FC_{y\ to\ x}$ is the mean food consumption from day y to x calculated as mean daily food consumption [g] on day x, multiplied by the number of days from day y to day x.

4. Ophtalmoscopy:
Before the start of the exposure period the eyes of all animals, and towards the end of the study (study day 22) the eyes of the animals of air control group, the acetone control group and high concentration group were subjected to ophthalmocopic examination.

5. Functional observation battery (FOB) and Motor activity:
A functional observational battery was performed in all animals towards the end of the administration period. At the day of examination, the respective groups animals were not exposed to the inhalation atmospheres. The FOB started with passive observations without disturbing the animals; followed by removal from the home cage, open field observations in a standard arena and sensorimotor tests as well as reflex tests. The findings were ranked according to the degree of severity, if applicable. The observations were performed at random.

The parameters determined are given in the table below. Detailed descriptions of the investigations are given in the report.

Home cage observations	Open field observations	Sensorimotor Tests/Reflexes:
1. posture	1. behaviour when removed from cage	1. approach response
2. tremor	2. fur	2. touch response
3. convulsions	3. skin	3. vision ("visual placing response")
4. abnormal movements	4. salivation	4. pupillary reflex
5. impairment of gait	5. nose discharge	5. pinna reflex
6. other findings	6. lacrimation	6. audition ("startle response")
	7. eyes / pupil size	7. coordination of movements ("righting response")
	8. posture	8. behaviour during "handling"
	9. palpebral closure	9. vocalisation
	10. respiration	10. pain perception ("tail pinch")
	11. tremors	11. grip strength of forelimbs
	12. convulsions	12. grip strength of hindlimbs
	13. abnormal movements	13. landing foot-splay test
	14. impairment of gait	14. other findings
	15. activity / arousal level	
	16. feces (number of fecal pellets / appearance / consistency) within two minutes	
	17. urine (appearance / quantity) within two minutes	
	18. number of rearings within two minutes	

Motor activity was measured on the same day as the FOB was performed. The measurement was performed in the dark using Polycarbonate cages with absorbent material and 4 infrared beams per cage. The number of beam interrupts were counted over 12 intervals, each lasting 5 minutes. The measurement was ended exactly 60 minutes after the first interuption of an infrared beam. During the measurements the animals received no food and no water.

6. Hematology and clinical chemistry:

Blood was collected from the retroorbital venous plexus in the morning from over-night fasted animals under isoflurane anaesthesia. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence. The following parameters were determined.

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count	✓	Prothrombine time
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count
✓	Hematocrit (Hct)	✓	Eosinophils (differential)		
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes	✓	Large unstained cells		

Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
✓	Magnesium	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Creatinine	✓	Serum-γ-glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Globulin		
✓	Sodium	✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

7. Urinalysis:

Not performed in this study

8. Sacrifice and pathology:

All animals were sacrificed under Narcoren anesthesia by exsanguination from the abdominal aorta and vena cava. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

Pathology:												
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: air control, acetone control and top concentration animals.												
C	W	H		C	W	H		C	W	H		
✓	✓	#	adrenals	✓	✓	#	kidneys	✓		#	salivary glands*	
✓		#	aorta	✓		✓	lachrymal glands (exorbital)	✓		#	seminal vesicles	
✓	✓	#	brain	✓		✓	larynx (3 levels)	✓		#	skin	
✓		#	bone marrow§	✓	✓	#	liver‡	✓		#	spinal cord (3 levels)@	
✓		#	caecum	✓	✓	✓	lung	✓	✓	#	spleen	
✓		#	colon	✓		#	lymph nodes#	✓		#	sternum w. marrow	
✓		✓	duodenum	✓		#	mammary gland (♀)	✓		#	stomach (fore- & glandular-)	
✓	✓	#	epididymides	✓		#	muscle, skeletal	✓	✓	#	testes	
✓		#	esophagus	✓		#	nerve, peripheral (sciatic n.)	✓	✓	#	thymus	
✓		#	eyes	✓		✓	nose†/nasal cavity (4 levels)	✓	✓	#	thyroid/parathyroid	
✓		#	femur (with knee joint)	✓	✓	#	ovaries and oviduct	✓		#	trachea&	
			gall bladder	✓		#	pancreas	✓		#	urinary bladder	
✓		✓	gross lesions	✓		#	pituitary	✓	✓	#	uterus	
✓	✓	#	heart	✓		#	pharynx	✓		#	vagina	
✓		#	ileum	✓		#	prostate		✓		body (anesthetized)	
✓		#	jejunum	✓		#	rectum					
§ from femur; # mandibular, mediastinal and mesenteric (no histopath from mandibular lymphnode); @ cervical, thoracic, lumbar); *submandibular and sublingual; ‡ each one slide of lobus dexter lateralis and l. sinister lateralis were fixed in Carony's solution; † whole head sampled; & ^longitudinal, with carina												

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

There were no treatment-related clinical signs of systemic toxicity in the air and aceton control groups as well as at the low (1 mg/m³) and mid (30 mg/m³) concentration levels.

At the high concentration level (300 mg/m³) treatment related clinical observations consisted of:

- slightly visually increased respiration during exposure in both sexes from study day 7 up to the end of the study,
- urinous odour in 4 males from day 27 to the end of the study and 7 females from study day 22 to the end of the study,
- piloerection in three females from day 19 up to day 24,
- injury on the head in one female from study day 15 up to day 27, and
- moderate labored respiration in 1 male on study day 21.

The animals that died developed visually increased respiration, urinous odour and piloerection before death.

2. Mortality

Four males and 3 females of the high concentration group (300 mg/m³) died during the study. Deaths were observed at days 10, 12, 21 and 22 in males and at days 7, 11 and 24 in females. Despite of the clinical signs mentioned above, there were no clinical biochemistry of histopathological findings indicating the cause of death.

3. Detailed clinical observations

The detailed clinical observations did not reveal any abnormalities in any of the groups, which were not described previously in the daily clinical observation.

4. FOB and Motor activity

No treatment-related findings were observed during the Functional Observation Battery examinations. These included the home cage and open field observations, the sensorimotor tests and reflexes as well as the quantitative observations (feces, rearing, grip strength, landing foot-splay).

Likewise, overall motor activity measurements did not reveal any statistically significant difference between controls and treated groups. If single 5-minute intervalls are considered there as a statistically significant increase of the activity in males exposed to the mid concentration during the 4th interval. Due to the single affected interval and in absence of a dose-response relationship this was considered to be incidental.

5. Ophthalmoscopy

No treatment-related ophthalmoscopical findings were noted in this study.

B. BODY WEIGHT

Body weight development was impaired in males at the high concentration (300 mg/m³) (see Figure 5.3.3-1). This was evident from significantly lower body weights at day 21 (-6.6%) and from significantly lower body weight gains throughout the study when compared to the acetone control group males. Overall body weight gain of high concentration males was decreased by 43%.

Differences between males at ≤ 30 mg/m³ as well as all treated females groups and acetone control groups did not attain statistical significance. Likewise, there were no significant differences between air and acetone control groups.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

There were no statistically significant differences between food consumption of acetone and treated groups except at day 7 in both sexes at 300 mg/m³. The overall average daily food intake of high concentration males tended to be slightly lower which is in line with the observed body weight effects in this group of animals (see Table 5.3.3-1).

Figure 5.3.3-1: Body weight development of rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

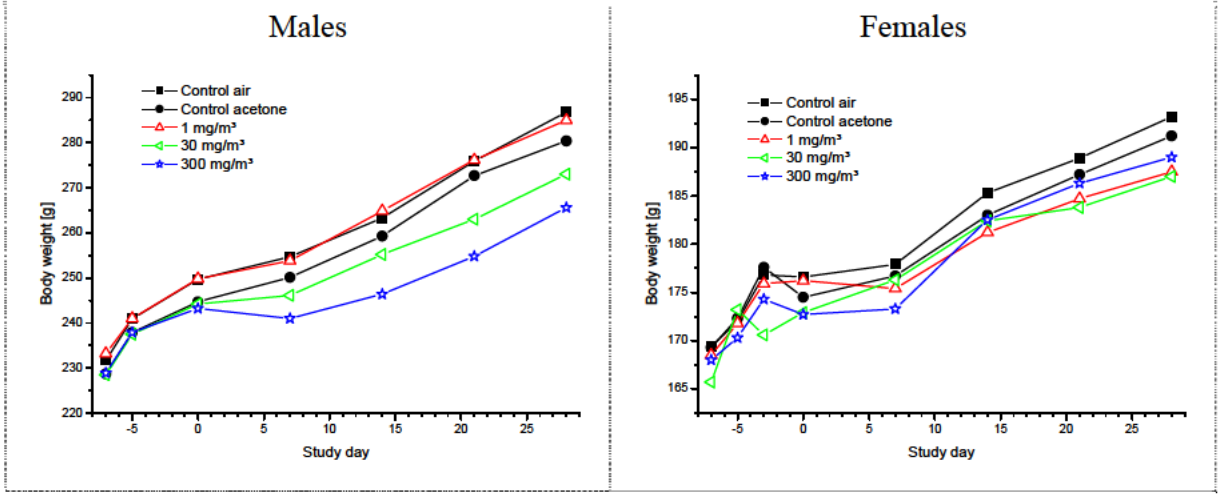


Table 5.3.3-1: Mean body weight and food consumption data of rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

Dose level [mg/m³]	0 (Air)	0 (Acetone)	1	30	300
Males					
Body weight [g]					
- Day 0	249.7	244.7	249.8	244.2	243.2
- Day 28	286.8	280.4	285.0	273.0	265.6
Δ% (compared to acetone control)			+1.6	-2.7	-5.3
Overall body weight gain [g]	37.1	35.7	35.2	28.8	20.4*
Δ% (compared to acetone control)			-1.4	-19.4	-43.0
Food consumption [g/day]					
- average week 1-4§	19.6	19.1	19.4	18.9	17.8
Δ% (compared to control)			+1.6	-0.9	-6.8
Females					
Body weight [g]					
- Day 0	176.6	174.5	176.2	172.9	172.7
- Day 28	193.2	191.2	187.5	187.0	189.0
Δ% (compared to acetone control)			-1.9	-2.2	-1.1
Overall body weight gain [g]	16.7	16.7	11.3	14.2	13.5
Δ% (compared to acetone control)			-32.3	-15.2	-19.2
Food consumption [g/day]					
- average week 1-4§	15.3	14.8	14.3	14.9	14.6
Δ% (compared to control)			-3.7	+0.5	-1.2

* $p \leq 0.05$ (Dunnett's test, two sided); § calculated from the weekly means

No statistically significant differences of weekly food efficiency between the acetone control and treated groups were observed except for day 21 in mid concentration males (30 mg/m³) and for days 7 and 21 in high concentration males.

D. BLOOD ANALYSIS

1. Hematological findings

Hematology examinations revealed a slight, but not statistically significant increase of white blood cell counts in high concentration males and females when compared to the acetone control groups. This correlated to increased polymorphonuclear neutrophil counts in the differential blood count (see Table 5.3.3-2). No treatment-related changes were seen in the other hematological parameters of both sexes. The only statistically significant differences were a slight increase in mean corpuscular volume (MCV) in females and a decrease of mean corpuscular hemoglobin concentration (MCHC), which in absence of effects on other red blood cell parameters is considered to be of no toxicological relevance.

Table 5.3.3-2: Selected hematology findings of rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

Dose [mg/m³]	0 (Air)		0 (Acetone)		1		30		300	
Males										
WBC [10^9/L]	6.12	±1.39	5.62	±1.49	5.71	±1.17	6.63	±2.03	**7.63**	**±3.50**
Neutrophils [%]	17.1	± 5.3	16.3	± 2.0	17.3	± 5.4	16.1	± 6.2	**31.0**	**±20.0**
Neutrophils [10^9/L]	1.04	±0.40	0.92	±0.29	1.00	±0.50	1.11	±0.78	**2.90**	**±3.70**
MCHC [mmol/L]	21.76	±0.65	22.10	±0.62	21.95	±0.54	21.87	±0.41	21.26*	±0.36
Females										
WBC [10^9/L]	3.98	±0.93	3.57	±0.99	3.72	±1.08	3.80	±1.20	**5.24**	**±1.35**
Neutrophils [%]	13.9	± 3.8	15.7	± 3.5	13.0	± 2.9	15.0	± 4.8	**21.6**	**± 5.9**
Neutrophils [10^9/L]	0.55	±0.20	0.56	±0.21	0.47	±0.11	0.56	±0.27	**1.13**	**±0.49**
RBC [10^{12}//L]	8.12	±0.41	7.81$^+$	±0.27	7.88	±0.28	7.83	±0.30	7.66	±0.42
MCV [fl]	51.4	± 1.9	53.2$^+$	± 1.1	52.6	± 1.1	52.6	± 1.2	55.0**	± 1.0
MCH [fmol]	1.13	±0.07	1.18$^+$	±0.05	1.17	±0.04	1.15	±0.04	1.20	±0.03
Platelets [10^{12}/L]	934	± 83	855$^+$	±133	934	± 95	865	±113	871	±100

comparison acetone control - treated groups: * $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis / iWilcoxon-test, two sided)
comparison air and acetone control: $^+$ $p \leq 0.05$ (Kruskal-Wallis / Wilcoxon-test, two sided)

No significant differences in hematology parameters were found between the air control and the vehicle control of the males. In female rats slight, but statistically significantly decreased red blood cell (RBC) and platelet counts and increased MCV and mean corpuscular hemoglobin (MCH) values were found in the peripheral blood of the animals exposed to the vehicle.

2. Clinical chemistry findings

No treatment-related differences were observed between treated and the acetone control groups. In absence of other treatment related changes the marginal, but statistically significant increase of alkaline phosphatase (ALP) in high concentration females was considered to be of no toxicological relevance (see Table 5.3.3-3).

There were a number of statistically significant differences between the air and acetone control. These consisted of decreased potassium, alanine and aspartate aminotransferase activities in female an of increased total bilirubin and total protein concentration in males of the acetone control. or lacked a dose-response relationship (increased total protein in low concentration females).

Table 5.3.3-3: Selected clinical chemistry findings of rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

Dose	[mg/m³]	0 (Air)		0 (Acetone)		1		30		300	
Males											
TBIL	[µmol/L]	2.64	±0.44	3.12*	±0.44	2.69	±0.51	2.95	±0.43	3.71	±0.61
TProt	[g/L]	65.99	±1.06	67.49*	±1.84	65.28	±2.65	65.43	±2.36	66.12	± 4.0
AST	[µkat/L]	2.08	±0.18	1.70^{++}	±0.37	1.67	±0.28	1.75	±0.53	1.77	±0.42
ALT	[µkat/L]	0.49	±0.13	0.39^{+}	±0.06	0.37	±0.04	0.36	±0.07	0.46	±0.07
ALP	[µkat/L]	0.68	±0.11	0.74	±0.11	0.69	±0.14	0.66	±0.12	0.98*	±0.25
K	[mmol/L]	4.77	±0.23	4.48	±0.25	4.57	±0.19	4.53	±0.20	4.47	±0.19

comparison acetone control - treated groups: * $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis / Wilcoxon-test, two sided)
comparison air and acetone control: $^{+}$ $p \leq 0.05$ (Kruskal-Wallis / Wilcoxon-test, two sided)

E. NECROPSY

Statistically significant differences of organ weights between the acetone control groups and treated groups were observed for thymus (absolute) and kidney, liver, lung, spleen and thymus (relative). For details see Table 5.3.3-4.
In absence of relevant morphologic findings, the significantly decreased mean absolute and relative thymus weights in high concentration females were not considered to be toxicologically relevant. The effect on thymus weight in females may be an indirect effect, as animals in this group had remarkable findings in the nasal cavity (atrophy/necrosis, see below). This might have caused stress to the animals which led to a decrease in the thymic absolute and relative weights.
Likewise, all other changes were considered to be incidental since they were not corroborated by any histopathological changes and the changes were not dose-related (relative lung weights in females and relative liver and lung weights in males). Especially, the statistically higher relative lung weights in females were probably due to very low lung weights in the acetone control group when compared to those in the respective air control.

In addition, absolute lung and relative liver and lung weights of aceton control males displayed a statistically significant difference when compared to the air control. These changes were not accompanied by any histopathological changes and were considered to be incidental.

Table 5.3.3-4: Selected absolute and relative organ weights of of rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

Sex		Males				Females			
	Concen-tration [mg/m³]	Absolute weight [mg]	Δ%	Relative weight [% of b.w.]	Δ%	Absolute weight [mg]	Δ%	Relative weight [% of b.w.]	Δ%
Terminal weight [g]	0	253.77				168.31			
	0	249.89				167.23			
	1	253.85	(1.6)[&]			165.38	(-1.1)		
	30	243.71	(-2.5)			165.34	(-1.1)		
	300	234.83	(-6.0)			165.21	(-1.2)		
Kidneys [g]	0	1.767		0.697		1.282		0.762	
	0	1.749		0.7		1.217		0.727	
	1	1.74	(-0.5)	0.686	(-2.0)	1.177	(-3.3)	0.711	(-2.2)
	30	1.703	(-2.6)	0.698	(-0.3)	1.169	(-3.9)	0.707	(-2.8)
	300	1.755	(0.3)	0.746	(6.6)	1.273	(4.6)	0.771*	(6.1)
Liver [g]	0	6.91		2.723		4.779		2.842	
	0	6.521		2.609*		4.515		2.7	
	1	6.587	(1.0)	2.594	(-0.6)	4.609	(2.1)	2.786	(3.2)
	30	6.506	(-0.2)	2.669	(2.3)	4.538	(0.5)	2.745	(1.7)
	300	6.483	(-0.6)	2.754	(5.6)	5.014	(11.1)	3.032**	(12.3)
Lung [g]	0	0.924		0.365		0.762		0.453	
	0	0.85		0.34*		0.708		0.423	
	1	0.886	(4.2)	0.349	(2.6)	0.762	(7.6)	0.461*	(9.0)
	30	0.887	(4.4)	0.363	(6.8)	0.700	(-1.1)	0.423	(0.0)
	300	0.875	(2.9)	0.374	(10.0)	0.777	(9.7)	0.471**	(11.3)
Spleen [g]	0	0.531		0.208		0.381		0.227	
	0	0.452		0.18		0.389		0.232	
	1	0.497	(10.0)	0.196	(8.9)	0.367	(-5.7)	0.222	(-4.3)
	30	0.463	(2.4)	0.19	(5.6)	0.382	(-1.8)	0.231	(-0.4)
	300	0.563	(24.6)	0.24**	(33.3)	0.429	(10.3)	0.26	(12.1)
Thymus [mg]	0	253.4		0.1		280.2		0.166	
	0	224.8		0.09		260.8		0.155	
	1	257.2	(14.4)	0.101	(12.2)	234.4	(-10.1)	0.141	(-9.0)
	30	219.3	(-2.4)	0.09	(0.0)	213.5	(-18.1)	0.128	(-17.4)
	300	223.5	(-0.6)	0.094	(4.4)	186.1**	(-28.6)	0.113**	(-27.1)

[&] % Difference compared to vehicle (acetone) control
* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis / Wilcoxon-test, two sided)

2. Gross and histopathology

The only treatment-related gross pathological findings consisted of discolored lung lobes in 2 males and 3 females which died prematurely.
There were a few additional gross lesions which were observed with single incidences in the air and acetone control groups as well as in treated male and female groups. Neither the type nor distribution indicated a relation to treatment.

Histopatholog identified the respiratory tract, i.e. the nasal cavity, the larynx and the lungs as well as the duodenum as target organs at the two higer dose levels. The histopathological findings in these organs are listed in Table 5.3.3-5.

Table 5.3.3-5: Incidence and severity of selected histopathological lesions in rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

Sex	Males					Females				
Concentration [mg/m³]	0 (air)	0 (acetone)	1	30	300	0 (air)	0 (acetone)	1	30	300
Animals in selected group	10	10	10	10	10	10	10	10	10	10
Nasal cavity, I **exam**	10	10	10	10	10	10	10	10	10	10
Hyperplasia , respiratory epithelium		2 [1.0]$			**9 [1.8]**		2 [1.5]			**9 [2.0]**
Inflammation, reactive					**2 [p]&**					**4 [p]**
Nasal cavity, II **exam**	10	10	10	10	10	10	10	10	10	10
Atrophy/necrosis, olfactory epithelium				**2 [1.0]**	**10 [2.5]**				**3 [1.0]**	**9 [2.1]**
Hyperplasia , respiratory epithelium					**2 [2.5]**					**7 [1.7]**
Inflammation, reactive		1 [p]	1 [p]		1 [p]					
Nasal cavity, III **exam**	10	10	10	10	10	10	10	10	10	10
Atrophy/necrosis, olfactory epithelium				**5 [1.0]**	**10 [2.4]**				**4 [1.8]**	**9 [2.3]**
Hyperplasia , respiratory epithelium					**10 [1.8]**				**1 [2.0]**	**7 [1.8]**
Metaplasia, squamous					**1 [1.0]**					**4 [1.8]**
Nasal cavity, IV **exam**	10	10	10	10	10	10	10	10	10	10
Atrophy/necrosis, olfactory epithelium				**9 [1.7]**	**10 [2.3]**				**8 [2.4]**	**9 [2.4]**
Regeneration/repair, olfactory epithel.					**9 [2.0]**				**1 [1.0**	**8 [1.9]**
Hyperplasia , respiratory epithelium	1 [2.0]				**8 [1.5]**				**1 [2.0]**	**7 [1.3]**
Larynx, level III **exam**	10	10	10	10	10	10	10			10
Hyperplasia , respiratory epithelium					3 [1.3]		1 [1.0]			
Lungs **exam**	10	10	10	10	10	10	10	10	10	10
Inflammation, perivascular	5 [1.2]	1 [1.0]	4 [1.5]	2 [1.0]	4 [1.8]	1 [1.5]	6 [1.0]	5 [1.0]	2 [1.0]	**6 [2.0]**
Histiocystiosis	1 [1.0]	1 [1.0]	3 [1.3]		**3 [2.0]**	1 [2.0]	1 [2.0]	2 [1.5]	**5 [1.0]**	**5 [1.0]**
Duodenum **exam**	10	10	10	10	10	10	10	10	10	10
Hyperplasia, mucosal, diffuse				**5 [1.0]#**	**7 [1.3]**	**1 [2.0]**	**1 [1.0]**		**5 [1.0]**	**10 [2.4]**

$ [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

[ ] The grading of mucosal hyperplasia was assessed by a quantitative mucosal area measurement performed by analySIS Doku 3.0. The following gradings were used: until an area of 6 mm² = grade 0, > 6-8 mm² = grade 1, >8-10 mm² = grade 2, >10-12 mm² = grade 3, > 12 mm² = grade 4.

& [p] finding present, no grading performed

A mild to moderate destruction of the olfactory epithelium in the nasal cavity was observed. These changes were characterized by (multi)focal atrophy and/or necrosis, (multi)focal reactive inflammation and signs of repair and regeneration as irregular architecture of the epithelium or gland-like structures, and karyomegaly of the sustentacular cells. These findings are histomorphological correlates of different stages and intensities of the compound-related irritant effect.
The changes in the respiratory epithelium were not as severe as those in the olfactory epithelium. There was minimal to moderate (multi)focal hyperplasia, especially in level I.
The minimal to slight hyperplasia of the larynx respiratory epithelium observed in three high concentration males was considered to be treatment-related, too.
In the lungs of all groups a minimal to slight perivascular infiltration of inflammatory cells was observed. As the severity was slightly higher in the high concentration females this was considered to be treatment-related. In addition, the number of histiocytes (severity) in the alveoli in was slightly higher at the high concentration males whereas the incidence was elevated females at the mid and high concentration.
Finally, a treatment related increase in the incidence and severity of diffuse mucosal hyperplasia in the duodenum was observed.

F. ATMOSPHERE ANALYSIS

The exposure conditions are summarized in Table 5.3.3-6.

Table 5.3.3-6: Exposure contitions

Test group	Supply air (conditioned) [m³/h]	Supply air (compressed) [m³/h]	Exhaust air [m³/h]	Atomizer pressure [bar]	Pump rate[1] [ml/h]	Acetone pump rate [ml/h]
0	6.0	-	5.4			
1	5.0	1.0	5.4	0.8 - 1.2		23 - 46
2	5.0	1.0	5.4	0.8 - 1.2	8 - 12.8	
3	5.0	1.0	5.4	0.8 - 1.2	184 - 368	
4	5.0	1.0	5.4	0.8 - 1.2	1840 - 3680	

[1] diluted test substance;
Conditioned supply air is activated charcoal filtered air conditioned to about 50% ± 20% relative humidity and 22°C ± 2°C;
Compressed air is filtered air pressurized to about 6 bar

The results of the atmosphere concentration analysis are depicted in Table 5.3.3-7.

Table 5.3.3-7: Measurement of pyraclostrobin and acetone concentrations

Test group	Target concentration [mg/m³]	Measured concentration (Pyraclostobin) [mg/m³]	Measured concentration (Acetone) [mg/m³]
0	Air control		
1	Aceton control		2.63 ± 0.94
2	1	1.17 ± 0.3	
3	30	30.4 ± 1.2	
4	300	299 ± 15	2.44 ± 0.23

The measured MMAD values for the pyraclostrobin containing aerosols are given in Table 5.3.3-8.

Table 5.3.3-8: Particle size measurement

Test group	MMAD [µm]	Geometric standard deviation	< 3 µm [%]
2	1.6 ± 0.3	4.0 ± 0.2	67.8 ± 3
3	1.9 ± 0.1	2.6 ± 0.1	70.3 ± 2
4	1.9 ± 0.1	2.5 ± 0.2	70.7 ± 2

III. CONCLUSIONS

Six hour subacute inhalatory exposure of rats to an aerosol of pyraclostrobin dissolved in acetone for 28-day on a 5 days per week basis resulted in the death of 4 male and 3 female rats at the high concentration of 300 mg/m³ (0.3 mg/L). This concentration is close to the acute 4h LC_{50} of 0.58 mg/L observed for a pyraclostrobin aerosol when dissolved in acetone. The respiratory tract (nasal cavity, larynx and lungs) and the duodenum were identified as target organs as indicated by dose dependent atrophy/necrosis and regeneration/repair of the olfactory epithelium, hyperplasia of the respiratory epithelium of nasal cavity and the larynx, perivascular inflammation of the lungs as well as hyperplasia of the duodenal mucosal. The low dose of 1 mg/m³ was identified as NOAEC.

Report:	CA 5.3.3/2 [redacted]2014a Bas 500 F (Pyraclostrobin) - Repeated dose 28-day inhalation toxicity study with recovery period in Wistar rats, aerosol exposure 2014/1003946
Guidelines:	OECD 413, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, EPA 870.3465
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 28-day inhalation toxicity study, four groups of Wistar rats (10/sex) were exposed to liquid aerosols of pyraclostrobin (COD-001236, purity 99.02%) dissolved in acetone at nominal concentrations of 3, 10 and 30 mg/m³ for 6 hours daily on a 5 applications per week basis. The control group was exposed to acetone. The study included a recovery group of 10 animals/sex treated either with acetone (vehicle control) or 30 mg/m³ for 28 day followed by a 4 weeks treatment-free recovery period.
No mortality was observed in any dose group. Fluctuations of body weight (body weight loss during inhalation exposure, body weight gain during the treatment free weekends) was observed in all groups including controls. The overall body weights and body weight gains did not display statistically significant differences. No treatment related clinical biochemistry findings were noted in treated groups. A weight increase of the duodenum in the mid and high dose groups was considered to be treatment-related, but an adaptive and not adverse effect, as no histopathological correlate was observed, which could explain the weight increase. Histopathology did not reveal any indication of systemic toxicity. However, a minimal to slight atrophy/necrosis of the olfactory epithelium in high concentration males and females indicated local irritant effects, which were reversible within the 4-week recovery period.
Based on the results of this study the 'No Observed Adverse Effect Concentration' (NOAEC) for systemic toxicity was 30 mg/m³, whereas based on the effects in the upper respiratory tract the NOAEC for local effect in nasal cavity was 10 mg/m^3.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin (BAS 500 F)

Description:	Solid melt / red-brown
Lot/Batch #:	COD-001236
Purity/content:	99.02%
Stability of test compound:	Stable; Expiry date: 01.10.2015

2. Vehicle and/or positive control: Acetone

3. Test animals:

Species:	Rat
Strain:	Wistar Crl:WI(Han)
Sex:	males and females
Age at dosing:	about 7 weeks
Weight at dosing:	260.0 ± 11.1 g (males), 178.7 ± 10.6 g (females)
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH; 97633 Sulzfeld
Acclimation period:	at least one week In order to accustom the animals to exposure they were treated with supply air for 6 hours under conditions comparable to exposure on 2 days before start of exposure (pre-exposure period).
Diet:	milled mouse/rat laboratory diet "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum except during exposure and motor activity measurements
Water:	Tap water ad libitum except during exposure and motor activity measurements
Housing:	five animals per cage in Polysulfon cages H-Temp (PSU) (Tecniplast, Hohenpeißenberg, Germany), floor area approx. 2065 cm².
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; 15 air changes per hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of work: 21-Jan-2014 to12-Jun-2014
(in-life dates: 21-Jan-2014 to 04-Apr-2014)

2. Animal assignment and treatment

Groups of 10 male and 10 female Wistar rats per test group were exposed in a head-nose inhalation system to atmospheres containing aerosols of BAS 500 F for 6 hours per working day for about 28 days on a 5 days/week basis (20 exposures). For generation of the aerosols the test substance (non volatile solid melt) was dissolved in acetone. The target concentrations were 0, 3, 10 and 30 mg/m³. Additionally, one group of male and female rats each was exposed to the vehicle acetone.

Test group	Target concentration [mg/m³]	No. of animals per sex	Males Animal No.	Females Animal No.
0	Acetone control	10	1-10	41-50
1	3	10	11-20	51-60
2	10	10	21-30	61-70
3	30	10	31-40	71-80

In order to check for any reversibility, progression or delay of toxic effects the following post-exposure observation groups were used. These animals were observed for about 4 weeks after the exposure period.

Test group	Target concentration [mg/m³]	No. of animals per sex	Males Animal No.	Females Animal No.
0	Acetone control	10	81-90	101-110
3	30	10	91-100	111-120

The animals were allocated to the treatment groups according to weight using suitable randomization software. The variation of individual weights of the animals used did not exceed 20% of the mean weight of each sex.

3. Aerosol generation and analysis

A head-nose inhalation system INA 60 (stainless-steel construction, BASF Aktiengesellschaft, volume V ≈ 90 L) was used. The animals were restrained in glass tubes and their snouts projected into the inhalation system.

A liquid aerosol was generated. The test substance was diluted 1:9 with acetone. The aerosol was produced by continuously pumping the test-substance solution to a two-component atomizer by means of a metering pump. Using compressed air, the aerosol was generated which was produced inside the exposure system. From study day 4 to day 15 the atmosphere generation procedure was changed for the low dose group due to variability of the test atmosphere concentration. In principle, a higher concentrated aerosol was generated, which was diluted with additional air to achieve the desired concentration of 3 mg/m³. Due to technical reasons, the initial generation setup for the low concentration group was restored from study day 18 onwards. This did not negatively affect the pyraclostrobin concentration in the inhalation atmosphere. In the control group, acetone was sprayed at the pump rate used for the high concentration group.

A positive pressure was maintained inside the exposure systems by adjusting the air flow of the exhaust air system. This ensured that the aerosol in the breathing zones of the animals was not diluted by laboratory air.

The concentrations of the inhalation atmospheres were analysed by gravimetry This analytical method is judged to be valid, because pyraclostrobin does not possess an appreciable vapour pressure. Daily means were calculated based on three samples for the mid and high concentration group. Due to the longer sampling time for the low concentration, two samples per day were analyzed only. For this a filtration equipment with a sampling probe of 7 mm diameter was used. Through pre-weight filters metered volumes of the aerosol drawn by means of a vacuum pump.

- Filter: MN 85/90 BF (d = 47 mm)
- Sampling position: immediately adjacent to the animals' noses
- Sampling flow: 3 L/min
- Sampling velocity: 1.25 m/s
- Sample volume: 360 L, 120 L and 45 L at target concentrations of 3, 10 and 30 mg/m³

The volatile solvent acetone was analyzed by gas chromatography in the control group based on one sample per exposure. Like for the treated groups the samples were withdrawn immediately adjacent to the animals' noses. The sampling probe had an inner diameter of 4 mm. Fifteen liter samples were withdrawn at a sampling velocity of 1.25 m/s and a flow rate of 1 L/min. The samples were drawn through 3 absorption vessels connected in series, each of which was filled with 2-propanol as absorption solvent. After the sampling, the content of the probe and the first 2 absorption vessels was eluted and pooled into a 50 mL graduated flask for individual analysis. The last absorption vessel served as control for absorbing efficiency during sampling.

The particle size analysis was done using a Stack Sampler Marple 298 (New Star Environmental) and a vacuum pump (Millipore). The sampling probe had an internal diameter 7 mm. Before sampling, the impactor was assembled with pre-weighed metal collecting discs and equipped with a backup particle filter. The impactor was connected to the vacuum pump and for each test group samples were taken from the breathing zone of the animals. Samples were withdrawn at a velocity of 1.25 m/sec and a volume of 3 L/min. Depending on the nominal atmosphere concentrations the sample volumes were 360, 120 and 45 L for test groups 1 to 3, respectively. After sampling the impactor was disassembled. The impactor stages and the backup particle filter were freed of acetone and re-weighed. Each four samples were taken per concentration level during the 4 weeks exposure period.

4. Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Parameter	Statistical test	References
body weight, body weight change (during exposure period)	Comparison of each group with the control group was performed using DUNNETT test (two-sided) for the hypothesis of equal means.	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096 – 1121 DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482 – 491
body weight, body weight change (recovery group during post-exposure period) and Food consumption (recovery group)	Comparison of the dose group with the control group was performed using the STUDENT'S t-test (two-sided) for the hypothesis of equal means.	WINER, B.J. (1971): Statistical principles in experimental design. McGraw-Hill, New York, 2nd edition
Blood parameters	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians For parameters with unidirectional changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) with Bonferroni-Holm adjustment for the hypothesis of equal medians	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York Holm (1979): A Simple Sequentially Rejective Multiple Test Procedure. Scand. J. Statist. 6, 65-70
Blood parameters (recovery group animals)	For parameters with bidirectional changes: WILCOXON-test (two-sided) for the hypothesis of equal medians For parameters with unidirectional changes: Pairwise WILCOXON-test (one-sided) for the hypothesis of equal medians	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York
weight parameters (pathology)	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each concentration group with the control group was performed using the WILCOXON test for the hypothesis of equal medians	HETTMANNSPERGER, T.P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-140. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3 MILLER, R.G. (1981): Simultaneous Statistical Inference Springer-Verlag New York Inc., 165-167 NIJENHUIS, A. and S.W. WILF (1978): Combinatorial Algorithms, Academic Press,New York, 32-33

C. METHODS

1. Clinical examinations:
The animals were examined for evident signs of toxicity or mortality twice daily on working days and once daily on weekends and public holidays.
The clinical condition of the test animals was recorded once on pre-exposure and on post-exposure observation days and at least 3 times (before, during and after exposure) on exposure days. During exposure only a group wise examination was possible.

2. Body weight:
The body weight of the animals was determined on start of the pre-exposure, at the start of the exposure period and then, as a rule, twice a week (Monday and Friday) as well as prior to gross necropsy. As a rule, the animals were weighed at the same time of the day.

3. Food consumption, food efficiency and compound intake:
Food consumption was determined weekly and calculated as mean food consumption in grams per animal and day.
The animals were maintained in social-housing cages, with 5 animals per cage, during the whole study period. Therefore, the food consumption was determined cage-wise. The food consumption per animal and day was calculated by dividing food consumption of the day of a respective cage by the 5 animals per cage. For test groups 1 and 2, the animals of each test group were housed in only two cages per sex, no statistical evaluation of food consumption is possible. Due to recovery group animals of test group 3 and control group statistical evaluation were done during exposure period, but not for the post-exposure observation period for the same reason mentioned above.

4. Ophtalmoscopy:
Before the start of the exposure period the eyes of all animals, and towards the end of the study (study day 27/26) the eyes of the animals of the acetone control group and high concentration group were subjected to ophthalmocopic examination. At the end of the observation period (day 56/55) the eyes of the animals of control group and test group 3 (high concentration) were examined, additionally.

5. Functional observation battery (FOB) and Motor activity:
Not performed in this study.

6. Hematology and clinical chemistry:
Blood was collected from the retroorbital venous plexus in the morning from over-night fasted animals under isoflurane anaesthesia. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence. The following parameters were determined.

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count	✓	Platelet count
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Prothrombin time
✓	Hematocrit (Hct)	✓	Eosinophils (differential)		
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes	✓	Large unstained cells		

Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Potassium	✓	Creatinine	✓	Serum-γ-glutamyl transpeptidase (γ-GT)
✓	Sodium	✓	Globulin		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

7. Urinalysis:
Not performed in this study.

8. Sacrifice and pathology:
All animals were sacrificed under pentobarbital anesthesia by exsanguination from the abdominal aorta and vena cava. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

Pathology:												
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: acetone control and top concentration animals.)												
C	W	H		C	W	H		C	W	H		
✓	✓	#	adrenals	✓	✓	#	kidneys	✓		#	salivary glands*	
✓		#	aorta	✓		#	lachrymal glands (exorbital)	✓		#	seminal vesicles	
✓	✓	#	brain	✓		✓	larynx (3 levels)	✓		#	skin	
✓			bone marrow§	✓	✓	✓	liver‡	✓		#	spinal cord (3 levels)@	
✓		#	caecum	✓	✓	✓	lung	✓	✓	#	spleen	
✓		#	colon	✓		#	lymph nodes#	✓		#	sternum w. marrow	
✓	✓	✓	duodenum	✓		#	mammary gland	✓		#	stomach (fore- & glandular-)	
✓	✓	#	epididymides	✓		#	muscle, skeletal	✓		#	teeth	
✓		#	esophagus	✓		#	nerve, peripheral (sciatic n.)	✓	✓	#	testes	
✓		#	eyes with optic nerve	✓		✓	nose/nasal cavity (4 levels)	✓	✓	#	thymus	
✓		#	femur (with knee joint)	✓	✓	#	ovaries	✓	✓	#	thyroid/parathyroid	
✓		✓	gross lesions	✓		#	pancreas	✓			tongue	
✓		#	Harderian glands	✓		#	pituitary	✓		#	trachea&	
✓	✓	#	heart	✓		✓	pharynx	✓		#	urinary bladder, ureter, urethra	
✓		#	ileum	✓		#	prostate	✓	✓	#	uterus	
✓		#	jejunum	✓		#	rectum		✓		body (anesthethized)	
§ from femur; # tracheobronchial, mediastinal and mesenteric; @ cervical, thoracic, lumbar cord; *mandibular and sublingual; ‡ each one slide of lobus dexter lateralis and l. sinister lateralis were fixed in Carony's solution; & one transverse section and one longitudinal through carina of the bifurcation of the extrapulmonary bronchi												

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

There were no treatment-related clinical signs of systemic toxicity in the aceton control groups as well as at the low (3 mg/m³) and mid (10 mg/m³) and high (30 mg/m³) concentration levels.

2. Mortality

No deaths occurred throughout the study.

3. Ophthalmoscopy

No treatment-related ophthalmoscopical findings were noted in this study.

B. BODY WEIGHT

Body weight development in all dose groups during exposure was not statistically significantly different from the control group (see Figure 5.3.3-1 and Table 5.3.3-9). This holds true for the high dose recovery group at days 27 (end of exposure) and 55/56 (end of recovery period).
Fluctuations of body weights were observed during the exposure period. These were more pronounced in males than females. These fluctuations were considered to be due to the stress associated with the exposure conditions as these fluctuations were observed in all groups independent of dose and occurred already after the first, pre-treatment exposures to air in the inhalation apparatus during the acclimatization period. Additionally, a recovery of body weights was generally observed during the treatment-free weekends.

In the report body weight gains were only calculated for the treatment periods; treatment-free weekends were not considered. Sporadically, statistically significant changes of body weight gain were observed, which were considered incidental due to a lack of a dose response (low dose females for the day 10 to 14 interval) or temporal inconsistency (recovery group males for the intervals day -3 to 0 and 0 to 1, recovery group females for the day 38 to 42 interval).

Considering the entire treatment period, respectively the treatment and recovery period for the recovery animals, a numerically, but not statistically significant effect on body weight gain was observed in high concentration males (see Table 5.3.3-9).
The overall body weight change (day 0 to 56) of high concentration males was decreased by 19.3%.

Figure 5.3.3-2: Body weight development of rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

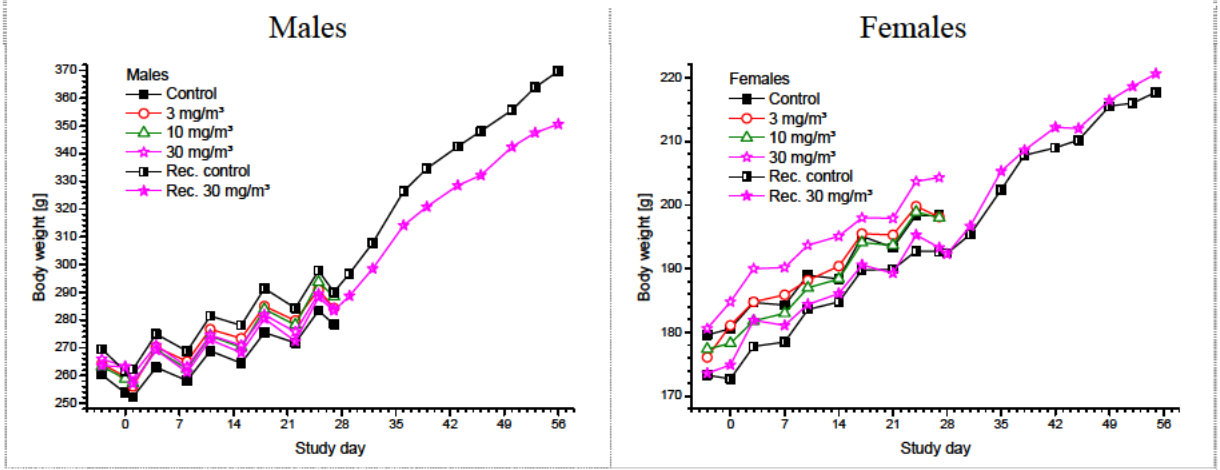


Table 5.3.3-9: Mean body weight and food consumption data of rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

Dose level [mg/m³]	0	3	10	30	0^R	30^R
Males						
Body weight [g]						
- Day 0	253.8	259.6	258.9	263.3	261.5	263.1
- Day 27	278.5	284.3	288.5	284.2	290.1	283.4
- Day 56					369.7	350.6
Δ% (compared to control day 27)		2.1	3.6	2.0		-2.3
Δ% (compared to control day 56)						-5.2
Overall body weight gain until day 27[g]	24.7	24.8	29.6	20.9	28.6	20.3
Δ% (compared to control)		0.3	19.7	-15.5		-29.0
Overall body weight gain until day 56[g]					108.3	87.4
Δ% (compared to control)						-19.3
Food consumption [g/day]						
- average week 1-4[§]	17.4	18.0	17.9	17.3	19.8[#]	19.8[#]
Δ% (compared to control)		3.8	3.2	-0.7		-0.3
Females						
Body weight [g]						
- Day 0	180.6	181.1	178.3	184.8	172.7	174.9
- Day 27	198.4	198.1	198.0	204.3	192.7	193.3
- Day 55					217.7	220.6
Δ% (compared to control day 27)		-0.2	-0.2	3.0		0.3
Δ% (compared to control day 55)						1.3
Overall body weight gain until day 27[g]	17.8	17.0	19.8	19.5	20.0	18.4
Δ% (compared to control)		-4.2	11.2	9.4		-8.0
Overall body weight gain until day 55[g]					45.0	45.6
Δ% (compared to control)		-4.5	10.7	9.6		1.5
Food consumption [g/day]						
- average week 1-4[§]	14.2	14.6	14.4	14.3	14.7[#]	14.7[#]
Δ% (compared to control)		3.0	1.3	0.6		-0.5

* $p \leq 0.05$ (Dunnett's test, two sided); [$] calculated from the weekly means; R = Recovery group; [#] average week 1-8
Values may not calculate exactly due to rounding of figures

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

There were no statistically significant differences between food consumption of acetone and treated groups. The overall average daily food intake was well comparable between the test and the control groups (see Table 5.3.3-9).

D. BLOOD ANALYSIS

1. Hematological findings

No treatment-related changes of hematological parameters were observed (see Table 5.3.3-10). In mid and high concentration females (10 and 30 mg/m³) a slight decrease in hemoglobin values was observed, which is considered incidental and not treatment-related because the group means were within historical control range (7.8 to 9.5 mmol/L). Slight changes in absolute and relative neutrophil cell counts and relative lymphocyte counts in 30 mg/m³ females were within historical control range and thus not considered to be affected by treatment. The control ranges were 0.37-0.88 Giga/L absolute neutrophil and 11.1-26.6% for relative neutrophil counts as well as 66.7-83.9% for relative lymphocyte counts.

Table 5.3.3-10: Selected hematology findings of rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

Dose [mg/m³]	0	3	10	30	0^R	30^R
Males						
HGB [mmol/L]	9.0 ± 0.3	8.8 ± 0.2	8.8 ± 0.2	8.7 ± 0.2	8.8 ± 0.2	8.9 ± 0.3
Neutrophils [%]	20.6 ± 4.1	22.4 ± 7.2	20.0 ± 5.8	22.9 ± 4.7	27.3 ± 6.0	25.2 ± 4.9
Neutrophils [10^9/L]	1.07 ±0.27	1.19 ±0.57	1.00 ±0.21	1.19 ±0.22	1.41 ±0.25	1.21 ±0.24
Lymphocytes [%]	75.1 ± 3.6	73.1 ± 6.9	75.7 ± 6.2	71.9 ±5.7	66.9 ± 6.8	69.5 ± 4.5
Females						
HGB [mmol/L]	8.6 ± 0.33	8.6 ± 0.2	8.3 ± 0.2*	8.2 ± 0.3*	8.4 ± 0.3	8.4 ± 0.1
Neutrophils [%]	19.0 ± 4.0	16.5 ± 3.9	17.7 ± 3.3	21.9 ± 6.5	25.0 ± 9.0	17.2 ± 5.1*
Neutrophils [10^9/L]	0.56 ±0.11	0.51 ±0.10	0.59 ±0.12	0.70 ±0.19	0.68 ±0.16	0.50 ±0.10**
Lymphocytes [%]	76.1 ± 4.8	79.4 ± 4.3	77.8 ± 3.7	73.5 ± 6.5	69.6 ± 8.9	77.5 ± 5.5*

* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis / Wilcoxon-test, two sided)
^{R} Recovery group, values measured on day 56

2. Clinical chemistry findings

Table 5.3.3-11: Selected clinical chemistry findings of rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

Dose [mg/m³]	0	3	10	30	0^R	30^R
Males						
Creatinine [µmol/L]	27.3 ± 2.1	27.9 ± 2.1	30.5 ± 2.4**	27.9 ± 3.6	26.8 ± 2.0	25.3 ± 2.1
Females						
ALT [µkat/L]	0.59 ±0.10	0.49 ± 0.05**	0.51 ±0.08	0.51 ±0.11*	0.58 ±0.10	0.58 ±0.08
Sodium [mmol/L]	141.5 ±1.1	141.6 ±0.9	140.3 ±1.0*	141.4 ±1.0	141.5 ±1.2	141.1 ±1.0

* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis / Wilcoxon-test, two sided)
R Recovery group, values measured on day 56

No treatment-related differences were observed between treated and the acetone control groups. In absence of dose a dependency the statistically significant changes of creatinine sodium levels and alanine aminotransferase (ALT) values were considered to be of incidental nature and not related to treatment (see Table 5.3.3-11). The latter values were additionally within the historical control range (0.43-0.80 µkat/L).

E. NECROPSY

1. Organ weight

Statistically significant differences of organ weights were restricted to the duodenum and spleen (see Table 5.3.3-12). In absence of a histopathological correlate the increase of absolute and relative duodenum weight at ≥ 10 mg/m³ se group was regarded as a treatment-related, but adaptive, non-adverse change.

In absence of corroborative histopathological changes, the increase of absolute spleen weights in high concentration females was not considered to be treatment-related.

Table 5.3.3-12: Selected absolute and relative organ weights of rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

Sex		Males				Females			
	Concen-tration [mg/m³]	Absolute weight [mg]	Δ%	Relative weight [% of b.w.]	Δ%	Absolute weight [mg]	Δ%	Relative weight [% of b.w.]	Δ%
Terminal weight [g]	0	252.66				174.19			
	3	258.32	(2)			174.79	(0)		
	10	261.12	(3)			174.92	(0)		
	30	257.44	(2)			181.45	(0)		
Duodenum [g]	0	0.371		0.147		0.364		0.209	
	3	0.393	(6)	0.152	(4)	0.363	(0)	0.207	(-1)
	10	**0.425****	**(15)**	**0.163****	**(11)**	**0.440****	**(21)**	**0.252****	**(21)**
	30	**0.502****	**(35)**	**0.195****	**(33)**	**0.508****	**(40)**	**0.281****	**(34)**
Spleen [g]	0	0.471		0.186		0.373		0.214	
	3	0.490	(4)	0.190	(2)	0.403	(8)	0.231	(8)
	10	0.519	(10)	0.199	(7)	0.381	(2)	0.218	(2)
	30	0.518	(10)	0.201	(8)	0.442*	(18)	0.243	(14)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis / Wilcoxon-test, two sided)

No altered organ weights were noted at the end of the recovery period.

2. Gross and histopathology

All macroscopic findings occurred either individually or were equally distributed between control and treatment groups. They were considered to be incidental or of spontaneous origin.

Histopathology identified the respiratory tract, i.e. the nasal cavity and the larynx as target organs. A minimal to slight (multi)focal loss and/or destruction (atrophy/necrosis) of the olfactory epithelium in the nasal cavity was observed at 30 mg/m³ (see Table 5.3.3-13). These histopathological changes were reversible within the 4 weeks recovery period.
In some treated and one control animal at the base of the epiglottis a small focal area was covered by flattened epithelium, which differed from the normal cuboidal to columnar laryngeal epithelium. This finding was still observed in recovery group in two control animals and two treated animals (see Table 5.3.3-14). This minimal and focal change was regarded to be an adaptive, non-adverse response to the inhalation procedure and occurs also in controls. No dysfunction of the larynx is to be expected (Kaufmann et al., 1st International ESTP Expert Workshop: ‘‘Larynx squamous metaplasia’’. A re-consideration of morphology and diagnostic approaches in rodent studies and its relevance for human risk assessment, ExpToxicol Pathol 61: 591-603, 2009).

Table 5.3.3-13: Incidence and severity of selected histopathological lesions in rats exposed to pyraclostrobin by inhalation for 28 days on a 5 days/week basis

Sex		Males				Females			
Concentration [mg/m³]		0	3	10	30	0	3	10	30
Animals in selected group		10	10	10	10	10	10	10	10
Nasal cavity, II	**exam**	10	10	10	10	10	10	10	10
Atrophy/necrosis					2 [1.0]$				7 [1.4]
Inflammation					1 [1.0]$				
Cyst(s)					1 p&			1 p&	
Nasal cavity, III	**exam**	10	10	10	10	10	10	10	10
Atrophy/necrosis, olfactory epithelium					3 [1.0]				7 [1.6]
Nasal cavity, IV	**exam**	10	10	10	10	10	10	10	10
Atrophy/necrosis, olfactory epithelium					5 [1.4]				10 [1.5]
Larynx, level I	**exam**	10	10	10	10	10	10	10	10
Epithelial alteration			2 [1.0]	4 [1.0]	3 [1.0]	1 [1.0]	4 [1.0]	2 [1.0]	3 [1.0]
Inflammatory cell infiltration								1 [2.0]	
Larynx, level II	**exam**	10	10	10	10	10	10	10	10
Metaplasia, squamous									1 [2.0]
Infiltration, lymphoid						1 [2.0]			

$ [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

& [p] finding present, no grading performed

Table 5.3.3-14: Histological findings in larynx in recovery group animals

Sex		Males		Females	
Concentration [mg/m³]		0	30	0	30
Animals in selected group		10	10	10	10
Larynx, level II	**exam**	10	0	10	10
Epithelial alteration		1 [1.0]$	2 [1.0]	1 [1.0]	0

$ [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

In the testes of each five control animals and high concentration group animals, tubular degeneration was observed. This finding was characterized by randomly affected (not stage specific) tubules with sloughed spermatogenic cells, vacuolation of the spermatogenic epithelium or missing germ cell layers. This effect in testis is known if rats are kept in tubes and try to evade. The equal incidence of this finding in control and treated animals indicates that this effect is not treatment-related. The histopathological changes observed in the epididymides (focal cibriform change, inflammatory cell or lymphoid infiltration, edema, oligospermia and debris) were regarded to be secondary to the above mentioned changes in the testes.

All other findings occurred either individually or were biologically equally distributed between control and treated groups and thus considered to be of incidental or spontaneous origin without any relation to treatment.

F. ATMOSPHERE ANALYSIS

The exposure conditions are summarized in Table 5.3.3-15.

Table 5.3.3-15: Exposure conditions

Test group	Supply air 1 (conditioned) [m³/h]	Supply air (compressed) [m³/h]	Supply air 2 (conditioned) [m³/h]	Exhaust Air 1 [m³/h]	Exhaust Air 2 [m³/h]	Acetone concentration [mg/m³]
0	4.5 ± 0.0	1.5-± 0.0		5.4		485.4 ± 181.5
1	5.0 ± 0.6	1.0 ± 0.6	4.0 ± 6.0*	5.4	4.0 ± 0.0*	
2	5.0 ± 0.6	1.0 ± 0.6		5.4		
3	5.0 ± 0.6	1.0 ± 0.6		5.4		

* from study day 4 to day 15;
Conditioned supply air is activated charcoal filtered air conditioned to about 30% to 70% relative humidity and 22°C ± 2°C;
Compressed air is filtered air pressurized to about 6 bar

The results of the atmosphere concentration analysis are depicted in Table 5.3.3-16.

Table 5.3.3-16: Measurement of pyraclostrobin and acetone concentrations

Test group	Target concentration [mg/m³]	Measured concentration (pyraclostobin) [mg/m³]	Measured concentration (Acetone) [mg/m³]
0	Aceton control		485.4 ±181.5
1	3	3.01 ± 0.8	n.d.
2	10	10.1 ± 1.5	n.d.
3	30	29.1 ± 2.9	n.d.

The measured MMAD values for the pyraclostrobin containing aerosols are given in Table 5.3.3-17.

Table 5.3.3-17: Particle size measurement

Test group	MMAD [µm]	Geometric standard deviation	< 3 µm [%]
1	0.6 – 2.3	2.8 – 6.0	56.3 – 95.0
2	0.9 – 3.8	2.7 – 5.2	44.6 - 89.3
3	0.8 – 3.1	2.7 – 4.5	49.3 - 89.9

The aerosols were highly respirable for rats and a very high proportion of the aerosol particles reached the lungs. The remaining fractions may have reached the upper respiratory tract and been deposited there.

III. CONCLUSIONS

Six hour subacute inhalatory exposure of rats to an aerosol of pyraclostrobin dissolved in acetone for 28-day on a 5 days per week basis did not result in any adverse effects indicating systemic toxicity. The respiratory tract (nasal cavity) was identified as target organ as indicated by atrophy/necrosis of the olfactory epithelium in the group treated with the highest concentration of 30 mg/m³. This change was reversible. Therefore, the 'No Observed Adverse Effect Concentration' (NOAEC) for systemic toxicity was 30 mg/m³ under the conditions of this study, whereas the NOAEC for local effect in nasal cavity was 10 mg/m^3.

Overall conclusion on subchronic inhalation toxicity

Regarding classification, the above described inhalation studies provide supportive evidence for the proposed classification of pyraclostrobin with STOT SE Category 3 (H335, May cause respiratory irritation). In absence of validated animal models that deal specifically with respiratory tract irritation, classification with STOT SE Cat. 3 is primarily based on human data. As discussed in MCA 5.9.3 there is evidence from direct observation that single exposure of humans to aerial spray mist of pyraclostrobin containing formulations can cause upper respiratory tract pain or irritation. The data of the subchronic inhalation studies provide supportive evidence for this classification. The observed inflammatory reactions of the (upper) respiratory tract (lung, nasal cavity and larynx at higher concentrations (first study), nasal cavity and larynx (second study)) are considered to represent local effects as similar effects in the respiratory tract were not observed in feeding or gavage studies. Reversibility of the observed irritant effects to the respiratory tract was observed in humans and in rats. In the occupational poisoning cases the severity was graded as being of low severity, which according to the US NIOSH (National Institute of Occupational Safety and Health) typically include illness or injury that resolves without treatment. Reversibility respiratory irritation was also demonstrated in the second 28-day inhalation study in rats as the atrophy / necrosis of the olfactory epithelium observed in the nasal cavity at the end of the treatment period was no longer detectable at the end of the 28-day recovery period.

The systemic effects observed in the inhalation studies do not justify a classification for STOT Repeated Exposure for the following reasons: The observed mortality at 0.3 mg/L (300 mg/m³) after repeated exposure for 6 hours is very close to the acute, 4h inhalation LC_{50} of 0.58 mg/L. In both cases for aerosol generation pyraclostrobin was dissolved in acetone. As pyraclostrobin is already classified as ‘toxic by inhalation’, a classification for the same end point after repeated exposure is not appropriate. The increases of white blood cell and neutrophil counts at 300 mg/m³ are considered to be unspecific responses to the local inflammation of the respiratory tract. Finally, in absence of signs of anemia, the minimal to moderate hyperplasia of the duodenal epithelium at $\geq$ 30 mg/m³ in the first subchronic inhalation study, respectively the increase of absolute and relative duodenal weight at $\geq$ 10 mg/m³ in the second subchronic inhalation study is not considered to represent an adverse finding.

CA 5.4 Genotoxicity Testing

Studies evaluated in the draft monograph of Rapporteur Member State Germany of August 1, 2001:
A sufficient data-package of in vitro genotoxicity studies in bacterial and mammalian cell systems and of in vivo genotoxicity studies conducted with pyraclostrobin is available. These studies as listed in Table 5.4.1-1 and Table 5.4.2-1 below have been evaluated by European authorities and Germany as Rapporteur Member State (European Commission Peer Review Program) and were considered to be acceptable. For the convenience of the reviewer, these studies are summarized below as extracted from the monograph.

Submission of not yet per-reviewed studies in this dossier:
No additional data on genotoxicity of pyraclostrobin was generated.

Pyraclostrobin has been tested both in vitro and in vivo in a series of mutagenic and genetic toxicity tests using bacterial and mammalian cells and whole animals to assess its potential to induce mutations and to damage chromosomes and DNA. All of these tests were clearly negative.

In summary the available data on genotoxicity of pyraclostrobin does not indicated a genotoxic potential.

Thus, the conclusion for relevant endpoints for the current re-registration remains as follows:

In vitro studies	Weight of evidence suggests no genotoxic concern
In vivo studies	Weight of evidence suggests no genotoxic concern
In vivo studies in germ cells	No data, not required

CA 5.4.1 In vitro studies

A summary of the in vitro genotoxicity studies evaluated for Annex I inclusion of pyraclostrobin is summarized in Table 5.4.1-1 below.

Table 5.4.1-1: In vitro mutagenicity studies with pyraclostrobin

Study type	Test System	With S-9 mix	Result	Reference (BASF DocID)
In vitro Mutagenicity in bacterial cells (Ames test)	*Salmonella thyphimurium* (TA 1535, 100, 1537, 98); *Escherichia coli* (WP2 uvrA); Concentration up to 5000 µg/plate	No Yes	Negative Negative	1997/10973
In vitro Mutagenicity in mammalian cells	CHO/HGPRT; Concentrations up to 20 µg/mL	No Yes	Negative Negative	1998/11422
In vitro Cytogenicity	Chromosome aberration in Chinese hamster V79 cells; Wide range of concentrations up to 25 µg/mL	No Yes	Negative Negative	1999/11403
In vitro DNA damage and repair	UDS, rat primary hepatocytes; Dose range: 0 – 1.0 µg/mL	No	Negative	1998/11421

CA 5.4.2 In vivo studies in somatic cells

A summary of the *in vivo* genotoxicity studies evaluated for Annex I inclusion of pyraclostrobin is summarized in Table 5.4.2-1 below.

Table 5.4.2-1: *In vivo* mutagenicity studies with pyraclostrobin

Study type	Test System	With S-9 mix	Result	Reference (BASF DocID)
In vivo Cytogenicity	Mouse Micronucleus test (0, 75, 150, 300 mg/kg bw); Oral gavage	Not applicable	Negative	1998/10460

A report amendment to the mouse micronucleus test was prepared, providing the results of the range finding toxicity test for the setting of the dose levels in the main study. In the following the data included in this report amendment are discussed.

Report:	CA 5.4.2/1 [redacted] 2016 a Amendment No 1: Cytogenetic study in vivo with BAS 500 F in the mouse micronucleus test. Single oral administration 2016/1309356
Guidelines:	OECD 474, EEC 92/69 B 12, EPA/TSCA 798.5395
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Administration of BAS 500 F (pyraclostrobin; batch: CP 026063; purity: 98.2%) to NMRI mice at dose levels of 125, 250, 300, 400, 500, 1000 and 2000 mg/kg bw resulted in dose dependent mortality at dose levels ≥ 400 mg/kg. In this study pyraclostrobin was dissolved in olive oil. Based on the mortality data, a LD_{50} of > 400 mg/kg bw, but < 500 mg/kg bw can be derived.

A. MATERIALS (as given in the main study, BASF DocID 1998/10460)

1.	**Test Material:**	BAS 500 F (pyraclostrobin)
	Description:	Red-brown melt
	Lot/Batch #:	CP 026063
	Purity:	98.2%
	Stability of test compound:	not explicitly indicated in the report
2.	**Vehicle and/or positive control:**	Olive oil
3.	**Test animals:**	
	Species:	Mice
	Strain:	NMRI
	Sex:	Male and female
	Age:	not indicated in the report
	Weight at dosing:	mean weight of males and female mice: 27.1 g
	Source:	Charles River GmbH, WIGA, Sulzfeld, Germany
	Acclimation period:	about 1 week
	Diet:	Kliba Haltungsdiät, Klingentalmühle, Kaiseraugst, Switzerland, ad libitum
	Water:	Drinking water from water bottles, ad libitum

Housing:	Group housing during acclimatization, single housing during the mutagenicity test in Makrolon M1 cages

Environmental conditions:

Temperature:	20 - 24°C (central air-conditioning)
Humidity:	30 - 70% (central air-conditioning)
Air changes:	not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: Not explicitly indicated in the report. According to the raw data, the in-life part of the study and the evaluation of the bone marrow was performed in July and August 1997.

2. Animal assignment and treatment:

In the range finding toxicity test either 2 animals/sex (2000 mg/kg bw) or 4 animals/sex/dose (500 and 1000 mg/kg bw) or 5 animals/sex/dose (125, 250, 300 and 400 mg/kg bw) were dosed and observed for mortality and clinical signs up to 5 days (information provided in the report amendment).

For the main study groups, 5 male and 5 female mice were administered BAS 500 F at dose levels of 75, 150, and 300 mg/kg bw. Additional groups of the same size were administered either the vehicle (olive oil) or the positive controls cyclophosphamide or vincristine. The animals were assigned to the treatment groups by means of a computer generated randomization list. The animals were killed either 24 or 48 hours after administration for the evaluation of the bone marrow.

3. Test item preparation:

Pyraclostrobin (BAS 500 F) was dissolved in olive oil. While the preparation of the solution was not described in detail in the study report, information in the study protocol and the raw data indicate that the test-item was dissolved by heating the vehicle to about 60 °C for 5 minutes.

II. RESULTS AND DISCUSSION (data of the range finding toxicity test only)

A. Observations

1. Clinical signs of toxicity

Dose-related clinical signs of systemic toxicity were noted at dose levels ≥ 250 mg/kg and consisted of reduced general state, irregular respiration, lateral position, piloerection, salutatory spasm and hunched posture. No clinical signs of toxicity were recorded for animals which died within 15 minutes. The duration of the clinical signs is likewise indicated in Table 5.4.2-2.

2. Mortality

A dose-related mortality was noted at dose levels ≥ 400 mg/kg bw. Mortality was observed as early as 15 minutes after administration up to 1 day [see Table 5.4.2-2].

Table 5.4.2-2: Results of the range finding toxicity test in mice for dose setting in the mouse micronucleus assay with BAS 500 F (pyraclostrobin)

Dose (mg/kg)	Toxicological results*	Time of death	Duration of signs#	Recovery of survivors	Type of signs [code]
Males					
2000	2/0/2	h 0.25	not observed	all dead	not observed
1000	4/3/4	h 0.25 to d 1	h 0.25 to d 1	all dead	3, 4, 8, 38
500	3/4/4	h 0.5 to d 2	h 0.25 to d 1	d 2	3, 4, 8, 10, 25, 38
400	1/5/5	d 1	h 1 to d 1	d 4	3, 10, 38
300	0/5/5	-	h 1 to d 1	d 4	10, 38
250	0/5/5	-	h 1.5 to h 3	d 1	10, 38
125	0/0/5	-	-	-	-
Females					
2000	2/0/2	h 0.25	not observed	all dead	not observed
1000	4/2/4	h 0.25 to d 1	h 0.25 to d 1	all dead	3, 4, 38
500	3/4/4	h 0.5 to d 2	h 0.25 to d 1	d 2	3, 4, 8, 10, 25, 38
400	1/5/5	d 1	h 1 to d 1	d 4	3, 10, 38
300	0/5/5	-	h 1 to d 1	d 4	10, 38
250	0/5/5	-	h 1.5 to h 3	d 1	10, 38
125	0/0/5	-	-	-	-

* Number of animals which died/number of animals with clinical signs/number of animals used
\# first respectively last time of observation
h, d: hour, day after administration; d 0 = day of administration
\- not observed
Codes for type of clinical signs:
3 reduced general state
4 irregular respiration
8 lateral position
10 piloerection
25 saltatory spasm
38 hunched posture

3. Dose selection for the main study

Based on the data of the range finding toxicity study, dose levels of 75, 150 and 300 mg/kg bw were selected for the main study. The highest dose of 300 represents 2/3 of the LD_{50} (see below).

4. Derivation of a LD_{50} in mice

Even though not designed as a study to determine a LD_{50} in mice, the mortality data are suitable to derive an acute oral toxicity value.
Based on the mortality data in Table 5.4.2-2 it is obvious that the LD_{50} of pyraclostrobin dissolved in olive oil is > 400 mg/kg, but < 500 mg/kg.

Including the mortality data generated in the course of the main study (no mortality in each 5 ♂ and 5 ♀ at 75 and 150 mg/kg; 1 case of mortality in 10 ♂ (24h und 48h groups) and no mortality in 10 ♀ at 300 mg/kg) acute LD_{50} values of 449, 453 and 451 mg/kg bw can be calculated for male mice, female mice and combined sexes, respectively. This calculation used the Probit analysis according to Finney (Finney, D.J. (1971): "Probit Analysis" Cambridge University Press, pp. 1 – 50) using SAS 9.3.

III. CONCLUSIONS

Based on the data of the preliminary toxicity test in NMRI mice dose levels of 75, 150 and 300 mg/kg were selected for the main part of the in-vivo mouse micronucleus assay with BAS 500 F (pyraclostrobin). The mortality data allow the calculation of a LD_{50} of 451 mg/kg bw for NMRI mice when data of male and female animals were combined. The calculated LD_{50} values for the individual sexes were 449 mg/kg bw for males and 453 mg/kg bw for females.

In order to prove the exposure of the target organ in the mouse micronucleus assay, i.e. the bone marrow, a study with radioactive pyraclostrobin was conducted in mice using the same vehicle as in the micronucleus assay.

Report:	CA 5.4.2/2 [redacted] 2016 c 14C-BAS 500 F- Study on the kinetics in mice 2016/1225931
Guidelines:	OECD 417, EPA 870.7485, Commission Regulation (EC) No 440/2008, JMAFF
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

^{14}C-BAS 500 F was administered once orally to four male mice at a target dose level of 300 mg/kg bw and an actual nominal dose of 296.7 mg/kg bw. Two hours after dosing, mice were sacrificed under isoflurane anaesthesia and defined organs and tissues (e.g. blood cells, plasma, bone marrow, liver) were prepared and worked up for the detection of the radioactive residues to demonstrate that the test substance and/or its metabolites reached the systemic circulation including organs/tissues. Corresponding samples of untreated mice from a parallel running study served as controls (see BASF DocID 2016/1225930).
The mean total radioactive residues (TRR) 2 h post dosing were 6.38 µg Eq/g in plasma and 6.54 µg Eq/g in bone marrow. In liver and blood cells, mean total radioactive residues of 44.63 and 1.81 µg Eq/g were found, respectively. Taken together, the data demonstrate that ^{14}C-BAS 500 F and/or its metabolites reach the systemic circulation including bone marrow and liver after single oral administration of the test substance at a target dose of 300 mg/kg bw.

(BASF DocID 2016/1225931)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material (labelled):	^{14}C-BAS 500 F
Lot/Batch #:	566-6101
Purity (radiochemical):	>95%
Purity (chemical):	98.1% (the CoA provided indicates a radiochemical purity of 98.9%)
Specific activity of AI:	5.59 MBq/mg
Stability of test compound:	The stability was verified within the current study.

2. Test Material (non-labelled):	BAS 500 F
Description:	Solidified melt
Lot/Batch #:	COD-001236
Purity:	99.02%
Stability of test compound:	The stability was guaranteed by the sponsor (expiry date: 01 Feb 2020).
3. Vehicle:	Olive oil
4. Test animals:	
Species:	Mouse
Strain:	Crl:NMRI
Sex:	Male
Age:	4 weeks
Weight at dosing:	33.0 ± 2.6 g
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld, Germany
Acclimation period:	At least 5 days
Diet:	Kliba lab diet (mouse / rat "GLP") pellets, Provimi Kliba SA, Kaiseraugst, Basel, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	During acclimatization: Individual housing in Polycarbonate Cages (Type III)
	During the experiment: Individual housing in Polycarbonate Cages (1291H; PC, 820 cm^2, Tecniplast) with steel wire mesh ground (7 x 7 mm mesh wire).
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	15 per h
Photo period:	Alternating 12 hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 19-July-2016 to 01-Aug-2016

2. Test substance preparation:

In order to achieve a homogenous test substance preparation (nominal concentration: 6.63 mg/g), a suspension of non-labelled test substance was prepared in olive oil. The preparation was stirred vigorously for about 2 h, sonicated at about 50°C for 15 min and afterwards stirred and sonicated at about 50°C to yield a homogenous preparation. In order to achieve the required specific activity, an appropriate amount of the radio-labelled test substance was taken, the solvent was evaporated to dryness under a stream of nitrogen and the residue was mixed with a defined weight of the previously prepared non-labelled test substance in olive oil. The preparation was homogenized by stirring with a magnetic stirrer for about 1 ½ hours, additional ultrasonication for about 15 minutes and repeating this homogenization process procedure once. On the administration day, the preparation was stirred for at least one hour before samples were taken.

Before start and at the end of the administration, samples were taken to determine the specific activity of the test substance in the preparation and to demonstrate the correct concentration of the test substance, its homogeneity and stability as well as its radiochemical purity.

Based on the current data, the nominal concentration of the test substance preparation was 32.9 mg/g, corresponding to 30.0 mg/mL and its nominal specific activity was 36.6 MBq/mL. The dosed amount of radioactivity per animal was between 10.9 and 13.4 MBq.

3. Homogeneity/Concentration and stability control:

The amounts of radioactivity, the amounts of ^{14}C-BAS 500 F and the total amount of BAS 500 F (sum of ^{14}C and non-radioactive) in the test substance preparation was determined by LSC, Radio-HPLC as well as HPLC-UV in samples that were taken before and after administration of the test substance preparation to the animals. The samples were prepared for analysis using conventional methods described in standard operating procedures. The analyses of these samples allow to demonstrate the homogeneity, correctness of the concentration and the stability of the test substance in the test substance preparation.

4. Study design and test groups:

The study was designed to obtain data to demonstrate that the test substance reaches the systemic circulation and defined organs / tissues. For this purpose, blood cells, plasma, bone marrow from the two femora, and the liver were collected.
Treated animals were sacrificed 2 hours after administration. Data from four untreated control animals, derived from another, parallel running study (BASF DocID 2016/1225930, see M-CA 5.8 of this dossier), were used for calculation of background levels.

The experiments were performed according to the following scheme:

Experiment 0 (BASF DocID 2016/1225930)	
Animals:	4 males
Radioactivity per animal:	untreated control
Dosing:	untreated
Analysis:	total radioactivity (for determination of the detection limit)
Experiment 1 (this study)	
Animals:	4 males
Radioactivity per animal:	about 10 MBq
Dosing:	1 oral dose, 300 mg/kg bw
Analysis:	total radioactivity

5. Experimental procedure and observations:

The animals were dosed at a volume of 10 mL/kg body weight by oral gavage.
A check for moribund and dead animals was conducted at least once daily. A cage side examination was conducted at least once daily for any signs of morbidity, pertinent behavioral changes and signs of overt toxicity.
The body weight was determined on the day of administration prior to dosing for the animals of experiment 1. The animals of experiment 0 were weighed in the parallel running study (BASF DocID 2016/1225930).

6. Sample work up and measurement:

Whole blood samples were inverted several times to ensure homogeneity and then separated into plasma and blood cells by centrifugation.
After weighing, aliquots of plasma were mixed with scintillation cocktail (Hionic Fluor, Perkin Elmer) and analyzed for radioactivity without any additional treatment.
The liver was homogenized. Subsequently 1 mL Soluene®-350 was added to aliquots of the suspensions and incubated over night at about 37°C and isopropanol was added afterwards. Bleaching was performed with 1 mL 30% H_2O_2 followed by incubation at room temperature at least over night. Hionic Fluor was added before measurement of radioactivity by LSC.
Blood cells were dissolved in Soluene®-350 and processed as described above. Bone marrow from the two femora was removed with a cotton ball, which transferred into a scintillation vial. After addition of Soluene®-350 the samples were processed as described for the liver.
All values given in the project and in the report are background corrected. The background values of LSC analyses were 10 to 170 cpm (see BASF DocID 2016/1225930). The quantification limit (LOQ) for LSC measurement was set twice the background. For further calculations (e.g. mean values and standard deviations) samples < LOQ were set to 0.

II. RESULTS AND DISCUSSION

A. ANALYTICS

Concentration control analysis confirmed the nominal concentration with means between 90 and 110 % related to the nominal value (109.1 ± 3.5; Mean ± RSD). The relative standard deviation below 5% demonstrates the homogenous distribution of the test substance in its preparation.
The measured specific activity of the test substance preparation compared to its target value is assessed to be acceptable within the precision of the method (36.7 and 41.2 MBq/mL for target and measured specific activity, respectively). Radio-HPLC analyses confirmed the stability of the radio-labelled test substance over the administration period by radiochemical purities of ^{14}C-BAS 500 F > 95%.

B. CLINICAL OBSERVATIONS AND MORTALITY

No mortality occurred during the study period. No test substance related relevant findings were observed.

C. BODY WEIGHT

As to be expected for a short-term experiment (2 h), no effects on body weight were noted.

D. BIOKINETICS

After a single administration of ^{14}C-BAS 500 F to male mice at a target dose level 300 mg/kg bw, the mean actual nominal dose level was 296.7 ± 5.9 mg/kg bw (see Table 5.4.2-3).
Mean radioactive residues resulted in 1.81 ± 0.56 µg Eq/g for blood cells and 6.38 ± 2.13 µg Eq/g for plasma. The mean radioactive residues in bone marrow and liver were 6.54 ± 3.57 and 44.63 ± 18.15 µg/g, respectively (see Table 5.4.2-3). Variability of the single animal data reflect inter-individual biological variability that are in a normal range for kinetic studies. Based on the current data, radioactivity was clearly detectable in blood cells, plasma, bone marrow and liver of all orally dosed animals.
Blood cells, plasma, bone marrow and liver from four untreated mice (untreated control, 30.9 – 36.1 g bw) were collected and measured for total radioactivity (background levels). Taking the specific activity of administered ^{14}C-BAS 500 F and the measured sample weights into account, double background levels corresponded to < 0.05 µg Eq/g for plasma, < 0.2 µg Eq/g for blood cells, < 4 µg Eq/g for bone marrow and < 4 µg Eq/g for liver, respectively.
These data demonstrate that the detected residue levels in blood cells, plasma, bone marrow and liver in mice dosed with ^{14}C-BAS 500 F at a dose level of 300 mg/kg bw were significantly above background.

Table 5.4.2-3: Radioactive residues (µg Eq/g) in plasma, blood cells, bone marrow and liver of male mice treated with ^{14}C-BAS 500 F at a target dose level of 300 mg/kg bw (2 hours post dosing)

Parameter	Animal No.	1	2	3	4	Mean	SD
Body weight	[g]	34.4	35.5	32.4	29.5	33.0	2.6
Specific activity	[MBq/g]	-	-	-	-	1252.5	-
Dose	[mg/kg bw]	289.5	301.5	301.7	294.1	296.7	5.9
Radioactive Dose	[MBq/animal]	12.47	13.40	12.24	10.87	12.25	1.05
Blood cells	[µg Eq/g]	1.98	1.58	1.18	2.49	1.81	0.56
Plasma	[µg Eq/g]	8.68	4.70	4.45	7.71	6.38	2.13
Bone marrow	[µg Eq/g]	3.84	3.75	7.26	11.29	6.54	3.57
Liver	[µg Eq/g]	31.50	53.67	27.61	65.72	44.63	18.15

It is worth to note that the mean plasma levels determined in mice two hours after administration of a dose of 300 mg/kg bw (6.38 µg/Eq/g plasma) are in good agreement to the plasma levels in rats at the same point in time after administration of 50 mg/kg bw (1.33 µg Eq/g plasma; see BASF DocID 1998/10997). Plasma levels in mice at a 6-fold higher dose were 4.8-fold higher.

III. CONCLUSION

The current study demonstrated that radioactive residues of ^{14}C-BAS 500 F are present in the systemic circulation, in bone marrow and in liver of mice, two hours after single oral administration of the test substance at a target dose level of 300 mg/kg bw.

CA 5.4.3 In vivo studies in germ cells

This is no data requirement and consequently no studies have been conducted, however, the following literature data was evaluated:

Report: CA 5.4.3/1
Cayir A. et al., 2012b
Micronuclei, nucleoplasmic bridges, and nuclear buds induced in human lymphocytes by the fungicide Signum and its active ingredients (Boscalid and Pyraclostrobin)
2012/1366624
Guidelines: none
GLP: no (literature study)

Executive Summary

Pyraclostrobin and the pyraclostrobin containing formulation Signum as well as the active substance boscalid were tested for their ability to induce micronuclei, nucleoplasmic bridges, and nuclear buds in human lymphocytes in vitro. The method used was similar to OECD guideline 487, but used the additional endpoints nucleoplasmic bridges, and nuclear buds, that are also indicative for a genotoxic effect. Both active ingredients used for the assays were of >99% purity and the formulation Signum was a commercial formulation containing 26.7% w/w boscalid and 6.7 % w/w pyraclostrobin.
Heparinized blood was obtained from 2 young donors that reported to be healthy but were not tested for their health status. 2 parallel cultures were tested for each test concentration and culture condition in the absence of metabolic activation only. In a G_0 phase protocol blood was treated with the test concentrations for 24 hours without phytohemagglutinin (PHA), then the test item was removed and PHA was added to the culture medium. After 44 hours cytochalasin B was added and cultures were harvested 72 hours after initiation. In the second protocol proliferating lymphocytes were treated with PHA for 44 hours before the test items were added. Cytochalasin B was added 48 hours after initiation and cells were harvested 72 hours after start. Signum and Boscalid were tested in 8 concentrations in both G_0 Phase and proliferating lymphocytes while pyraclostrobin was tested in 7 concentrations in G_0 Phase lymphocytes and only 3 concentrations in proliferating lymphocytes. Cells were fixed and slides stained with Giemsa before microscopic evaluation. 1000 binucleated cells were scored for each culture resulting in a total of 4000 binucleated cells per concentration. Parameters evaluated were micronuclei, nuclear buds and nucleoplasmic bridges as a measure of genotoxicity and cytokinesis-block proliferation index (CBPI) and %cytostasis as a measure of cytotoxicity. Mitomycin C served as a positive control and yielded a statistically significant increase in micronuclei in all experiments.

For all 3 test items an increase in micronuclei compared to the concurrent vehicle control was observed both in G_0 and proliferating cells. This increase was not dose dependent for both boscalid and Signum with only single concentrations reaching statistical significance. The study authors attribute the missing dose response for micronucleus induction to the increasing level of %cytostasis, nevertheless all concentrations tested were well below the cytostasis limit of 55%. For pyraclostrobin a more pronounced effect on micronucleus formation was noted that was dose-related even though levels of cytostasis were enhanced compared to Signum and boscalid. The authors do not indicate their historical control data for the study design employed. On this basis it is difficult to assess the biological relevance of the effects observed. Nevertheless, it can be concluded that under the conditions described, boscalid, pyraclostrobin and the formulated product Signum induce clastogenic or aneugenic effects in vitro.

This is not considered relevant information since for both, pyraclostrobin and boscalid, comprehensive in vitro and in vivo data for genotoxicity are already available and have been evaluated for Annex I inclusion. The relevant follow up assay for a positive in vitro micronucleus assay in either mammalian cell lines or human lymphocytes is a rodent bone marrow micronucleus assay addressing both aneugenic and clastogenic effects. This study has been performed for boscalid and was clearly negative (BASF DocID 1999/11048). For pyraclostrobin a chromosomal aberration assay in mice was likewise negative (see above). The author`s recommendation to perform a Comet assay in vivo is not considered useful since this assay addresses clastogenic and mutagenic but not aneugenic effects and would not lead to a better understanding of the genotoxic profile.

Genotoxicity testing of formulated products is not a data requirement in the EU and is in general not considered useful since testing of the undiluted active substance for a genotoxic hazard is considered more appropriate. Therefore, no in vivo studies on the formulation Signum (BAS 516 07 F) are available. Nevertheless, for product registrations in Brazil in vivo micronucleus tests in mouse bone marrow have been performed with altogether 12 different formulations containing pyraclostrobin and with one formulation containing boscalid. **All these studies were negative, thus proving that the presence of formulation auxiliaries does not alter the genotoxic profile of the active substances.** Table 5.4.3-1 shows a list of in vivo micronucleus studies available for formulated products containing either pyraclostrobin or boscalid.

Table 5.4.3-1: In vivo micronucleus assays with formulated products containing pyraclostrobin or boscalid

Study type	Test System	Formulation tested	Result	Reference*
In vivo Cytogenicity	Mouse Micronucleus test (0, 312.5, 625, 1250 mg/kg bw); Oral gavage	WG-type 6.7g/kg pyraclostrobin	Negative	2010/1141998 ** &2013/1403103 **
In vivo Cytogenicity	Mouse Micronucleus test (0, 200, 400, 800 mg/kg bw); Oral gavage	TK-type 40% pyraclostrobin	Negative	2005/1006741
In vivo Cytogenicity	Mouse Micronucleus test (0, 75, 150, 300 mg/kg bw); Oral gavage	DS-type 50g/L pyraclostrobin	Negative	2006/1015913
In vivo Cytogenicity	Mouse Micronucleus test (0, 450, 900, 1800 mg/kg bw); Oral gavage	FS-type 50g/L pyraclostrobin	Negative	2006/1019777
In vivo Cytogenicity	Mouse Micronucleus test (0, 75, 150, 300 mg/kg bw); Oral gavage	EC-type 130 g/L pyraclostrobin	Negative	2007/1057132
In vivo Cytogenicity	Mouse Micronucleus test (0, 25, 50, 100 mg/kg bw); Oral gavage	SC-type 260 g/L pyraclostrobin	Negative	2008/1027419
In vivo Cytogenicity	Mouse Micronucleus test (0, 250, 500, 1000 mg/kg bw); Oral gavage	EC-type 81 g/L pyraclostrobin	Negative	2010/1005652
In vivo Cytogenicity	Mouse Micronucleus test (0, 175-750 mg/kg bw); Oral gavage	SC-type 250 g/L pyraclostrobin	Negative	2010/1033921
In vivo Cytogenicity	Mouse Micronucleus test (0, 500, 1000, 2000 mg/kg bw); Oral gavage	WG-type 20 g/kg pyraclostrobin	Negative	2010/1123697
In vivo Cytogenicity	Mouse Micronucleus test (0, 62.5, 125, 250 mg/kg bw); Oral gavage	SC-type 333 g/L pyraclostrobin	Negative	2010/1130232
In vivo Cytogenicity	Mouse Micronucleus test (0, 500, 1000, 2000 mg/kg bw); Oral gavage	FS-type 8.3 g/L pyraclostrobin	Negative	2010/1201610
In vivo Cytogenicity	Mouse Micronucleus test (0, 75, 150, 300 mg/kg bw); Oral gavage	SC-type 125 g/L pyraclostrobin	Negative	2013/1188674
In vivo Cytogenicity	Mouse Micronucleus test (0, 93.75-750 mg/kg bw); Oral gavage	WG-type 200 g/L boscalid	Negative	2008/1037578

* study reports available on request
** study summaries are given below

Based on negative genotoxicity datasets for both, pyraclostrobin and boscalid, and a large number of negative in vivo micronucleus assays in mice with different formulated products, the in vitro findings reported by Cayir et al. are not considered to have any relevance in vivo.

Category: Supplemental information

The formulation listed first in Table 5.4.3-1 (BAS 536 02 F) has a composition which very similar to the composition of Signum (BAS 516 07 F). The content of pyraclostrobin is identical and with the exception of the second active substance (dimethomorph instead of boscalid) both formulations differ only in the type of carrier used. A comparison of the composition of both formulations (BAS 516 07 F and BAS 536 02 F) is provided in the confidential Document JCP of BAS 516 07 F.
In the following the mutagenicity studies available for the formulation BAS 536 02 F are presented. These are considered as further strong evidence that BAS 516 07 F does not have any mutagenic potential in vivo.

Report:	CA 5.4.3/2 Schulz M., Landsiedel R., 2010 a BAS 536 02 F - Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and preincubation test) 2010/1059892
Guidelines:	OECD 471 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13 No. L 142, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium (strains TA98, TA 100, TA 1535 and TA 1537) and E. coli strain WP2 uvrA were exposed to BAS 536 02 F; batch: 3481; containing 12.2% dimethomorph and 7.0% pyraclostrobin) using DMSO as a solvent in the presence and absence of metabolic activation in two independent experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.

The test substance was tested in concentrations between 33 and 5000 µg/plate. A weak bacteriotoxic effect was observed in the preincubation assay at 5000 µg/plate. No precipitation of the test substance was found with and without S9 mix.

No biologically relevant increase in the number of revertant colonies was observed in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.

The test substance BAS 536 02 F is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2010/1059892)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 536 02 F

Description:	Solid, brown
Lot/Batch #:	3481
Purity:	Formulation, contents of active ingredients BAS 550 F (Dimethomorph): 12.2% BAS 500 F (Pyraclostrobin): 7.0%
Stability of test compound:	The test substance was stable over the study period under the storage conditions and guaranteed until 01 Jan 2012.
	The homogeneity of the test substance was ensured by mixing prior to preparation of test substance solutions.
Solvent used:	DMSO

2. Control Materials:

Negative control:	In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).
Vehicle control:	The vehicle control with and without S9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60 µg/plate

To demonstrate the efficacy of the S9 mix in this assay, the S9 batch was further characterized with benoz(a)pyrene in the strains TA 100 and TA 98 as required in OECD 471.

3. Activation:

S9 was produced from the livers of induced male Wistar rats. The rats received on three consecutive days intraperitoneal injections of 80 mg phenobarbital and 80 mg β-naphthoflavone orally per kg body weight. 24 hours after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9 mix, was kept on ice until used.

The S9 mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay:	In the first experiment triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without S9) for all tester strains indicated above.
Pre-incubation assay:	In the second experiment the test article / vehicle / positive control substance, bacterial and S9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains.

B. TEST PERFORMANCE:

1. Dates of experimental work: 20-Apr-2010 to 29-Apr-2010

2. Plate incorporation assay:

To test tubes containing 2 mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates). In the experiments with E. coli the warm soft agar contains 0.5 mM tryptophan instead of histidine + biotin.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.
After incubation in the dark for 48 to 72 hours at 37°C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects, the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37°C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in at least two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable in the vehicle DMSO and in water over a period of 4 hours.

B. TOXICITY

No bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction of titer) was observed in the standard plate test up to the highest concentration tested.

In the preincubation assay bacteriotoxicity (reduced his^+ or trp^+ background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed depending on the strain and test conditions from about 5000 µg/plate onwards.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiment with and without metabolic activation a biologically relevant increase in number of revertants was observed in any strain tested [see Table 5.4.3-2]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

No test substance precipitation was observed with and without S9 mix.

Table 5.4.3-2: Bacterial gene mutation assay with BAS 536 02 F - Mean number of revertants

Experiment 1: Plate incorporation assay*										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	41 ± 6	31 ± 3	127 ± 9	114 ± 9	18 ± 3	18.0 ± 2	8 ± 3	8 ± 2	36 ± 4	31 ± 4
Test substance										
33 µg/plate	40 ± 5	32 ± 6	148 ± 12	110 ± 5	20 ± 1	19 ± 3	9 ± 1	6 ± 2	37 ± 4	31 ± 7
100 µg/plate	29 ± 8	27 ± 3	141 ± 10	136 ± 11	16 ± 2	19 ± 2	7 ± 4	6 ± 3	33 ± 3	33 ± 5
333 µg/plate	37 ± 7	29 ± 4	139 ± 20	129 ± 15	20 ± 3	20 ± 2	7 ± 2	8 ± 1	39 ± 2	33 ± 3
1000 µg/plate	27 ± 1	29 ± 8	136 ± 11	101 ± 18	20 ± 4	19 ± 1	6 ± 1	7 ± 4	30 ± 2	30 ± 2
2500 µg/plate	33 ± 7	33 ± 5	120 ± 5	97 ± 12	20 ± 3	17 ± 2	7 ± 1	6 ± 2	40 ± 4	34 ± 9
5000 µg/plate	30 ± 7	29 ± 3	123 ± 7	96 ± 13	15 ± 1	14 ± 4	7 ± 1	6 ± 3	30 ± 5	32 ± 4
Pos. control§	606 ± 33	442 ± 22	733 ± 66	811 ± 73	128 ± 18	613 ± 48	140 ± 9	362 ± 26	220 ± 27	702 ± 30
Experiment 2: Preincubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	27 ± 2	26 ± 4	128 ± 11	121 ± 11	19 ± 4	18 ± 2	7 ± 2	7 ± 2	29 ± 4	31 ± 4
Test substance										
33 µg/plate	23 ± 5	24 ± 5	97 ± 12	132 ± 16	21 ± 6	19 ± 1	6 ± 4	8 ± 2	26 ± 3	26 ± 3
100 µg/plate	29 ± 2	23 ± 3	113 ± 16	150 ± 26	20 ± 1	18 ± 5	7 ± 3	8 ± 2	29 ± 4	30 ± 3
333 µg/plate	27 ± 6	26 ± 2	112 ± 21	139 ± 33	23 ± 8	20 ± 1	9 ± 2	7 ± 2	30 ± 4	30 ± 4
1000 µg/plate	24 ± 1	21 ± 3	134 ± 11	125 ± 21	22 ± 5	26 ± 5	7 ± 2	8 ± 3	30 ± 3	30 ± 3
2500 µg/plate	23 ± 3	21 ± 6	120 ± 12	139 ± 9	19 ± 6	20 ± 5	7 ± 2	6 ± 2	29 ± 3	28 ± 4
5000 µg/plate	19 ± 6	19 ± 1	126 ± 23	137 ± 8	23 ± 6	24 ± 2	5 ± 2	3 ± 1	26 ± 9	26 ± 9
Pos. control§	669 ± 32	501 ± 23	888 ± 60	837 ± 37	136 ± 25	676 ± 88	139 ± 23	361 ± 21	221 ± 18	641 ± 117

*: Numbers may differ from original data due to rounding; P: precipitation;
§ = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSION

According to the results of the present study the test substance BAS 536 02 F is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.4.3/3 [redacted] 2010 a BAS 536 02 F - Micronucleus test in bone marrow cells of the mouse 2010/1141998
Guidelines:	OECD 474, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 5.4.3/4 [redacted] 2014 a Amendment No. 1 to the report - BAS 536 02 F - Micronucleus test in bone marrow cells of the mouse 2013/1403103
Guidelines:	OECD 474, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Please note: The Amendment provides detailed information on the clinical observation made in the preliminary dose range finding study.

Executive Summary

BAS 536 02 F (batch: 3481, containing 12.2% dimethomorph and 7.0% pyraclostrobin) was tested for chromosomal damage (clastogenicity) and for the ability to induce spindle poison effects (aneugenic activity) in NMRI mice using the micronucleus test method. For this purpose, the test substance was administered once orally to groups of 5 male mice at dose levels of 312.5, 625 and 1250 mg/kg body weight. The vehicle (deionized water) served as negative and cyclophosphamide and vincristine sulfate as positive controls. The animals were sacrificed 24 or 48 (additional high dose and vehicle group) hours after the administration and the bone marrow was prepared and investigated for micronuclei.

The oral administration of BAS 536 02 F did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing either small or large micronuclei. The rate of micronuclei was mostly close to the concurrent negative control and was within the range of the historical control data. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur.

Both of the positive control chemicals, i.e. cyclophosphamide for clastogenic effects and vincristine for induction of spindle poison effects, led to the expected increase in the rate of polychromatic erythrocytes containing small (cyclophosphamide) or small and large (vincristine sulphate) micronuclei, thus demonstrating the sensitivity of the test system.

BAS 536 02 F does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study, i.e. is devoid of clastogenic or aneugenic activity in vivo.

(BASF DocID 2010/1141998)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 536 02 F

Description:	Solid, brown
Lot/Batch #:	3481
Purity:	Formulation, contents of active ingredient BAS 550 F (dimethomorph): 12.2% BAS 500 F (pyraclostrobin): 7.0%
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Jan 2012 as indicated by the sponsor. Homogeneity of the test substance was guaranteed on account of the high purity and was ensured by mixing before test substance preparation.
Vehicle used:	Deionized water

2. Control Materials:

Negative:	No negative control was employed in this study.
Vehicle control:	Deionized water
Positive control:	Cyclophosphamide (CPP) 20 mg/kg for the determination of clastogenic effects Vincristine sulphate (VCR) 0.15 mg/kg for the determination of aneugenic effects

3. Test animals:

Species:	Albino mice
Strain:	Crl:NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	5 - 8 weeks
Mean body weight at dosing:	29.78 g
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	3 males and female/dose
Micronucleus assay:	5 males/dose
Acclimation period:	At least 5 days
Diet:	Standardized pelleted feed (Maus/Ratte Haltung "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland)
Water:	Drinking water in bottles, ad libitum
Housing:	During the study the mice were housed individually in Makrolon cages, type MII.

4. Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	frequency not indicated (fully air-conditioned rooms)
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	1000, 1500 and 2000 mg/kg
Micronucleus assay:	312.5, 625 and 1250 mg/kg The test substance was administered once by oral gavage using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 12-Jul-2010 to 06-Oct-2010

2. Preliminary range finding test:

Groups of 3 male and 3 female NMRI mice were treated once by oral gavage with a test substance at dose levels of 1000, 1500 or 2000 mg/kg bw.

3. Micronucleus test:

Treatment and sampling:	Groups of 5 male mice were treated once with either the vehicle or 312.5, 625 or 1250 mg test substance/kg bw by oral gavage. Additional test groups treated with the vehicle control and the high dose were used for the second sampling period. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substances CPP and VCR were administered once by oral gavage (CPP) or i.p. injection (VCR). The animals were observed for evident clinical signs of toxicity throughout the study. Twenty-four hours after the administration the mice were sacrificed and the two femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 300xg for 5 minutes. The supernatant was discharged and the pellet re-suspended in about 50 µl fresh FCS. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide preparation:	One drop of the suspension was applied on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May-Grünwald solution, rinsed, and finally stained with Giemsa solution (7.5%). After rinsing and clarifying in xylene, the preparations were mounted. The slides were coded prior to microscopic evaluation.
Slide evaluation:	In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored. The increase in the number of micronuclei in polychromatic erythrocytes of treated animals as compared to the vehicle control group provides an index of a chromosome-breaking (clastogenic) effect or of a spindle activity (aneugenic) of the substance tested.

In addition, the number of small micronuclei (d < D/4) and of large micronuclei (d ≥ D/4) (d = diameter of micronucleus, D = cell diameter) was determined: The size of micronuclei may indicate the possible mode of action of the test substance, i.e. a clastogenic or a spindle poison effect.

The ratio of polychromatic to normochromatic erythrocytes was calculated. An alteration of this ratio indicates a toxic effect on erythropoieses and thus, that the test substance actually reached the target organ.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test (modified rank test according to Wilcoxon). Here, the relative frequencies of cells with micronuclei of each animal were used.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and significant increase in the number of micronucleated polychromatic erythrocytes was observed.
- The proportion of cells containing micronuclei exceeded both the values of the concurrent vehicle control range and the vehicle historical control range.

A test substance is generally considered negative in this test system if:

- There was no significant increase in the number of micronucleated polychromatic erythrocytes at any dose above concurrent control frequencies.
- The frequencies of cells containing micronuclei were within the historical control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle was verified analytically. The homogeneity of the test substance in the vehicle was guaranteed by constant stirring during the removal and administration of the test substance formulation and by analytical determination of 3 individual samples of each concentration. The mean test substance concentrations were determined as 33.75, 71.31 and 139.69 mg/mL at nominal concentrations of 31.25, 62.50, and 125.00 mg/mL, respectively. These values correspond to 108 to 114.1% of the nominal concentration. The recovery rates of the mid and high dose group (114.1 and 111.7%) was slightly higher than the expected range (90-110%). However, these recovery rates are well within the internationally accepted range of 80 - 120%.
The homogeneity of the test substance in the vehicle was guaranteed by constant stirring during the removal and administration of the test substance formulation and indirectly by analytical determination of 3 individual samples of each concentration.

B. PRELIMINARY RANGE FINDING TEST

In the pretest in males and females, deaths were observed down to 1500 mg/kg bw. At 1000 mg/kg bw, all animals survived displaying piloerection and hunched posture as signs of general toxicity up to the end of the observation period of 2 days. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment. Based on the mortality down to 1500 mg/kg bw and the clinical signs observed throughout the observation period of 2 days at 1000 mg/kg bw, the dose of 1250 mg/kg bw was considered as maximum tolerated dose (MTD) for the main study.

C. MICRONUCLEUS ASSAY

Treatment of mice with BAS 536 02 F did not lead to a biologically relevant increase in the rate of micronuclei (see Table 5.4.3-3). Micronucleus frequencies of treated animals were near to the concurrent vehicle control values and within the historical control range (0.4 to 3.0% for the vehicle water). The number of normochromatic or polychromatic erythrocytes containing small or large micronuclei did not deviate from the vehicle control value and was within the historical control range.

The PCE/NCE ratio was not affected by treatment with the test substance. Thus, there was no indication that erythropoiesis was inhibited.

The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE containing almost exclusively small micronuclei (21.7‰). The administration of the spindle poison vincristine resulted in an incidence of micronuclei in polychromatic erythrocytes of 75.8‰. This included 22.7‰ of PCE with large micronuclei. The positive controls thus demonstrated the sensitivity of the test system.

The administration of the test substance led to distinct clinical signs of toxicity. At 625 mg/kg body weight, all animals displayed piloerection from 1 to 4 h after injection. At 1250 mg/kg all animals from the 24 hour and 48 hour treatment groups displayed piloerection and hunched posture at the 1, 2 and 4 h observation intervals.

No signs of systemic toxicity were observed in any of the animals treated with the positive control substances or the vehicle.

Table 5.4.3-3: Micronucleus test in mice administered BAS 536 02 F by oral gavage

Test group	Sacrifice interval [h]	Animal No.	Micronuclei in PCE		Number of NCE[c]
			total[a] [‰]	large[b] [‰]	
Vehicle control deionized water	24	5	1.2	0.0	2734
Test substance 312.5 mg/kg bw	24	5	1.7	0.0	2386
Test substance 625 mg/kg bw	24	5	1.5	0.1	2424
Test substance 1250 mg/kg bw	24	5	1.1	0.0	2520
Positive control Cyclophosphamide 20 mg/kg bw	24	5	21.7**	0.1	4037
Positive control Vincristine sulfate 0.15 mg/kg bw	24	5	75.8**	22.7**	4888
Vehicle control deionized water	48	5	2.0	0.0	3603
Test substance 1250 mg/kg bw	48	5	1.1	0.0	2592

** $p \leq 0.01$ (Wilcoxon Test; one sided)
PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes
[a] sum of small and large micronuclei
[b] large micronuclei (indication of spindle poison)
[c] number of NCEs observed when scoring 10 000 PCEs

The exposure of the target organ, i.e. bone marrow was not determined in this study. However, as demonstrated in a separate study, radioactive pyraclostrobin administered to mice by oral gavage was detected at quantifiable concentrations in bone marrow and plasma (see BASF DocID 2016/1225931). Despite of the use of different vehicles (olive oil vs. a mixture of Pluriol E 200 (= polyethylene glycol) and 0.5% aqueous Tylose®) and dose levels (300 mg/kg bw vs. 50 mg/kg bw) plasma levels were in good agreement in mice and rats (see BASF DocID 1998/10997), respectively. Based on this independence of vehicle (and strain) used, it can be reasonably assumed that pyraclostrobin formulated in BAS 536 02 F likewise reached the blood circulation and the highly blood perfused bone marrow.

III. CONCLUSION

Based on the result of this study BAS 536 02 F does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study, i.e. is devoid of clastogenic or aneugenic activity in vivo.

CA 5.5 Long-Term Toxicity and Carcinogenicity

Studies evaluated in the draft monograph of Rapporteur Member State Germany of August 1, 2001:

A chronic toxicity study in rats and two carcinogenicity studies in rats and mice have been evaluated by European authorities and Germany as Rapporteur Member State and were considered to be acceptable. For the convenience of the reviewer, these studies are summarized below as extracted from the monograph. The results are summarized in Table 5.5-1.

Table 5.5-1: Summary of long-term toxicity/carcinogenicity studies conducted with pyraclostrobin

Study	Dosages (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Main adverse effect	Reference (BASF DocID)
24-month chronic toxicity Wistar rats	M: 1.1, 3.4 and 9.0 F: 1.5, 4.6 and 12.3 [0, 25, 75, 200 ppm]	3.4/4.6 m/f [75 ppm]	9.0/12.3 m/f [200 ppm]	Systemic toxicity: Reduced body weight Oncogenicity: No increase in tumour incidences	1999/11672 *2002/100511 3*[#]
24-month carcinogenicity Wistar rats	M: 1.2, 3.4 and 9.2 F: 1.5, 4.7 and 12.6 [0, 25, 75, 200 ppm]	3.4/4.7 m/f [75 ppm]	9.2/12.6 m/f [200 ppm]	Systemic toxicity: Reduced body weight, reduced food consumption (m), liver cell necrosis (m) Oncogenicity: No evidence of carcinogenictity	1999/11868 *2002/1005114* [#]
18-month carcinogenicity B6C3F1 mice	M: 1.4, 4.1 and 17.2 F: 1.6, 4.8, 20.5 and 32.8 [0, 10, 30, 120 ppm (m, f), 180 ppm (f)]	4.1/4.8 m/f [30 ppm]	17.2/20.5 m/f [120 ppm] & 32.8 f [180 ppm]	Systemic toxicity: Reduced body weight Oncogenicity: No evidence of carcinogenictity	1999/11871

[#] References (BASF DocIDs) in italic are submitted the first time in this supplemental dossier. As these report amendment do not affect the outcome and interpretation of the studies they are listed in this table.

The results of the chronic toxicity study in rats (24 months) indicated that a maximum tolerated dose was met at the high dose of 200 ppm (ca. 9.0 mg/kg bw/day for males and 12.3 mg/kg bw/day for females). This is demonstrated by a body weight gain depression in the second part of the study. No further substance related adverse effects were observed. There was no evidence of treatment related increase in neoplasms.

A carcinogenicity study in rats was conducted up to 200 ppm, which represents the maximum tolerated dose as evidenced by significant body weight gain depression in males and females, which was accompanied by reduced food consumption in males only. Furthermore, liver cell necrosis occurred in males. Nevertheless, there was no evidence of a pyraclostrobin related carcinogenic response.

A carcinogenicity study in mice was conducted up to 180 ppm for females and 120 ppm for males, which represents the maximum tolerated dose as evidenced by significant body weight gain depression. No further substance related effects were observed in this study. There was no evidence of a treatment related increase in neoplasms.

In summary, long-term feeding studies with pyraclostrobin in rats and mice demonstrated that the primary toxic treatment-related effect is body weight decrease. No evidence of a carcinogenic potential could be established.

Based on the available studies, the following endpoints were determined in the Annex I listing of pyraclostrobin.

Target/critical effect:	Reduced body weight; (rat & mouse); liver cell necrosis (rat)
Lowest relevant NOAEL	24-months, rat/mouse: 75/30 ppm (4 mg/kg bw/d)
Carcinogenicity:	No evidence of carcinogenicity

Submission of not yet per-reviewed studies in this dossier:
In this dossier amendments to the already submitted long-term studies as well as supplementary long term studies are submitted.

The amendments to the chronic and carcinogenicity studies in rats further evaluated the histopathology of mid and low dose animals previously not completely examined. These examinations were performed on request of US EPA in order to potentially detect additional histiocytic sarcoma or systemic infiltrations of these tumors. But these additional evaluations revealed no further cases of histiocytic sarcoma. The examination of the full set of preserved organs of additional, previously not completely examined low and mid dose male rats revealed a low incidence of additional neoplastic and non-neoplastic lesions. These additional findings did not affect the original interpretation of the rat chronic and carcinogenicity studies.

The supplementary studies using higher dose levels were mostly conducted in parallel to the main long-term feedings studies. An exception is one carcinogenicity study in female B6C3F1 mice, which was performed on request of US EPA in 2004/2005. In essence, all studies confirmed that the main studies were dosed up to the Maximum Tolerated Dose (MTD). Accordingly, the studies were terminated pre-term and the examination and reporting was restricted to in-life parameters (clinical observations, body weight and food consumption, if appropriate clinical biochemistry investigations). No histopathological examinations were performed.

Thus, the conclusion for relevant endpoints for the current re-registration remains as follows:

Target/critical effect	Reduced body weight (rat & mouse); liver cell necrosis (rats) No classification required
Lowest relevant NOAEL / NOEL	24-months, rat/mouse: 75/30 ppm (4 mg/kg bw/d)
Carcinogenicity	No evidence for carcinogenicity No classification required

Note: The rat chronic toxicity and carcinogenicity study reports (CA 5.5/1 and CA 5.5/3) were already submitted and reviewed in the course of the initial registration of pyraclostrobin. These studies are submitted for completeness as the basis for the assessment of report amendments submitted under CA 5.5/2 and CA 5.5/4. Both amendments provide the results of additional histopathological evaluations on the low and mid dose animals to specifically determine the incidence of histiocytic sarcomas. In addition, all tumors as well as any significant non-neoplastic findings observed in the intermediate dose groups were recorded. Taking into account that the majority of the data were already reviewed, the following study summary will be more comprehensive than for other studies already reviewed, but will be less detailed than the summary of new studies.

Report:	CA 5.5/1 [redacted] 1999d BAS 500 F - Chronic toxicity study in Wistar rats. Administration in the diet for 24 months 1999/11672
Guidelines:	EEC 87/302, OECD 452, EPA 83-1, JMAFF
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 5.5/2 [redacted] 2002a Amendment No. 1 to the report: BAS 500 F - Chronic toxicity study in Wistar rats. Administration in the diet for 24 months 2002/1005113
Guidelines:	EEC 87/302, OECD 452, EPA 83-1, JMAFF
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Material and Methods:
Pyraclostrobin was administered to groups of 20 male and 20 female Wistar rats at dietary concentrations of 0, 25, 75 and 200 ppm for 24 months. Food consumption and body weight were determined once a week during the first 13 weeks, thereafter at 4-week intervals. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week. Ophthalmological examinations were carried out prior to the start and towards the end of dosing. Urinalysis, clinico-chemical and hematological examinations were carried out 3, 6, 12, 18 and 24 months after start of the administration period. The animals were subjected to gross pathological assessment, followed by histopathological examinations.

Findings:
The stability of the test substance itself and the homogeneous distribution, stability and correct concentration of the test substance in the diet were confirmed by analysis.

There was no test substance related increase in mortality or clinical signs of toxicity in this study. No relevant effects on food consumption were observed at any dose level (see Table 5.5-2). In females, there were isolated statistically significant deviations (increased or decreased values) in all dose groups. Due to the isolated occurrence and the lack of a dose-response relationship, these deviations were assessed as being incidental and not related to treatment.

Figure 5.5-1: Body weight development of rats administered pyraclostrobin for 24 months - chronic toxicity study

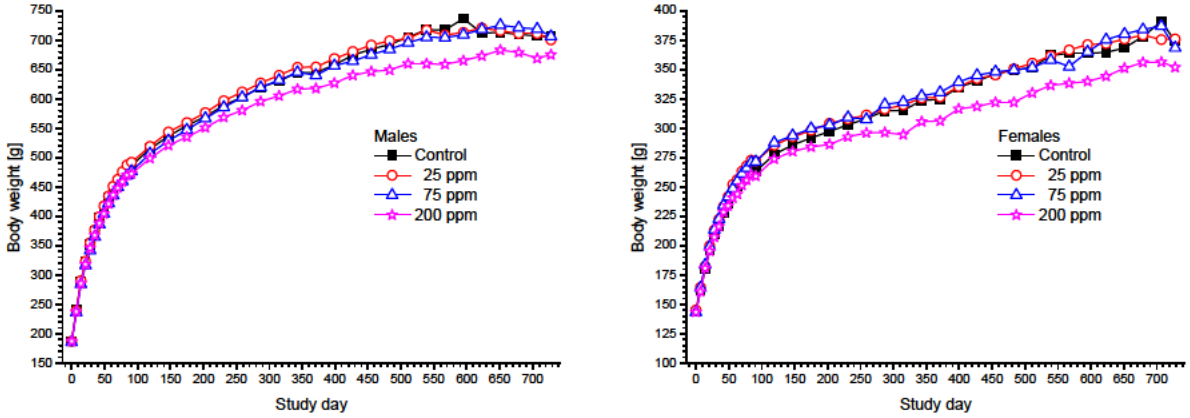


Table 5.5-2: Mean body weight and food consumption of rats administered pyraclostrobin for 24 months

Males				
Dose level [ppm]	0	25	75	200
Body weight [g]				
- Day 0	187.1	187.1	185.5	187.1
- Day 728	706.0	700.3	706.2	675.0
Δ% (compared to control) #		-0.8	0.0	-4.4
Overall body weight gain [g]	518.9	513.2	519.1	487.9
Δ% (compared to control) #		-1.1	0.0	-6.0
Food consumption [g/animal/day]				
Average daily food consumption§	25.6	26.0	25.7	24.9
Females				
Body weight [g]				
- Day 0	143.6	145.1	143.2	143.3
- Day 728	373.9	375.9	368.4	351.8
Δ% (compared to control) #		0.5	-1.5	-5.9
Overall body weight gain [g]	230.3	230.8	225.2	208.5
Δ% (compared to control) #		0.2	-2.2	-9.5
Food consumption [g/animal/day]				
Average daily food consumption§	18.9	19.3	19.3	18.2

Values may be not calculated exactly due to rounding of mean values
§ Calculated from the weekly means- Time weighted average.

In both sexes, the terminal body weight was somewhat lower than in the control group. During the course of the study, the maximum differences between the control and the high dose group achieved values of 10% (males) and 9% (females). Body weight gain was also reduced in top dose males and females, mainly in the second part of the study. Maximum differences in body weight change between the control and high dose group achieved 11% (males) or 14% (females). However, statistical significance (ANOVA and Dunnett´s test) was reached in males only occasionally (i.e. on study days 7 and 539) and in females only on study day 483 (see Figure 5.5-1and Table 5.5-2).

Clinico-chemical investigations demonstrated, at the high dose level only, a statistically significant decrease in alkaline phosphatase activity in both sexes, and alanine aminotransferase activity in the males (see Table 5.5-3). The slight, but not statistically significant decrease in protein and globulin values in high dose males mentioned in the DAR was marginal. In addition, some statistically significant differences of clinical chemistry parameters were noted. These were not dose dependent and/or not consistent over time and thus not considered to be related to treatment.

Table 5.5-3: Selected clinical chemistry parameters of rats administered pyraclostrobin for two years

Week	Study day	ALAT [µKatal/L]		ALP [[µKatal/L]]		Total protein [g/L]		Globulin [g/L]	
Males									
	Day 92	1.13	±0.22	5.81	±0.93	69.90	±3.47	35.54	±2.66
	Day 187	1.02	±0.17	5.57	±0.73	64.39	±3.20	31.90	±2.22
Control	Day 362	0.97	±0.20	4.67	±0.81	68.95	±3.55	35.67	±2.49
	Day 551	1.06	±0.18	4.89	±1.13	68.22	±3.73	38.15	±2.97
	Day 722	1.08	±0.24	4.72	±0.75	64.09	±4.56	37.89	±3.76
	Day 92	0.93§	± 0.11	5.18*	±0.86	67.59	±3.19	33.30	±2.29
	Day 187	0.90#	±0.13	4.55§	±0.56	62.35	±2.23	29.86#	±1.66
200 ppm	Day 362	0.83#	±0.14	3.90§	±0.38	68.09	±2.56	34.66	±1.94
	Day 551	0.90§	±0.11	4.02#	±0.51	67.46	±3.31	37.34	±2.57
	Day 722	0.93#	±0.29	4.28*	±0.72	63.32	±2.17	36.77	±1.73
Females									
	Day 93	0.85	±0.12	4.34	±0.72	68.84	±3.82	32.54	±2.32
	Day 188	0.89	±0.09	3.95	±0.71	65.89	±2.76	29.63	±1.43
Control	Day 363	0.82	±0.11	3.20	±0.52	76.31	±3.40	37.10	±1.74
	Day 554	1.05	±0.19	3.22	±0.79	71.18	±5.40	38.11	±3.37
	Day 723	1.12	±0.21	3.72	±0.83	75.29	±5.06	43.02	±3.86
	Day 93	0.78	± 0.12	3.78#	±0.74	69.65	±3.55	32.84	±2.16
	Day 188	0.86	±0.15	3.31#	±0.49	67.48	±2.84	30.32	±1.64
200 ppm	Day 363	0.82	±0.12	2.55§	±0.46	78.40	±3.01	37.44	±1.94
	Day 554	1.00	±0.11	2.68*	±0.57	72.82	±3.65	38.83	±2.58
	Day 723	0.94	±0.18	2.91#	±0.72	74.71	±3.39	42.89	±2.83

* $p \leq 0.05$, # $p \leq 0.02$, § $p \leq 0.002$ (Kruskal-Wallis + Mann-Whitney u tests, two-sided)

Hematological examinations, ophthalmoscopy and urinalysis did not reveal test substance related effects.

Table 5.5-4: Selected mean absolute and relative organ weights of rats administered pyraclostrobin 2 years – chronic toxicity group

Sex		Males				Females			
Organ weight [mg]	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ% #	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ% #
Terminal weight [g]	0	676.994				354.677			
	25	669.631	(-1.1)			357.550	(0.8)		
	75	675.526	(-0.2)			349.471	(-1.5)		
	200	648.335	(-4.2)			332.650	(-6.2)		
Testes [g]	0	19.29		0.666					
	25	19.285	(0.0)	0.653	(-2.0)				
	75	19.273	(-0.1)	0.722	(8.4)				
	200	19.449	(0.8)	0.758	(13.8)				
Kidney [g]	0	4.103		0.618		2.858		0.730	
	25	3.989	(-2.8)	0.600	(-2.9)	2.711	(-5.1)	0.762	(4.4)
	75	4.152	(1.2)	0.621	(0.5)	2.729	(-4.5)	0.800	(9.6)
	200	4.083	(-0.5)	0.632	(2.3)	2.796	(-2.2)	0.855	(17.1)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may be not calculated exactly due to rounding of figures

The pathological investigations did not demonstrate any test substance related adverse effect. A slight, not statistically significant increase of absolute and relative testis weights, and kidney weights in females at 200 ppm were regarded as toxicologically not significant (see Table 5.5-4). The increase of relative kidney weights was due to female #553, which displayed a mass of 18 mm diameter at the kidney, which later was identified as a metastasis of an uterus adeno-carcinoma.
In males, there were higher, but not dose-related incidences of tubular degeneration (1/7/7/6) and tubular mineralization (1/4/6/4) in the testes of all treated groups. However, these findings were often associated with tubular atrophy (9/5/5/6) and Leydig cell tumours (9/12/11/8), and they are hence not regarded as treatment-related findings, but being secondary events to atrophy and/or Leydig cell tumours.

There were no test substance related effects at dietary dose levels of 75 and 25 ppm.

By request of the US EPA, detailed histopathological evaluations of all male animals of the low and mid dose groups, which were killed scheduled at study termination and which exhibited no gross lesions in any of the organs with lymphoma infiltrates during necropsy, were performed specifically to determine the incidence of occult histiocytic sarcomas. This request was based on an apparent increase of histiocytic sarcoma of the hemolymphoreticular system in high dose males of the carcinogenicity study (see BASF DocID 1999/11868). In addition, all neoplastic and non-neoplastic findings in previously not histopathologically investigated organs of intermediate dose level males were recorded. The results of this additional analysis are presented in the Report Amendment No. 1.

The pathological investigations to determine histiocytic sarcomas revealed no additional malignant systemic tumors related to pyraclostrobin treatment in any group. Likewise, no 'sarcoma infiltrates' indicating the presence of a histiocytic sarcoma were observed in any organ investigated. The detailed histopathological evaluation of male animals of the low and mid dose groups identified only a marginal amount of additional neoplastic findings (see Table 5.5-5) which either displayed no dose-response relationship (e.g. mesenteric lymph node hemangioma in mid dose males) or were observed in comparable incidences in all groups. Thus these neoplasms were considered to be of incidental nature. Likewise, the total incidence of neoplastic findings was not altered in a way indicative for a treatment-related effect on carcinogenicity (see Table 5.5-6).

Table 5.5-5: Incidence of neoplastic findings in rats after examination of all intermediate dose males (affected organs only) – chronic toxicity group

Dose group	**0 ppm**	**25 ppm**	**75 ppm**	**200 ppm**
Animals in the selected group	20	20	20	20
Hemolymphoreticular system				
Histiocytic sarcoma	1	0	1	0
Heart				
Malignant endocardial schwannoma	0	1 (0)	0	0
Mesenteric lymph node				
Lymphangioma	0	1 (0)	1 (0)[a]	0
Hemangioma	1	1	5 (3)	0
Pancreas				
Islet-cell adenoma	2	0	1 (0)	0
Pituitary gland				
Adenoma	5	1	5 (3)	4
Prostate gland				
Adenoma	2	2 (1)	2 (1)	3
Thyroid glands				
Follicular adenoma	0	1 (0)	0	0

[a] Values in brackets indicate incidences of the histopathological investigation of the main report if different from those in the report amendment

Table 5.5-6: Overview of neoplastic findings in rats after administration of pyraclostrobin for 2-years – chronic toxicity group

Sex	Males				Females			
Dose [ppm]	0	25	75	200	0	25	75	200
Animals in group	20	20	20	20	20	20	20	20
Number of animals with:								
- neoplasms	17	18	20 (19)[a]	15	20	18	20	20
- 1 primary neoplasm	5	4 (6)	4	5	4	6	6	4
- 2 and > primary neoplasms	12	14 (12)	16 (15)	10	16	12	14	16
Number of animals with:								
- benign neoplasms	16	17	20 (19)	11	19	17	19	20
- benign neoplasms only	12	8 (9)	12 (11)	9	12	14	12	17
- malignant neoplasms	5	10 (9)	8	6	8	4	8	3
- malignant neoplasms only	1	1	0	4	1	1	0	1
- systemic neoplasms	2	0	1	0	0	0	0	1
- metastasized neoplasm	0	2	0	2	1	0	0	1
Total number of:								
- primary neoplasms	41	48 (44)	47 (40)	33	49	37	40	48
- benign neoplasms	36	36 (33)	38 (31)	26	41	33	30	44
- malignant neoplasms	5	12 (11)	9	7	8	4	10	4
- systemic neoplasms	2	0	1	0	0	0	0	1
- metastasized neoplasm	0	2	0	2	1	0	0	1

[a] Values in brackets indicate incidences of the histopathological investigation of the main report if different from those in the report amendment

Conclusion:

The no observed adverse effect level (NOAEL) in this 24-month chronic toxicity study in rats was 75 ppm (3.4 mg/kg bw/d in males and 4.6 mg/kg bw/d in females), based on a slight decrease in body weight at 200 ppm. There was no indication of a carcinogenic potential in rats.

Report:	CA 5.5/3 [redacted] 1999e BAS 500 F - Carcinogenicity study in Wistar rats. Administration in the diet for 24 months 1999/11868
Guidelines:	EEC 87/302, OECD 451, EPA 83-2, JMAFF
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 5.5/4 [redacted] 2002b Amendment No. 1 to the report: BAS 500 F - Carcinogenicity study in Wistar rats. Administration in the diet for 24 months 2002/1005114
Guidelines:	EEC 87/302, OECD 451, EPA 83-2, JMAFF
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Material and Methods:
Pyraclostrobin was administered to groups of 50 male and 50 female Wistar rats at dietary concentrations of 0, 25, 75 and 200 ppm for 24 months. Food consumption and body weight were determined once a week during the first 13 weeks, thereafter at 4-week intervals. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week. Differential blood counts were determined for all surviving animals at the end of the study and also from all animals sacrificed moribund during the study. After about 24 months, the animals were subjected to gross-pathological assessment, followed by histopathological examinations.

Statistical analysis of the results was based on the Dunnett's test (two-sided), the F-test (ANOVA, two-sided), the Kruskal-Wallis test (two-sided) and the Wilcoxon test.

Findings:
The stability of the test substance itself and the homogeneous distribution, stability and correct concentration of the test substance in the diet were confirmed by analysis.

There was no test substance related increase in mortality or clinical signs of toxicity in this study.

Impaired body weight development was observed in male and female rats at 200 ppm (see Figure 5.5-2). Body weights were significantly decreased in males from day 7 throughout day 567 and in females from study day 147 onwards. Decreases of cumulative body weight gain approached 10% in males and 22% in females (see Table 5.5-7).

Figure 5.5-2: Body weight development of rats administered pyraclostrobin for 24 months – carcinogencity study

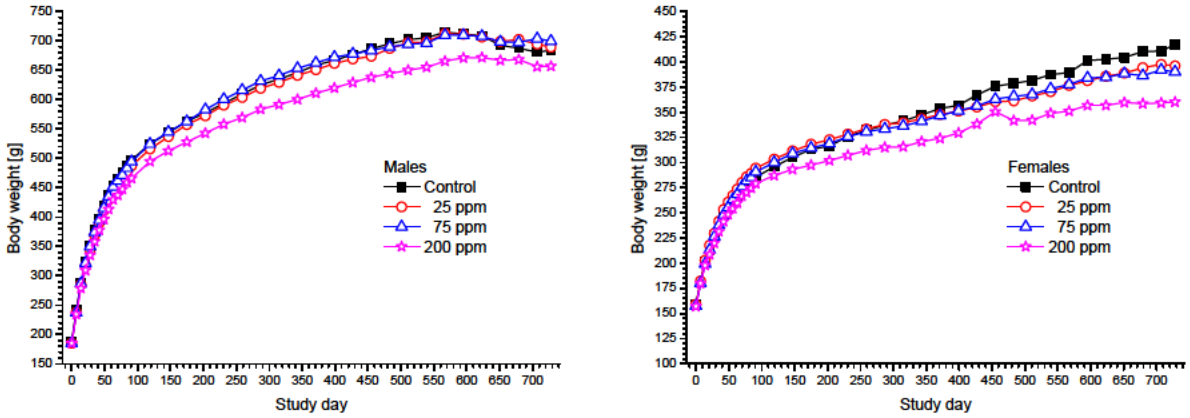


Table 5.5-7: Mean body weight and food consumption of rats administered pyraclostrobin for 24 months

Males				
Dose level [ppm]	0	25	75	200
Body weight [g]				
- Day 0	187.5	185.1	185.6	185.2
- Day 371	658.6	650.8	662.8	**610.8****
Δ% (compared to control) #		-1.2	0.6	-7.3
Overall body weight gain [g]	471.1	465.7	477.2	**425.6****
Δ% (compared to control) #		-1.1	1.3	-9.7
- Day 728	684.0	689.4	699.7	656.8
Δ% (compared to control) #		0.8	2.3	-4.0
Overall body weight gain [g]	496.5	504.3	514.1	471.6
Δ% (compared to control) #		1.6	3.5	-5.0
Food consumption [g/animal/day]				
Average daily food consumption (Day 7 to 91)	26.2	26.0	26.5	**25.3***
Average daily food consumption (entire study)§	25.7	26.1	26.4	25.2
Females				
Body weight [g]				
- Day 0	159.2	159.1	157.4	157.1
- Day 371	353.7	347.7	346.7	**324.1****
Δ% (compared to control) #		-1.7	-2.0	-8.4
Overall body weight gain [g]	194.5	188.6	189.3	**167.0****
Δ% (compared to control) #		-3.0	-2.7	-14.1
- Day 728	471.4	396.1	390.2	**360.2****
Δ% (compared to control) #		-5.1	-6.5	-13.7
Overall body weight gain [g]	258.2	237.0	232.8	**203.1****
Δ% (compared to control) #		-8.2	-9.8	-21.3
Food consumption [g/animal/day]				
Average daily food consumption (Day 7 to 91)	19.6	19.8	20.1	19.5
Average daily food consumption§	20.1	19.8	20.5	19.6

Values may not calculate exactly due to rounding of mean values
§ Calculated from the weekly means (time weighted).

Statistically significant reductions of food consumption were frequently observed in males during the first three month of treatment (see Table 5.5-7). Additionally, isolated cases of statistically significant altered food consumption were observed in all treated groups, which due to their sporadic occurrence were considered to be incidental.

Relative kidney weights were increased in males at 200 ppm and in females at the high (200 ppm) and the mid (75 ppm) dose level (see Table 5.5-8). Restricted to the high dose group, histopathological investigation of the kidneys revealed higher incidences of tubular casts and tubular atrophy in males and tubular atrophy in females. However, these findings show high spontaneous incidences. Additionally, lower incidences in kidney findings were observed, concerning pyelitis and chronic nephropathy in high dose males.

Absolute liver weights were decreased in females at the high dose level. Histopathological investigations revealed an increased incidence of liver cell necrosis (1/2/2/10) in males. Furthermore, at 200 ppm, lower incidences of adrenal cortex hyperplasia in males (37/34/31/19) and ovarial pigment deposition in females (33/26/32/17) were noted.

Table 5.5-8: Selected mean absolute and relative organ weights of rats administered pyraclostrobin 2 years – carcinogenicity group

Sex		Males				Females			
Organ weight [mg]	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ% #	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ% #
Terminal weight [g]	0	658.131				393.898			
	25	656.483	(-0.3)			378.39	(-3.9)		
	75	668.0	(1.5)			367.853	(-6.6)		
	200	627.538	(-4.6)			338.617**	(-14.0)		
Liver [g]	0	19.809		3.016		12.857		3.282	
	25	19.839	(0.2)	3.048	(1.1)	12.746	(-0.9)	3.364	(2.5)
	75	19.063	(-3.8)	2.865	(-5.0)	12.504	(-2.7)	3.403	(3.7)
	200	19.195	(-3.1)	3.047	(1.0)	11.527**	(-10.3)	3.408	(3.8)
Kidney [g]	0	4.006		0.621		2.704		0.701	
	25	4.169	(4.1)	0.643	(3.5)	2.771	(2.5)	0.74	(5.6)
	75	4.413	(10.2)	0.613	(-1.3)	2.798	(3.5)	0.77*	(9.8)
	200	4.545	(13.5)	0.675*	(8.7)	2.798	(3.5)	0.836**	(19.3)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may not calculate exactly due to rounding of figures

Based on an apparent increase of histiocytic sarcoma of the hemolymphoreticular system observed in high dose males, US EPA requested detailed histopathological evaluations of all male animals of the low and mid dose groups to determine the significance of these tumors. In addition, all neoplastic and non-neoplastic findings in previously not histopathologically investigated organs of intermediate dose level males were recorded. The results of this additional analysis are presented in the Report Amendment No. 1.

Table 5.5-9: Incidence of neoplastic findings in rats after examination of all intermediate dose males – carcinogenicity group

Dose group	0 ppm	25 ppm	75 ppm	200 ppm
Animals in the selected group	20	20	20	20
Brain				
Granular cell tumor	3	4 (3)[a]	3	0
Hemolymphoreticular system				
Histiocytic sarcoma	1	0	1	0
Lymphoma	0	2 (1)	0	1
Mesenteric lymph node				
Lymphangioma	1	2 (0)	1 (0)	0
Hemangioma	3	1 (0)	1	8
Pancreas				
Islet-cell adenoma	2	2 (1)	0	0
Acinar adenoma	2	0	1 (0)	1
Parathyroid				
Adenoma	2	2 (0)	0	0
Pituitary gland				
Adenoma	16	9 (6)	8 (6)	8
Prostate gland				
Adenoma	4	4 (1)	3 (2)	2
Thyroid glands				
Follicular adenoma	2	0	4 (3)	0

[a] Values in brackets indicate incidences of the histopathological investigation of the main report if different from those in the report amendment

The pathological investigations to determine histiocytic sarcomas revealed no additional malignant systemic tumors related to BAS 500 F treatment in any group. The detailed histopathological evaluation of intermediate dose males identified a low incidence of additional neoplastic findings (see Table 5.5-9) which did not affect the overall tumor incidence (see Table 5.5-10).

Table 5.5-10: Overview of neoplastic findings in rats after administration of pyraclostrobin for 2-years – carcinogenicity group

Sex	Males				Females			
Dose [ppm]	0	25	75	200	0	25	75	200
Animals in group	50	50	50	50	50	50	50	50
Number of animals with:								
- neoplasms	44	48 (45)[a]	46 (45)	47	50	46	50	48
- 1 primary neoplasm	6	14 (12)	11	14	13	12	20	8
- 2 and > primary neoplasms	38	34 (33)	35 (34)	33	37	34	30	40
Number of animals with:								
- benign neoplasms	42	41 (39)	44 (42)	41	49	42	48	48
- benign neoplasms only	22	25 (24)	25 (24)	27	38	25	38	30
- malignant neoplasms	22	23 (21)	21	20	12	21	12	18
- malignant neoplasms only	2	7 (6)	2	6	1	4	2	0
- systemic neoplasms	1	3 (2)	2	6	1	2	3	0
- metastasized neoplasm	4	3	5	3	2	3	3	2
Total number of:								
- primary neoplasms	120	116 (102)	116 (110)	114	119	108	101	123
- benign neoplasms	96	87 (75)	89 (83)	89	106	80	87	102
- malignant neoplasms	24	29 (27)	27	25	13	28	14	21
- systemic neoplasms	1	3 (3)	2	6	1	2	3	
- metastasized neoplasm	4	3	5	3	2	3	3	2

[a] Values in brackets indicate incidences of the histopathological investigation of the main report if different from those in the report amendment

There were only a few additional pre-neoplastic or non-neoplastic findings in the low and mid dose groups (see Table 5.5-11). These additional non-neoplastic findings did not alter the respective conclusions in the original report.

Table 5.5-11: Incidence of neoplastic findings in rats after examination of all intermediate dose males (affected organs only) – chronic toxicity group

Dose group	0 ppm	25 ppm	75 ppm	200 ppm
Animals in the selected group	20	20	20	20
Heart				
Peri-/arteritis	2	2 (1)	0	0
Pancreas				
Islet (cell) hyperplasia	0	1 (0)	0	0
Acinar (cell) hyperplasia	1	0	1 (0)	0
Prostate gland				
Prostatitis	36	16 (14)	16 (14)	35
Focal hyperplasia	17	10 (2)	14 (6)	15
Pituitary glands				
Hyperplasia	11	9 (6)	8 (2)	5
Thyroid glands				
C-cell hyperplasia	29	14 (3)	6 (3)	26
Cystic follicles	1	4 (0)	1 (0)	0
Nodular hyperplasia	1	4 (1)	4 (0)	1
Parathyroid glands				
Hyperplasia	13	4 (2)	4	7
Bone Marrow				
Myeloid hyperplasia	5	2 (1)	5	7
Mandibular lymph node				
Lymphoid hyperplasia	19	3 (1)	1 (0)	9
Plasmacytosis	37	5	6 (4	35
Submandibular glands				
Sialoadenitis	0	0	1 (0)	0

[a] Values in brackets indicate incidences of the histopathological investigation of the main report if different from those in the report amendment.

Overall, there were no test substance related effects at 75 and 25 ppm. Likewise, there was no evidence of a carcinogenic response at any dose level.

Conclusion:

The no observed adverse effect level (NOAEL) in this 24-month carcinogenicity study in rats was 75 ppm (3.4 mg/kg bw/d for males and 4.7 mg/kg bw/d for females), based on decreased body weights in both sexes and increased incidence of liver cell necrosis in males at 200 ppm. There was no indication of carcinogenic potential in rats.

In the following the supplementary chronic toxicity and carcinogenicity studies in rats conducted in parallel to the main studies are summarized. These studies were previously not submitted.

Report:	CA 5.5/5 [redacted] 2002c BAS 500 F - First supplementary chronic toxicity study in Wistar rats - Administration in the diet for 24 months 2002/1004125
Guidelines:	EEC 87/302 B, OECD 452, EPA 83-1, JMAFF
GLP:	yes (partially) (during the in-life phase up to the pre-term termination regular QAU audits were performed for this GLP study. However, the report was not QAU audited and thus the study has no full GLP status)

Executive Summary

This supplementary study was conducted in parallel to the chronic feeding study in male and female Wistar rats (see BASF DocID 1999/11672). BAS 500 F (pyraclostrobin) was administered to groups of 20 male and 20 female Wistar rats at dietary concentrations of 0 and 400 ppm. Since in the main study the maximum tolerated dose (MTD) was obtained at 200 ppm, the study was terminated after 405 study days without any further examinations. During the study period no animal died. A number of clinical chemistry parameters were altered, several of them were similar to effects already seen in the shorter-term studies at higher dose levels. Due to pre-term termination of the study, no organs were preserved.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 F

Description:	viscous melt / reddish-brown, clear
Lot/Batch #:	J. No. 27882/191/c (Tox. III/part 1)
Purity:	97.09%
Stability of test compound:	Stable until May 1997 (The same batch of test material was used in the main study (see BASF DocID 1999/11672). This material was reanalyzed after end of the in-life phase of the latter study and found to be stable.)

2. Vehicle and/or positive control: Rodent diet

3. Test animals:

Species:	Rat
Strain:	Wistar, Chbb:THOM (SPF)
Sex:	Male and female
Age:	42 days
Weight at dosing:	mean males: ca. 194 g; mean females: ca. 146 g
Source:	Dr. Karl Thomae GmbH, Biberach a. d. Riss, Germany
Acclimation period:	9 days
Diet:	Kliba maintenance diet rat-mouse-hamster 343 meal, Klingentalmühle AG, Kaiseraugst, Switzerland, ad libitum
Water:	Drinking water, ad libitum
Housing:	Group housing (1 animals/cage) housed in wire cages, type DK-III, Becker & Co. (Castrop-Rauxel, Germany)
Environmental conditions:	
Temperature:	20 - 24°C (central air-conditioning)
Humidity:	30 - 70% (central air-conditioning)
Air changes:	not reported
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: 24-Feb-1997 to 14-Apr-1998
(Inlife dates: 5-Mar-1997 (start of administration) - 14-Apr-1998 (necropsy of last female animals)

2. Animal assignment and treatment:
BAS 500 F was administered to groups of 20 male and 20 female Wistar rats at dietary concentrations of 0 and 400 ppm for 405 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights. At day 405 all animals were sacrificed without any further examination.

3. Test substance preparation and analysis:
The containers of test substance required for the feed mixtures in each case were cooled to approx. -20°C in the freezer so that they can be treated mechanically. Subsequently, the test substance was comminuted mechanically and stored at -20°C, unlike the usual storage conditions, to prevent it from becoming conglutinated again. For preparation of the feed mixes an acetone solution was prepared with the weighed test substance in the specific concentration. This solution was sprayed onto approx. 3.0 kg feed in a rotary evaporator (Büchi, Rotapor R 153) under partial vacuum. Subsequently, the solvent was removed under suction while heating to approx. 40°C for at least 30 minutes. Then, the premix was adjusted to the concentration desired with the appropriate amount of feed and mixed in a laboratory mixer of GEBR. LÖDIGE for approx. 10 minutes. The preparation frequencies were selected to ensure the stability of the test substance concentrations in the diet.

The stability of the test substance in the diet over 43 days at room temperature was verified analytically in the study 08B0376/956006 (analytical report (Amendment No. 1) of Jul. 5, 1996). Analyses to detect the homogeneous test substance distribution in the diet were carried out in samples of the parallel carcinogenicity studies (8250494/96086 (see BASF DocID 1999/11672) and 8250494/96096 (see BASF DocID 2002/1004124). For the concentration control analysis of the test substance preparations, samples were sent to the analytical laboratory at the beginning of the study, then at approx. 3-month intervals and approx. 4 weeks before the end of the study.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	Student's t-test (two-sided) for the hypothesis of equal means

Statistics of clinical chemistry

Parameter	Statistical test
hematological examinations	Mann-Whitney u-test (two-sided)
urinalysis	Fisher's exact test (one- and two-sided)

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality once daily.
A general cage side observation of the animals with regard to their outer appearance and behavior was carried out at least once daily. In addition to the general daily observation, each animal was subjected to an exact clinical check (including palpation).

2. Body weight:

The body weight of the animals was determined once weekly up to the 13th week of administration, then at 4-week intervals.

3. Food consumption, food efficiency and compound intake:

Food consumption was determined weekly during the first 13 weeks of administration, thereafter at 4-week intervals.
Even though not mentioned in report it is assumed that the food efficiency and substance intake was calculated as in the main study (see BASF DocID 1999/11672).
Food efficiency was calculated based upon individual values for body weight and food consumption:

$$Food\ efficiency\ at\ day\ x = \frac{BW_x - BW_y}{FC_{y\ to\ x}} x\,100$$

with BW_x and BW_y body weight (g) on day x and day y (last weighing date before day x), $FC_{y\ to\ x}$ as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days from day y to day x.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$Substance\ intake\ for\ day\ x = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the concentration in food on day x (mg/kg = ppm) and BW_x as body weight on day x of the study (g).

4. Water consumption:

No water consumption values were recorded.

5. Ophthalmoscopy:

On day 13 after the beginning of administration period, the eyes of all animals were examined with an ophthalmoscope (HEINE OPTOTECHNIK HERRSCHING, Germany) after administration of a mydriatic (Pharma Stulln). The ophthalmoscopy planned to be performed in all animals before the beginning of administration was inadvertently not carried out until day 13 of the study. The shift of the date has no adverse effect on the results of the study as the eye findings of the animals did not show any abnormalities on day 13.

6. Hematology and clinical chemistry:

Blood was taken in the morning from non-fasted animals from the retro-orbital venous plexus. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.
The following hematological and clinical chemistry parameters were determined after 3, 6 and 12 months of the administration period:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Hepato Quick's test – HQT)
✓	Hemoglobin (HGB)	✓	Neutrophils (differential)		
✓	Hematocrit (HCT)	✓	Eosinophils (differential)		
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Platelets (PLT)				
✓	Reticulocytes	✓	Large unstained cells		

Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
✓	Magnesium	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Creatinine	✓	γ-glutamyl transferase (SGGT)
✓	Potassium	✓	Globulin	✓	Serum Cholinesterase (SCHE)
✓	Sodium	✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

7. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.
The following parameters were determined after 3, 6 and 12 months of the administration period:

Urinalysis					
	Quantitative parameters:		*Semiquantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilinogen
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

8. Sacrifice and pathology:

No gross or histopathological examinations were performed as the study was terminated pre-term on study day 405.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No treatment related clinical signs of toxicity were observed in treated rats (see Table 5.5-12). All clinical observations were either observed in single animals only or displayed no dose-response relationship. Thus, they were not considered to be related to treatment.

Table 5.5-12: Clinical observations in rats administered pyraclostrobin for about 405 days – First supplemental chronic toxicity study

Sex	Male		Female	
Dose [ppm]	0	400	0	400
Animals in group	20	20	20	20
Hindlimb(s) swelling	2	0	-	-
Eyelid, crusts	1	0	0	1
Cataract	2	0	-	-
Vagina, discharge, red	-	-	0	1
Skin, palpable mass	-	-	0	1
Anogenital region smeared, red	-	-	0	1
Abdomen, palpable mass	-	-	0	1
paleness	-	-	0	1
Feces, discolored	-	-	0	1

2. Mortality

No mortality was observed during the study period.

3. Ophthalmoscopy

No treatment-related ophthalmoscopical findings were noted.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight development was impaired in dosed males as indicated by statistically significant lower body weights from study day 7 onwards. Body weight development of females was impaired when administered test substance as indicated by statistically significant lower body weights from study day 21 onwards (see Table 5.5-13 and Figure 5.5-3). Cumulative body weight gain was significantly lower throughout the major part of the study for males and females and was decreased by 18.4% in males and 21.4% in females at study day 399.

Figure 5.5-3: Body weight development of rats administered pyraclostrobin for 405 days –First supplemental chronic toxicity study

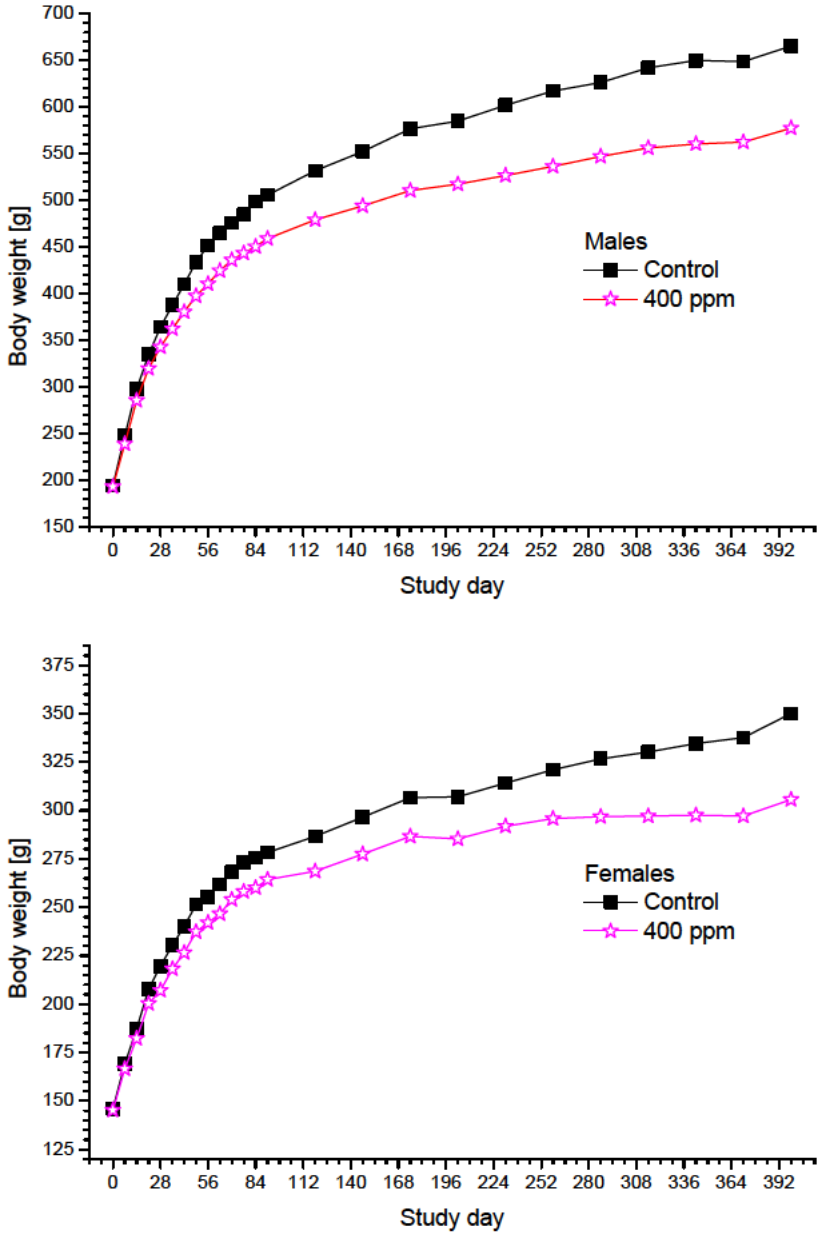


Table 5.5-13: Mean body weight of rats administered pyraclostrobin for 405 days – First supplemental chronic toxicity study

	Males		Females	
Dose level [ppm]	0	400	0	400
Body weight [g]				
- Day 0	194.4 ± 5.7	193.1 ± 6.6	146.0 ± 5.5	145.3 ± 6.3
- Day 91	505.7 ± 34.9	**458.9 ± 28.9*****	278.3 ± 25.8	**264.3 ± 12.8***
Δ% (compared to control)		- 9.3		- 5.0
- Day 231	602.0 ± 40.2	**526.6 ± 40.9*****	314.2 ± 36.2	**291.9 ± 15.5***
Δ% (compared to control)		- 12.5		- 7.1
- Day 399	665.4 ± 44.4	**577.4 ± 52.6*****	350.1 ± 43.2	**305.6 ± 20.9*****
Δ% (compared to control)		- 13.2		- 12.7
Overall body weight gain [g]				
- Day 91	311.3 ± 31.7	**265.8 ± 25.5*****	132.2 ± 21.8	**118.9 ± 8.8***
Δ% (compared to control)		- 14.6		- 10.1
- Day 231	407.6 ± 36.9	**333.5 ± 38.1*****	168.1 ± 33.0	**146.6 ± 11.8****
Δ% (compared to control)		- 18.2		- 12.8
- Day 399	471.0 ± 41.1	**384.3 ± 50.1*****	204.0 ± 40.0	**160.3 ± 18.1*****
Δ% (compared to control)		- 18.4		- 21.4

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (Student's t-test. two sided)

C. FOOD AND COMPOUND INTAKE

For treated males and females lower mean daily food intakes were observed frequently throughout the study period (see Figure 5.5-4 and ,Table 5.5-14). This was statistically significant in males and females at 19/24 and 15/24 determinations, respectively.

Figure 5.5-4: Mean daily food intake of rats administered pyraclostrobin for 405 days – First supplemental chronic toxicity study

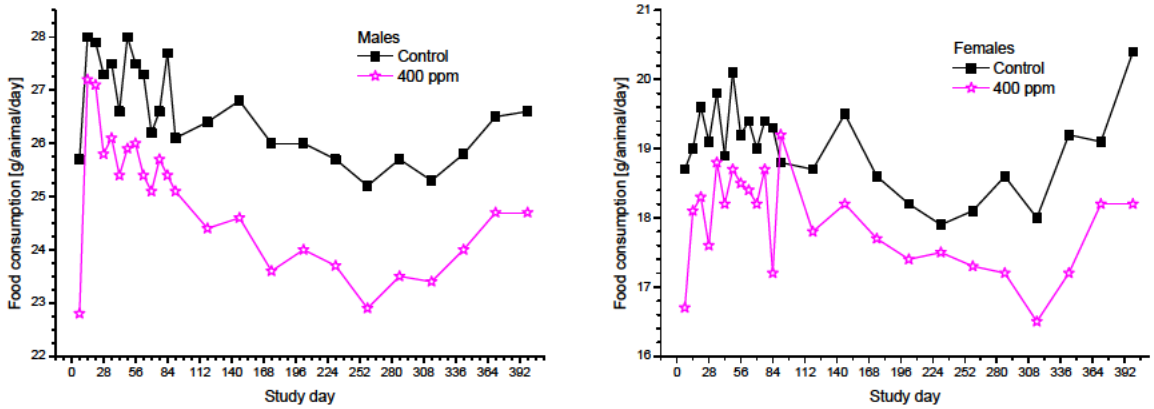


Table 5.5-14: Average daily food consumption of rats administered pyraclostrobin for 405 days – First supplemental chronic toxicity study

	Males		Females	
Dose level [ppm]	0	400	0	400
Average daily food consumption [g/animal]				
Day 0 to 90#	27.1	25.6	19.3	18.2
Δ% (compared to control)		-5.5		-5.5
Day 0 to 399[a]	26.3	24.3	18.9	17.7
Δ% (compared to control)#		-7.3		-6.1

Values were calculated based on weekly means. Values may not calculate exactly due to rounding of mean values

[a] Time weighted average

The time weighted mean daily test substance intake was calculated to have been 20.3 and 26.1 mg/kg bw/day in males and females, respectively.

D. BLOOD ANALYSIS

1. Hematological findings

A number of statistically significant differences to controls were observed. Most of these changes were not seen to all time points and were of marginal degree and thus were considered to be of incidental nature. These included increased MCV, WBC and prothrombin time and decreased MCHC values in females. In males only an increased prothrombin time was observed on day 187 (see Table 5.5-15).

Table 5.5-15: Selected hematology findings in rats administered pyraclostrobin for 405 days – first supplemental chronic toxicity study

	Study day	MCV [fL]	MCHC [mmol/L]	WBC [giga/L]	HQT [sec.]
Males					
	Day 96	49.8 ± 1.4	22.41 ± 0.47	8.07 ± 1.63	27.8 ± 1.6
Control	Day 187	50.8 ± 1.1	21.54 ± 0.47	6.84 ± 1.72	28.9 ± 2.0
	Day 362	52.0 ± 1.5	21.49 ± 0.50	6.48 ± 1.60	30.8 ± 1.9
	Day 96	49.9 ± 1.4	22.25 ± 0.38	7.82 ± 1.39	28.4 ± 1.2
400 ppm	Day 187	50.6 ± 1.5	21.67 ± 0.38	7.42 ± 1.36	**30.5 ± 0.9*****
	Day 362	51.8 ± 1.6	21.66 ± 0.68	6.26 ± 1.15	31.7 ± 3.1
Females					
	Day 96	50.0 ± 1.0	22.93 ± 0.31	4.83 ± 1.11	25.2 ± 1.0
Control	Day 187	51.6 ± 1.2	21.97 ± 0.48	3.53 ± 0.98	26.3 ± 0.9
	Day 362	52.1 ± 1.2	22.02 ± 0.81	3.79 ± 1.07	25.8 ± 2.6
	Day 96	**51.3 ± 1.5 ****	**22.58 ± 0.48 ****	5.18 ± 1.25	25.8 ± 1.3
400 ppm	Day 187	51.9 ± 1.6	21.87 ± 0.58	**4.38 ± 0.94****	**27.4 ± 1.6****
	Day 362	**53.3 ± 1.8***	**21.79 ± 0.56***	4.30 ± 1.15	**27.6 ± 2.2***

* $p \leq 0.05$; ** $p \leq 0.02$; *** $p \leq 0.002$ (Mann-Whitney u-test, two sided)

2. Clinical chemistry findings

A number of statistically significant and treatment-related clinical chemistry findings were observed (see Table 5.5-16). Some of the changes were similar to those seen in the 28-day and 90-day studies (e.g. decreased ALT, AL(A)T, serum cholinesterase, cholesterol, glucose) and may be related to the impaired nutritional state of the animals.

Table 5.5-16: Selected clinical chemistry findings in rats administered pyraclostrobin for 405 days (group means) – First supplemental chronic toxicity study

	day	Males				Females			
Dose [ppm		0		400		0		400	
ALT [µkat/l	96	1.17	±0.20	**0.93**	**±0.24*** **	1.09	±0.19	**0.84**	**±0.14*** **
	187	0.99	±0.15	**0.79**	**±0.12*** **	0.94	±0.12	**0.74**	**±0.11*** **
	362	0.82	±0.15	**0.70**	**±0.11****	0.84	±0.15	0.78	±0.20
ALP [µkat/l	96	6.36	±0.99	**5.40**	**±0.83****	4.55	±0.52	**3.85**	**±0.85*** **
	187	5.68	±1.12	**4.44**	**±0.64*** **	4.14	±0.66	**3.17**	**±0.74*** **
	362	5.32	±1.03	**3.7**	**±0.55*** **	3.33	±0.59	**2.22**	**±0.41*** **
SChE [µkat/l	96	18.63	±4.06	18.00	±2.99	74.03	±12.78	**60.45**	**±9.07*** **
	187	17.37	±3.99	17.31	±3.15	73.99	±11.59	**65.35**	**±9.44***
	362	22.24	±5.67	22.02	±3.47	77.77	±15.72	73.27	±11.78
K^+ [mmol/l	96	6.91	±0.38	**6.65**	**±0.36****	6.40	±0.48	6.37	±0.48
	187	6.86	±0.39	6.65	±0.37	6.37	±0.38	6.36	±0.46
	362	6.65	±0.33	6.74	±0.41	6.16	±0.51	6.28	±0.37
Cl^- [mmol/l	96	104.5	±1.6	105.2	±1.4	105.9	±1.3	**107.1**	**±0.34****
	187	107.3	±0.9	107.6	±1.2	108.7	±1.6	**109.7**	**±1.2***
	362	104.3	±1.4	**105.7**	**±1.2****	104.5	±2.0	**106.6**	**±1.7****
PO_4^{3-}, inorg. [mmol/l	96	2.54	±0.16	**2.33**	**±0.26*** **	2.13	±0.21	2.21	±0.34
	187	2.21	±0.17	2.10	±0.20	1.74	±0.18	1.84	±0.23
	362	2.16	±0.17	**1.68**	**±0.27*** **	1.70	±0.35	**1.35**	**±0.44****
Ca^{2+} [mmol/l	96	2.93	±0.09	**2.85**	**±0.08****	2.76	±0.09	2.77	±0.09
	187	2.87	±0.06	**2.78**	**±0.07*** **	2.77	±0.08	2.74	±0.09
	362	2.90	±0.06	**2.83**	**±0.05*** **	2.89	±0.07	**2.80**	**±0.11****
Glucose [mmol/l	96	7.69	±0.49	7.50	±0.64	7.98	±0.72	**7.42**	**±0.69****
	187	7.47	±0.77	**7.06**	**±0.44****	7.60	±0.71	7.35	±0.66
	362	7.05	±0.76	6.96	±0.52	6.71	±0.97	6.69	±0.59
Bilirubin, tot. [µmol/l	96	2.51	±0.72	**2.89**	**±0.52***	2.64	±0.44	**2.98**	**±0.66***
	187	1.74	±0.80	1.90	±0.58	1.58	±0.59	**2.25**	**±0.71****
	362	1.90	±0.69	2.09	±0.52	1.60	±0.64	**2.39**	**±0.51*** **
Creatinin [µmol/l	96	52.8	±4.9	**50.0**	**±3.4***	52.6	±3.5	51.1	±3.0
	187	50.2	±4.0	**45.6**	**±3.1*** **	50.4	±4.9	48.9	±4.3
	362	53.2	±5.1	**47.6**	**±4.1*** **	52.3	±3.9	50.9	±4.2
Protein, total [g/l	96	66.85	±4.10	**63.72**	**±3.21****	64.81	±3.51	63.40	±3.37
	187	68.83	±3.02	**65.32**	**±2.25*** **	70.13	±4.26	**66.32**	**±3.37****
	362	69.63	±3.57	**65.38**	**±3.00*** **	75.02	±4.30	**71.45**	**±4.94****
Urea [mmol/l	96	7.16	±0.69	7.19	±0.49	7.81	±0.97	7.70	±1.26
	187	6.71	±0.60	6.78	±0.46	7.42	±0.91	7.51	±1.43
	362	6.43	±0.55	**6.11**	**±0.49***	7.13	±1.35	7.09	±2.15
Globulin [g/l	96	35.34	±2.85	**32.24**	**±2.24*** **	31.48	±1.92	**29.99**	**±2.23***
	187	34.22	±2.53	**30.49**	**±1.71*** **	31.50	±2.53	**28.55**	**±2.16*** **
	362	35.14	±3.00	**30.85**	**±2.40*** **	33.38	±2.30	**30.82**	**±3.12****
Mg^{2+} [mmol/l	96	0.90	±0.05	0.87	±0.07	0.92	±0.06	0.94	±0.05
	187	0.86	±0.05	**0.82**	**±0.04****	0.91	±0.06	0.89	±0.07
	362	0.87	±0.07	**0.79**	**±0.05*** **	0.92	±0.05	0.90	±0.07
Cholesterol [mmol/l	96	2.15	±0.33	**1.85**	**±0.24****	2.00	±0.27	1.90	±0.31
	187	2.06	±0.29	**1.84**	**±0.30****	2.10	±0.28	**1.76**	**±0.23*** **
	362	2.35	±0.29	**2.00**	**±0.41*** **	2.50	±0.43	**2.15**	**±0.41****

* $p \leq 0.05$; ** $p \leq 0.02$; *** $p \leq 0.02$ (Mann-Whitney u-test, two sided)

3. Urinalysis

No treatment related changes of urinary parameters were observed.

III. CONCLUSIONS

Dietary administration of pyraclostrobin to rats at dietary dose levels of 0 and 400 ppm for 405 days resulted in an impairment of body weight development in males and females fed with 400 ppm test substance.
Treatment with pyraclostrobin did not affect the survival of rats. A number of treatment-related clinical chemistry parameters were altered, similar to those observed in shorter-term studies. As the study was terminated pre-term after a MTD was obtained in the main study no histological examination of the animals was performed.

Report:	CA 5.5/6 [redacted] 2002d BAS 500 F - Second supplementary chronic toxicity study in female Wistar rats - Administration in the diet for 24 months 2002/1004126
Guidelines:	EEC 87/302 B, OECD 452, EPA 83-1, JMAFF
GLP:	yes (partially) (during the in-life phase up to the pre-term termination regular QAU audits were performed for this GLP study. However, the report was not QAU audited and thus the study has no full GLP status)

Executive Summary

This 2nd supplementary study was conducted in parallel to the chronic feeding study female Wistar rats (see BASF DocID 1999/11672). BAS 500 F (pyraclostrobin) was administered to groups of 20 female Wistar rats at dietary concentration of 600 ppm. Control animal data were taken from the parallel conducted main study (see BASF DocID 1999/11672). Since in the main study the maximum tolerated dose (MTD) was obtained at 200 ppm, the study was terminated after 433 study days without any further examinations.
Treatment neither affected the survival of rats nor elicited clinical signs. Treatment–related changes of leukocyte counts were observed throughout the study. Even though other hematology parameters were changed in a way consistent to the changes seen in short-term studies at higher concentrations, these changes were either not consistent over time or – despite of their statistical significance – of marginal degree. Thus their toxicological significance remains unclear. Conversely, a number of clinical chemistry changes were consistent with previous studies and probably treatment-related.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 F

Description:	viscous melt / reddish-brown, clear
Lot/Batch #:	J. No. 27882/191/c (Tox. III/part 1)
Purity:	97.09%
Stability of test compound:	Stable until May 1997. (The same batch of test material was used in the main study (see BASF DocID 1999/11672). This material was reanalyzed after end of the in-life phase of the latter study and found to be stable.)

2. Vehicle and/or positive control: Rodent diet

3. Test animals:

Species:	Rat
Strain:	Wistar, Chbb:THOM (SPF)
Sex:	Female
Age:	42 days ± 1 day
Weight at dosing:	mean females: ca. 144 g
Source:	Dr. Karl Thomae GmbH, Biberach a. d. Riss, Germany
Acclimation period:	9 days
Diet:	Kliba maintenance diet rat-mouse-hamster 343 meal, Klingentalmühle AG, Kaiseraugst, Switzerland, ad libitum
Water:	Drinking water, ad libitum
Housing:	Group housing (1 animals/cage) housed in wire cages, type DK-III, Becker & Co. (Castrop-Rauxel, Germany)
Environmental conditions:	
Temperature:	20 - 24°C (central air-conditioning)
Humidity:	30 - 70% (central air-conditioning)
Air changes:	not reported
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: 10-Feb-1997 to 14-Apr-1998
Inlife dates: 19-Feb-1997 (start of administration) to 14-Apr-1998 (sacrifice of animals)

2. Animal assignment and treatment:
BAS 500 F was administered to a group of 20 female Wistar rats at dietary concentration 600 ppm for 433 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights. At day 433 all animals were sacrificed without any further examination. The data of control animals were taken from the parallel conducted main study (see BASF DocID 1999/11672).

3. Test substance preparation and analysis:
The containers of test substance required for the feed mixtures in each case were cooled to approx. -20°C in the freezer so that they can be treated mechanically. Subsequently, the test substance was comminuted mechanically and stored at -20°C, unlike the usual storage conditions, to prevent it from becoming conglutinated again. For preparation of the feed mixes an acetone solution was prepared with the weighed test substance in the specific concentration. This solution was sprayed onto approx. 3.0 kg feed in a rotary evaporator (Büchi, Rotapor R 153) under partial vacuum. Subsequently, the solvent was removed under suction while heating to approx. 40°C for at least 30 minutes. Then, the premix was adjusted to the concentration desired with the appropriate amount of feed and mixed in a laboratory mixer of GEBR. LÖDIGE for approx. 10 minutes. The preparation frequencies were selected to ensure the stability of the test substance concentrations in the diet.

The stability of the test substance in the diet over 43 days at room temperature was verified analytically in the study 08B0376/956006 (analytical report (Amendment No. 1) of Jul. 5, 1996). Analyses to detect the homogeneous test substance distribution in the diet were carried out in samples of the parallel carcinogenicity studies (8250494/96086 (see BASF DocID 1999/11672) and 8250494/96096 (see BASF DocID 2002/1004124). For the concentration control analysis of the test substance preparations, samples were sent to the analytical laboratory at the beginning of the study, then at approx. 3-month intervals and approx. 4 weeks before the end of the study.

4. Statistics:
Means and standard deviations of each test group were calculated for several parameters. The data of control animals were taken from the parallel study (see BASF DocID 2002/1004125). Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	Student's t-test (two-sided) for the hypothesis of equal means

Statistics of clinical chemistry

Parameter	Statistical test
hematological examinations	Wilcoxon test (two-sided)
Urinalysis	Fisher's exact test (one- and two-sided)

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality once daily. A general cage side observation of the animals with regard to their outer appearance and behavior was carried out at least once daily. In addition to the general daily observation, each animal was subjected to an exact clinical check (including palpation).

2. Body weight:

The body weight of the animals was determined once weekly up to the 13th week of administration, then at 4-week intervals.

3. Food consumption, food efficiency and compound intake:

Food consumption was determined weekly during the first 13 weeks of administration, thereafter at 4-week intervals.
Even though not mentioned in report it is assumed that the food efficiency and substance intake was calculated as in the main study (see BASF DocID 1999/11672). Food efficiency was calculated based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \text{ x } 100$$

with BW_x and BW_y body weight (g) on day x and day y (last weighing date before day x), $FC_{y\ to\ x}$ as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days from day y to day x.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the concentration in food on day x (mg/kg = ppm) and BW_x as body weight on day x of the study (g).

4. Water consumption:

No water consumption values were recorded.

5. Ophthalmoscopy:

Before the beginning of administration period, the eyes of all animals were examined with an ophthalmoscope after administration of a mydriatic (Pharma Stulln).

6. Hematology and clinical chemistry:

Blood was taken in the morning from non-fasted animals from the retro-orbital venous plexus. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.
The following hematological and clinical chemistry parameters were determined after 3, 6 and 12 months of the administration period:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (HQT)
✓	Hemoglobin (HGB)	✓	Neutrophils (differential)		
✓	Hematocrit (HCT)	✓	Eosinophils (differential)		
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Large unstained cells		
✓	Platelets (PLT)	✓	Monocytes (differential)		
✓	Reticulocytes				
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
✓	Magnesium	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Creatinine	✓	γ-glutamyl transferase (SGGT)
✓	Potassium	✓	Globulin	✓	Serum Cholinesterase (SCHE)
✓	Sodium	✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

7. Urinalysis:

For urinalysis the individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.
The following parameters were determined after 3, 6 and 12 months of the administration period:

Urinalysis					
	Quantitative parameters:		*Semiquantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilinogen
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

8. Sacrifice and pathology:

No pathology was done since the study was terminated on day 433.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No treatment related clinical signs of toxicity were observed in treated rats.

2. Mortality

No mortality was observed during the study period.

3. Ophthalmoscopy

No data given in the report concerning ophthalmoscopy.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight development was impaired in dosed females as indicated by statistically significant lower body weights from day 28, 56, 77, 84 and 147 onwards (see Figure 5.5-5 and Table 5.5-17).
Cumulative body weight gain was significantly lower throughout the major part of the study for females.

Figure 5.5-5: Body weight development of female rats administered pyraclostrobin for 433 days – Second supplemental chronic toxicity study

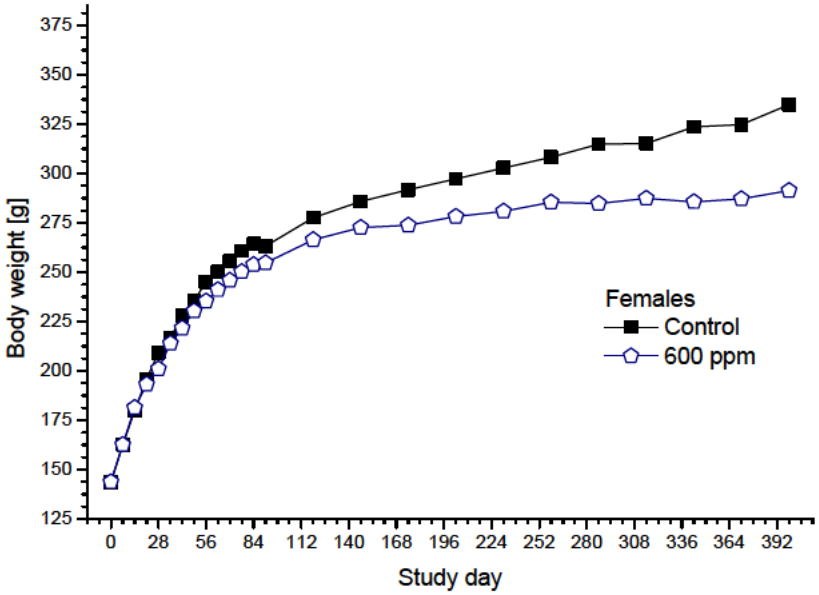


Table 5.5-17: Mean body weight of female rats administered pyraclostrobin for 433 days- Second supplemental chronic toxicity study

	Females	
Dose level [ppm]	0	600
Body weight [g]		
- Day 0	143.6 ± 5.4	143.8 ± 4.8
- Day 91	263.2 ± 19.1	254.7 ± 12.7
Δ% (compared to control)		-3.2
- Day 231	302.8 ± 26.2	**280.8 ± 15.5****
Δ% (compared to control)		**-7.3**
- Day 399	344.9 ± 31.4	**291.3 ± 14.9****
Δ% (compared to control)		**-13.0**
Overall body weight gain [g]		
- Day 91	119.6 ± 16.7	110.9 ± 10.9
Δ% (compared to control)		-7.3
- Day 231	159.1 ± 23.5	**137.0 ± 12.9****
Δ% (compared to control)		**-13.9**
- Day 399	191.3 ± 30.7	**147.5 ± 13.0****
Δ% (compared to control)		**-22.9**

* $p \leq 0.05$; ** $p \leq 0.01$ (Student's t-test. two sided)

C. FOOD & WATER CONSUMPTION AND COMPOUND INTAKE

For treated females statistically lower mean daily food intakes were observed frequently throughout the study period (see Figure 5.5-6 and Table 5.5-18). This was statistically significant at 12/24 determinations.

Figure 5.5-6: Food consumption of female rats administered pyraclostrobin for 433 days – Second supplemental chronic toxicity study

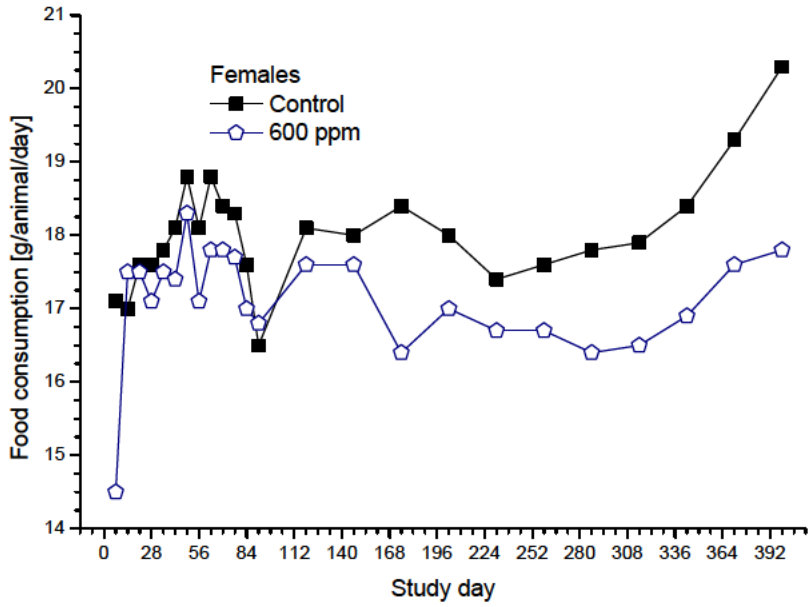


Table 5.5-18: Food consumption of rats administered pyraclostrobin for 433 days – Second supplemental chronic toxicity study

	Females	
Dose level [ppm]	0	600
Mean food consumption [g/animal]		
Day 0 to 90#	17.82	17.23
Δ% (compared to control)#		-3.3
Day 0 to 399	18.18	17.07
Δ% (compared to control)#		-6.1

Values were calculated based on mean individual daily food. Values may not calculate exactly due to rounding of mean values

The mean daily test substance intake was calculated to be 38.8 mg/kg bw/day in females at a dietary dose level of 600 ppm.

D. BLOOD ANALYSIS

1. Hematological findings

Table 5.5-19: Selected hematology findings in rats administered pyraclostrobin for 433 days – Second supplemental chronic toxicity study

		day	Females			
Dose	[ppm]		0		600	
WBC	[giga/L]	92/93	4.33	±1.17	**7.28**	**±1.70****
		187/188	4.21	±1.10	**6.56**	**±1.28****
		362/363	3.88	±0.63	**5.71**	**±1.50****
RBC	[tera/L]	92/93	7.97	±0.53	8.00	±0.22
		187/188	8.24	±0.26	**7.91**	**±0.28****
		362/363	7.75	±0.32	7.74	±0.28
HGB	[mmol/L]	92/93	9.16	±0.54	9.28	±0.31
		187/188	9.50	±0.25	**8.92**	**±0.28****
		362/363	9.05	±0.36	8.96	±0.28
HCT	[L/L]	92/93	0.41	±0.03	0.41	±0.01
		187/188	0.43	±0.01	**0.41**	**±0.01****
		362/363	0.40	±0.02	0.41	±0.01
MCV	[fL]	92/93	51.00	±0.84	**51.61**	**±1.07***
		187/188	51.60	±0.76	51.84	±1.06
		362/363	52.15	±0.70	52.56	±0.80
MCH	[fmol]	92/93	1.15	±0.05	1.16	±0.03
		187/188	1.15	±0.02	**1.13**	**±0.03****
		362/363	1.17	±0.03	1.16	±0.03
MCHC	[mmol/L]	92/93	22.55	±0.88	22.48	±0.34
		187/188	22.34	±0.34	**21.78**	**±0.31****
		362/363	22.39	±0.28	**22.04**	**±0.39****
PLT	[giga/L]	92/93	783.00	±73.89	**724.90**	**±64.49***
		187/188	816.85	±96.94	762.30	±81.79
		362/363	809.16	±88.74	758.63	±78.03
HQT	[µmol/L]	92/93	25.67	±1.33	26.05	±1.55
		187/188	27.21	±1.40	**28.84**	**±1.61****
		362/363	27.66	±1.84	27.83	±2.35

* $p \leq 0.05$; ** $p \leq 0.01$ (Wilcoxon-test. two sided)

The increased leucocytes counts (WBC) at all time-points are considered to be treatment-related (see Table 5.5-19). These are consistent with the changes seen in the 1st supplemental study (see BASF DocID 2002/1004125) and the 90-day study in rats at higher dose levels (see BASF DocID 1999/10195). A number of additional parameters displayed statistically significant differences. Most of these changes were not consistent over time and partially of marginal degree. Thus they are considered to be of incidental nature rather than related to treatment.

2. Clinical chemistry findings

A number of statistically significant and treatment-related clinical chemistry findings were observed (see Table 5.5-20). Some of the changes were similar to those seen in the 28-day and 90-day studies (e.g. decreased ALT, AL(A)T, serum cholinesterase, cholesterol, glucose), are consistent to those observed at 400 ppm in the first supplemental chronic toxicity study (see BASF DocID 2002/1004125) and may be related to the impaired nutritional state of the animals.

Table 5.5-20: Selected clinical chemistry findings in rats administered pyraclostrobin for 433 days (group means)

	day	Females			
Dose [ppm]		0		600	
ALT [µkat/L]	92/93	0.85	±0.12	0.79	±0.15
	187/188	0.89	±0.09	**0.76**	**±0.16****
	362/363	0.82	±0.11	**0.75**	**±0.13***
ALP [µkat/L]	92/93	4.34	±0.72	3.80	±0.91
	187/188	3.95	±0.71	**2.99**	**±0.90****
	362/363	3.20	±0.52	**2.10**	**±0.49****
SChE [µkat/L]	92/93	72.75	±7.32	**54.42**	**±9.71****
	187/188	73.29	±7.99	**61.21**	**±9.26****
	362/363	69.81	±10.27	75.33	±10.65
Na^+ [mmol/L]	92/93	142.49	±1.97	**143.39**	**±1.45***
	187/188	142.53	±1.34	142.77	±0.93
	362/363	142.12	±1.52	**144.29**	**±1.15****
K^+ [mmol/L]	92/93	6.05	±0.53	6.33	±0.51
	187/188	6.13	±0.37	6.32	±0.35
	362/363	6.14	±0.50	**6.56**	**±0.41****
Cl^- [mmol/L]	92/93	106.11	±1.69	105.88	±1.53
	187/188	106.38	±2.08	105.76	±1.12
	362/363	105.44	±2.27	**108.62**	**±1.44****
PO_4^{3-}. inorg. [mmol/L]	92/93	1.98	±0.33	**2.25**	**±0.26****
	187/188	1.73	±0.18	**1.95**	**±0.25****
	362/363	1.68	±0.36	1.64	±0.37
Ca^{2+} [mmol/L]	92/93	2.71	±0.10	2.75	±1.29
	187/188	2.63	±0.07	**2.70**	**±0.09****
	362/363	2.72	±0.08	**2.78**	**±0.06****
Glucose [mmol/L]	92/93	7.32	±1.25	7.39	±0.62
	187/188	7.21	±0.45	**6.77**	**±0.77***
	362/363	6.76	±0.87	6.78	±0.63
Bilirubin. tot. [µmol/L]	92/93	2.07	±0.50	**2.86**	**±0.71****
	187/188	1.64	±0.44	3.37	±0.59
	362/363	1.98	±0.67	2.37	±0.54
Crea [µmol/L]	92/93	46.76	±4.17	45.66	±4.12
	187/188	48.97	±4.98	46.98	±3.32
	362/363	51.32	±3.81	**46.22**	**±3.83****
Globulin [g/L]	92/93	32.54	±2.32	31.70	±1.80
	187/188	29.63	±1.43	**28.19**	**±2.01***
	362/363	37.10	±1.74	**35.27**	**±2.83***
Mg^{2+} [mmol/L]	92/93	0.91	±0.06	0.88	±0.04
	187/188	0.86	±0.04	0.84	±0.03
	362/363	0.90	±0.04	**0.83**	**±0.05****
Cholesterol [mmol/L]	92/93	1.88	±0.32	2.00	±0.23
	187/188	1.95	±0.26	1.95	±0.37
	362/363	2.60	±0.40	**2.37**	**±0.49***

* $p \leq 0.05$; ** $p \leq 0.01$ (Wilcoxon test. two sided)

3. Urinalysis

No treatment related changes of urinary parameters were observed.

III. CONCLUSIONS

Dietary administration of pyraclostrobin to female rats at dietary dose levels of 600 ppm for 433 days resulted in an impairment of body weight development in females fed with 600 ppm test substance. Treatment neither affected the survival of rats nor elicited clinical signs. Treatment–related changes of leukocyte counts were observed throughout the study. Even though other hematology parameters were changed in a way consistent to the changes seen in short-term studies at higher concentrations, these changes were either not consistent over time or – despite of their statistical significance – of marginal degree. Conversely, a number of clinical chemistry changes were consistent with previous studies and probably treatment-related.
Since the study was terminated pre-term, no histopathological examination was performed.
Based on the design of the study it was not intended to determine a NOAEL.

Report:	CA 5.5/7 [redacted] 2002e BAS 500 F - First supplementary carcinogenicity study in Wistar rats - Administration in the diet for 24 months 2002/1004123
Guidelines:	EEC 87/302 B, OECD 451, EPA 83-2, JMAFF
GLP:	yes (partially) (during the in-life phase up to the pre-term termination regular QAU audits were performed for this GLP study. However, the report was not QAU audited and thus the study has no full GLP status)

Executive Summary

This supplementary study was conducted in parallel to the carcinogenicity study in male and female Wistar rats (see BASF DocID 1999/11868). Pyraclostrobin (BAS 500 F) was administered to groups of 50 male and 50 female Wistar rats at dietary concentrations of 0 and 400 ppm. Since in the main study the maximum tolerated dose (MTD) was obtained at 200 ppm, the study was terminated after 399 study days without any further examinations. During the study period one male animal died in the treated group and 2 females and 1 female died in the control or treated group, respectively. These deaths are not assumed to be treatment related. No relevant clinical signs were observed. Due to termination of the study, no organs were preserved.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 F

Description:	viscous melt / reddish-brown, clear
Lot/Batch #:	J. No. 27882/191/c (Tox. III/part 1)
Purity:	97.09%
Stability of test compound:	Stable until May 1997. (The same batch of test material was used in the main study (see BASF DocID 1999/11672). This material was reanalyzed after end of the of the latter study and found to be stable.)

2. Vehicle and/or positive control: Rodent diet

3. Test animals:

Species:	Rat
Strain:	Wistar, Chbb:THOM (SPF)
Sex:	Male and female
Age:	42 days
Weight at dosing:	mean males: ca. 183 g; mean females: ca. 145 g
Source:	Dr. Karl Thomae GmbH, Biberach a. d. Riss, Germany
Acclimation period:	8 days
Diet:	Kliba maintenance diet rat-mouse-hamster 343 meal, Klingentalmühle AG, Kaiseraugst, Switzerland, ad libitum
Water:	Drinking water, ad libitum
Housing:	Group housing (1 animals/cage) housed in wire cages, type DK-III, Becker & Co. (Castrop-Rauxel, Germany)
Environmental conditions:	
Temperature:	20 - 24°C (central air-conditioning)
Humidity:	30 - 70% (central air-conditioning)
Air changes:	not reported
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: 03-Mar-1997 to 14-Apr-1998
(Inlife dates: 11-Mar-1997 (start of administration) to 14-Apr-1998

2. Animal assignment and treatment:
BAS 500 F was administered to groups of 50 male and 50 female Wistar rats at dietary concentrations of 0 and 400 ppm for 399 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights. At day 399 all animals were sacrificed without any further examination.

3. Test substance preparation and analysis:
The containers of test substance required for the feed mixtures in each case were cooled to approx. -20°C in the freezer so that they can be treated mechanically. Subsequently, the test substance was comminuted mechanically and stored at -20°C, unlike the usual storage conditions, to prevent it from becoming conglutinated again. For preparation of the feed mixes an acetone solution was prepared with the weighed test substance in the specific concentration. This solution was sprayed onto approx. 3.0 kg feed in a rotary evaporator (Büchi, Rotapor R 153) under partial vacuum. Subsequently, the solvent was removed under suction while heating to approx. 40°C for at least 30 minutes. Then, the premix was adjusted to the concentration desired with the appropriate amount of feed and mixed in a laboratory mixer of GEBR. LÖDIGE for approx. 10 minutes. The preparation frequencies were selected to ensure the stability of the test substance concentrations in the diet.

The stability of the test substance in the diet over 43 days at room temperature was verified analytically in the study 08B0376/956006 (analytical report (Amendment No. 1) of Jul. 5, 1996). Analyses to detect the homogeneous test substance distribution in the diet were carried out in samples of the parallel carcinogenicity studies (8250494/96086 (see BASF DocID 1999/11672)) and 8250494/96096 (see BASF DocID 2002/1004124)). For the concentration control analysis of the test substance preparations, samples were sent to the analytical laboratory at the beginning of the study, then at approx. 3-month intervals and approx. 4 weeks before the end of the study.

4. Statistics:
Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
mortality	Fisher's exact test (two-sided)
food consumption, body weight, body weight change, food efficiency	Student's t-test (two-sided) for the hypothesis of equal means

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality once daily.
A general cage side observation of the animals with regard to their outer appearance and behavior was carried out at least once daily. In addition to the general daily observation, each animal was subjected to an exact clinical check (including palpation).

2. Body weight:

The body weight of the animals was determined once weekly up to the 13th week of administration, then at 4-week intervals.

3. Food consumption, food efficiency and compound intake:

Food consumption was determined weekly during the first 13 weeks of administration, thereafter at 4-week intervals.
Even though not mentioned in report it is assumed that the food efficiency and substance intake was calculated as in the main study (see BASF DocID 1999/11868).
Food efficiency was calculated based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

with BW_x and BW_y body weight (g) on day x and day y (last weighing date before day x), $FC_{y\ to\ x}$ as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days from day y to day x.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the concentration in food on day x (mg/kg = ppm) and BW_x as body weight on day x of the study (g).

4. Water consumption:

No water consumption values were recorded.

5. Ophthalmoscopy:

No ophthalmoscopy was performed in this study.

6. Hematology and clinical chemistry:

In this study, no blood smears were prepared.

7. Urinalysis:

No urinalysis was performed in this study.

8. Sacrifice and pathology:

No pathology was done since the study was terminated on day 399.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No treatment related clinical signs of toxicity were observed in treated rats (see Table 5.5-21). All clinical observations were either observed in single animals only and displayed no dose-response relationship.

Table 5.5-21: Clinical observations in rats administered pyraclostrobin for 399 days – First supplemental carcinogenicity study

Sex	Male		Female	
Dose [ppm]	0	400	0	400
Animals in group	50	50	50	50
Sacrificed moribund	0	1	2	1
Skin, lesion	1	1	0	1
Skin, palpable mass	2	0	2	1
piloerection	0	1	1	0
Anogenital region, smeared with urine	0	1	0	1
Anogenital region, smeared	-	-	1	0
chromodacryorrhea	-	-	1	0
Conjunctivitis	1	0	1	0
Cornea opacity	-	-	0	1
Eye, discharge	-	-	1	1
Eyelid, crusts, red	0	1	-	-
Eyelid, closure	-	-	1	0
Eyelid, swelling	-	-	1	0
cataract	1	0	-	-
Reduced general state	0	1	1	0
alopecia	-	-	2	1
Abdomen, extended	-	-	0	1
Abdomen, palpable mass	-	-	1	0
icterus	-	-	0	1
paleness	-	-	1	0
hypothermia	-	-	1	0
Urine, discolored	-	-	1	1
Feces, discolored	-	-	1	0

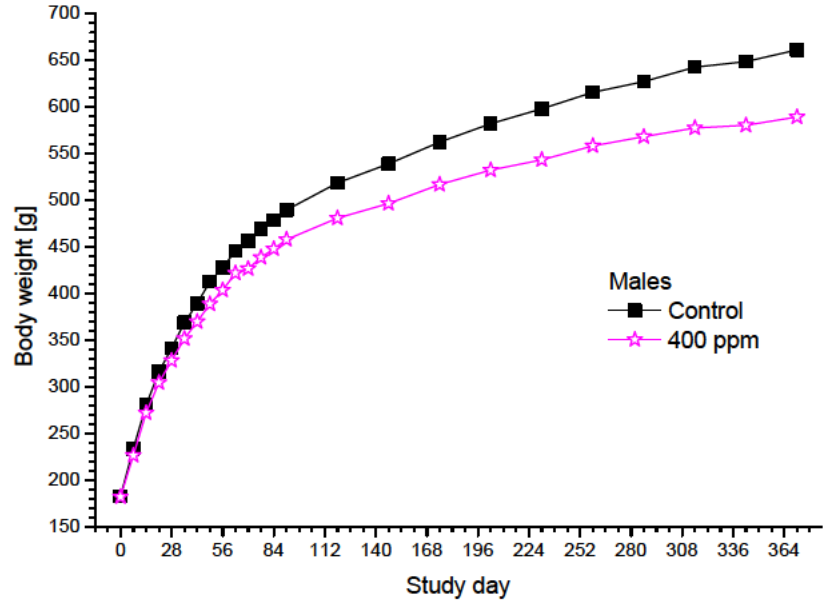
2. Mortality

The low incidence of mortality (one 400 ppm male and 2 and 1 females at 0 and 400 ppm, respectively) was not indicative of a treatment-related effect.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight development was impaired in dosed males as indicated by statistically significant lower body weights from study day 7 onwards. Likewise, body weight development of females was impaired, however body weights were significantly lower from study day 70 onwards only (see Table 5.5-22 and Figure 5.5-7). Cumulative body weight gain was significantly lower throughout the major part of the study for males and females (see Table 5.5-22).

Figure 5.5-7: Body weight development of rats administered pyraclostrobin for 399 days – First supplemental carcinogenicitiy study



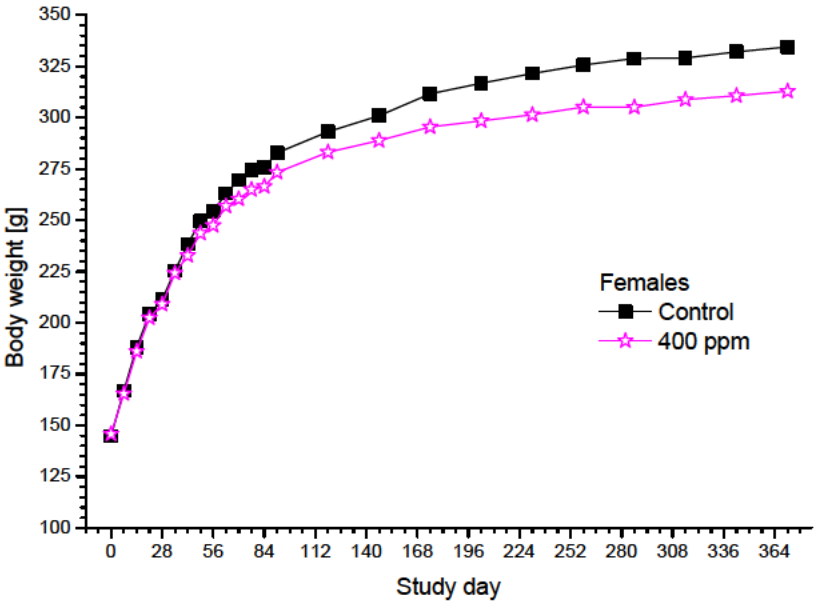


Table 5.5-22: Mean body weight of rats administered pyraclostrobin for 399 days – First supplemental carcinogenicity study

	Males		Females	
Dose level [ppm]	0	400	0	400
Body weight [g]				
- Day 0	182.8 ± 10.2	182.2 ± 8.2	144.8 ± 8.2	145.8 ± 6.6
- Day 91	489.6 ± 34.0	**458.0 ± 25.7*** **	282.8 ± 20.7	**273.3 ± 18.0***
Δ% (compared to control)		**-6.5**		**-3.4**
- Day 231	598.1 ± 50.2	**543.4 ± 39.2*** **	321.4 ± 27.3	**301.3 ± 22.6*** **
Δ% (compared to control)		**-9.1**		**-6.3**
- Day 371[a]	661.2 ± 63.8	**589.3 ± 47.2*** **	334.4 ± 28.1	**312.8 ± 25.9*** **
Δ% (compared to control)		**-10.9**		**-6.5**
Overall body weight gain [g]				
- Day 91	306.9 ± 33.3	**275.8 ± 23.6*** **	138.3 ± 17.0	**127.5 ± 15.0****
Δ% (compared to control)		**-10.2**		**-7.8**
- Day 231	415.3 ± 49.2	**361.2 ± 38.7*** **	176.9 ± 23.4	**155.5 ± 19.7*** **
Δ% (compared to control)		**-13.0**		**-12.1**
- Day 371	478.4 ± 62.6	**407.1 ± 47.3*** **	190.3 ± 25.6	**167.1 ± 22.1*** **
Δ% (compared to control)		**-14.9**		**-12.2**

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (Student's t-test. two sided)
[a] last study day with data reported

C. FOOD & WATER CONSUMPTION AND COMPOUND INTAKE

Consistently lower food consumption was observed for males throughout and for females during major parts of the study. This was statistically significant for males at all and for females at 15/23 determinations (see Figure 5.5-8 and Table 5.5-23).

Figure 5.5-8: Mean daily food consumption in rats administered pyraclostobin for 399 days – First supplemental carcinogenicity study

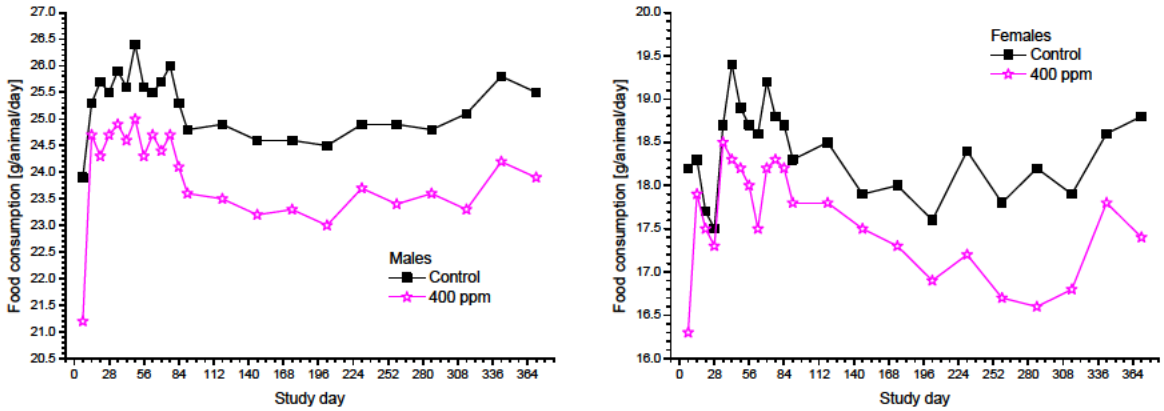


Table 5.5-23: Average food consumption of rats administered pyraclostrobin for 399 days – First supplemental carcinogenicity study

	Males		Females	
Dose level [ppm]	0	400	0	400
Food consumption [g/animal]				
Day 0 to 90#	25.5	24.2	18.5	17.8
Δ% (compared to control) #		-4.8		-3.7
Day 0 to 371#	25.1	23.7	18.3	17.4
Δ% (compared to control) a		-5.6		-4.9

Values were calculated based on mean individual daily food. Values may not calculate exactly due to rounding of mean values

a Time weighted

The time weighted mean daily test substance intake was determined to have been 19.7 and 25.0 mg/kg bw/day for males and females, respectively.

III. CONCLUSIONS

Dietary administration of pyraclostrobin to rats at dietary dose levels of 0 and 400 ppm for 399 days resulted in an impairment of body weight development in males and females. Treatment did not affect the survival of rats and no relevant clinical signs were observed. Based on the study design it was not intended to determine a NOAEL.

Report:	CA 5.5/8 [redacted] 2002f BAS 500 F - Second supplementary carcinogenicity study in female Wistar rats - Administration in the diet for 24 months 2002/1004124
Guidelines:	EEC 87/302 B, OECD 451, EPA 83-2, JMAFF
GLP:	yes (partially) (during the in-life phase up to the pre-term termination regular QAU audits were performed for this GLP study. However, the report was not QAU audited and thus the study has no full GLP status)

Executive Summary

This supplementary study was conducted in parallel to a carcinogenic feeding study in male and female Wistar rats (see BASF DocID 1999/11672). Pyraclostrobin (BAS 500 F) was administered to groups of 50 female Wistar rats at a dietary concentration of 600 ppm. Control animal data were taken from the parallel conducted main study (see BASF DocID 1999/11672). Since in the main study the maximum tolerated dose (MTD) was obtained at 200 ppm, the study was terminated after 426 study days without any further examinations.

No mortality or relevant clinical signs were observed during the study. Food consumption and body weight development was impaired throughout the study.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 F

Description:	viscous melt / reddish-brown, clear
Lot/Batch #:	J. No. 27882/191/c (Tox. III/part 1)
Purity:	97.09%
Stability of test compound:	Stable until May 1997. (The same batch of test material was used in the main study (see BASF DocID 1999/11672). This material was reanalyzed after end of the in-life phase of the latter study and found to be stable.)

2. Vehicle and/or positive control: Rodent diet

3. Test animals:

Species:	Rat
Strain:	Wistar, Chbb:THOM (SPF)
Sex:	Female
Age:	43 days ± 1 day
Weight at dosing:	mean females: ca. 164 g
Source:	Dr. Karl Thomae GmbH, Biberach a. d. Riss, Germany
Acclimation period:	8 days
Diet:	Kliba maintenance diet rat-mouse-hamster 343 meal, Klingentalmühle AG, Kaiseraugst, Switzerland, ad libitum
Water:	Drinking water, ad libitum
Housing:	Group housing (1 animals/cage) housed in wire cages, type DK-III, Becker & Co. (Castrop-Rauxel, Germany)
Environmental conditions:	
Temperature:	20 - 24°C (central air-conditioning)
Humidity:	30 - 70% (central air-conditioning)
Air changes:	not reported
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: 03-Feb-1997 to 14-Apr-1998
Inlife dates: 12-Feb-1997 (start of administration) to 14-Apr-1998 (necropsy of last female animals)

2. Animal assignment and treatment:
BAS 500 F was administered to a group 50 female Wistar rats at a dietary concentrations 600 ppm for 426 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights. At day 426 all animals were sacrificed without any further examination. The data of control animals were taken from the parallel study with the Doc ID 1999/11868.

3. Test substance preparation and analysis:
The containers of test substance required for the feed mixtures in each case were cooled to approx. -20°C in the freezer so that they can be treated mechanically. Subsequently, the test substance was comminuted mechanically and stored at -20°C, unlike the usual storage conditions, to prevent it from becoming conglutinated again. For preparation of the feed mixes an acetone solution was prepared with the weighed test substance in the specific concentration. This solution was sprayed onto approx. 3.0 kg feed in a rotary evaporator (Büchi, Rotapor R 153) under partial vacuum. Subsequently, the solvent was removed under suction while heating to approx. 40°C for at least 30 minutes. Then, the premix was adjusted to the concentration desired with the appropriate amount of feed and mixed in a laboratory mixer of GEBR. LÖDIGE for approx. 10 minutes. The preparation frequencies were selected to ensure the stability of the test substance concentrations in the diet.

The stability of the test substance in the diet over 43 days at room temperature was verified analytically in the study 08B0376/956006 (analytical report (Amendment No. 1) of Jul. 5, 1996). Analyses to detect the homogeneous test substance distribution in the diet were carried out in samples of the parallel carcinogenicity studies (see BASF DocID 1999/11672 and BASF DocID 2002/1004124). For the concentration control analysis of the test substance preparations, samples were sent to the analytical laboratory at the beginning of the study, then at approx. 3-month intervals and approx. 4 weeks before the end of the study.

4. Statistics:
The data of control animals were taken from the main study (see BASF DocID 1999/11672). Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	Student's t-test (two-sided) for the hypothesis of equal means

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality once daily.
A general cage side observation of the animals with regard to their outer appearance and behavior was carried out at least once daily. In addition to the general daily observation, each animal was subjected to an exact clinical check (including palpation).

2. Body weight:

The body weight of the animals was determined once weekly up to the 13th week of administration, then at 4-week intervals.

3. Food consumption, food efficiency and compound intake:

Food consumption was determined weekly during the first 13 weeks of administration, thereafter at 4-week intervals.
Even though not mentioned in report it is assumed that the food efficiency and substance intake was calculated as in the main study (see BASF DocID 1999/11672).
Food efficiency was calculated based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \times 100$$

with BW_x and BW_y body weight (g) on day x and day y (last weighing date before day x), $FC_{y\ to\ x}$ as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days from day y to day x.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the concentration in food on day x (mg/kg = ppm) and BW_x as body weight on day x of the study (g).

4. Water consumption:

No water consumption values were recorded.

5. Ophthalmoscopy:

No ophthalmoscopy was performed in this study.

6. Hematology and clinical chemistry:

In this study, no blood smears were prepared.

7. Urinalysis:

No urinalysis was performed in this study.

8. Sacrifice and pathology:

No pathology was done since the study was terminated on day 426.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No treatment related clinical signs of toxicity were observed in treated rats (see Table 5.5-24). All clinical observations were observed in single animals only.

Table 5.5-24: Clinical observations in rats administered pyraclostrobin for 426 days – Second supplemental carcinogenicity study

Sex	Female
Dose [ppm]	600 pmm
Animals in group	50
Oblique head posture	1
Skin, palpable mass	2
Forelimb(s), injury	1
Loss	1
Mamma, induration	2
Piloerection	2
Mydriasis	1
Cataract	1
Eyelid, crusts, red	1
Reduced general state	1

2. Mortality

No mortality was observed during the study period.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight development was impaired in dosed females as indicated by statistically significant lower body weights from study day 35 onwards. (see Figure 5.5-9 and Table 5.5-25).
Cumulative body weight gain was significantly lower throughout the major part of the study for females.

Figure 5.5-9: Body weight development of rats administered pyraclostrobin for 426 days – Second supplemental carcinogenicity study

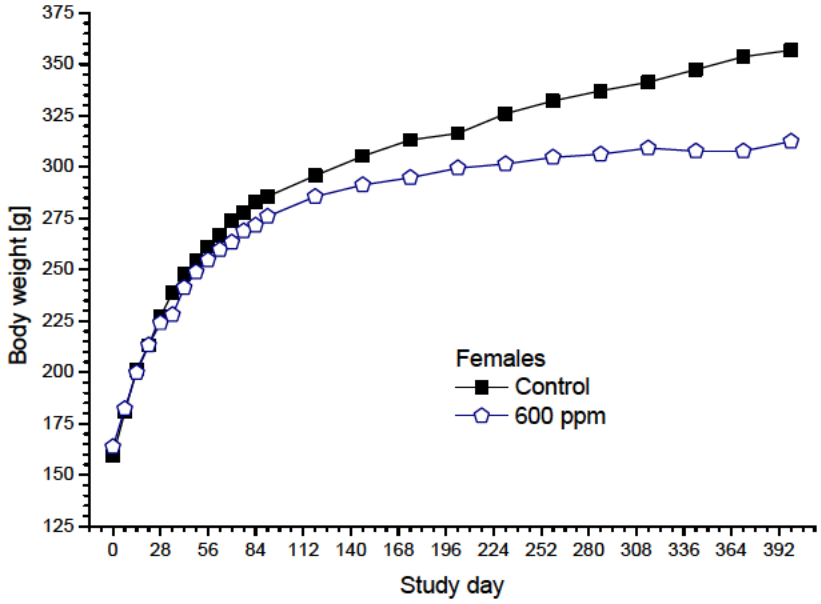


Table 5.5-25: Mean body weight of rats administered pyraclostrobin for 426 days – Second supplemental carcinogenicity study

	Females	
Dose level [ppm]	0	600
Body weight [g]		
- Day 0	159.2 ± 6.6	163.7 ± 4.8**
- Day 91	285.7 ± 21.4	**275.9 ± 14.3****
Δ% (compared to control)		**-3.4**
- Day 231	326.0 ± 25.2	**301.5 ± 16.1****
Δ% (compared to control)		**-7.5**
- Day 399	356.9 ± 33.4	**321.5 ± 21.2****
Δ% (compared to control)		**-12.4**
Overall body weight gain [g]		
- Day 91	126.5 ± 19.4	**112.3 ± 13.2****
Δ% (compared to control)		**-11.2**
- Day 231	166.8 ± 23.4	**135.8 ± 16.5****
Δ% (compared to control)		**-17.4**
- Day 399	197.7 ± 31.9	**148.8 ± 20.4****
Δ% (compared to control)		**-24.7**

* $p \leq 0.05$; ** $p \leq 0.01$ (Student's t-test. two sided)

C. FOOD & WATER CONSUMPTION AND COMPOUND INTAKE

Food consumption of treated females was statistically lower almost throughout the entire study (see Figure 5.5-10 and Table 5.5-26).

Figure 5.5-10: Food consumption of female rats administered pyraclostrobin for 426 days – Second supplemental carcinogenicity study

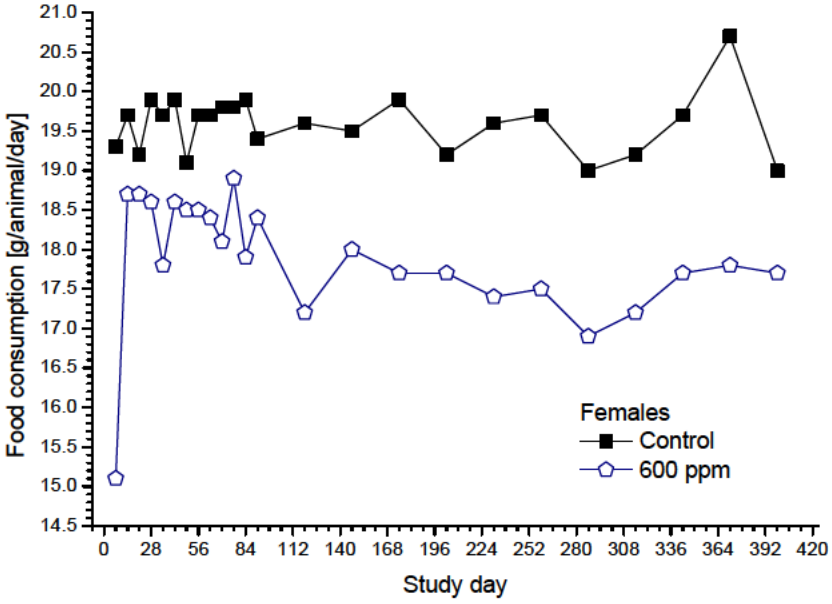


Table 5.5-26: Mean food consumption of rats administered pyraclostrobin for 426 days

	Females	
Dose level [ppm]	0	600
Food consumption [g/animal]		
Day 0 to 90[#]	19.6	18.2
		-7.4
Day 0 to 399	19.6	17.7
Δ% (compared to control)[#]		-9.7

[#] Values were calculated based on mean individual daily food. Values may not calculate exactly due to rounding of mean values

The time weighted mean daily test substance intake was calculated to have been 37.4 mg/kg bw/day in females at the dietary dose level of 600 ppm.

III. CONCLUSIONS

Dietary administration of pyraclostrobin to female rats at dietary dose levels of 600 ppm for 426 days resulted in lower food consumption and an impairment of body weight development Treatment neither affected survival nor elicited relevant clinical signs. Based on the design of the study it was not intended to determine a NOAEL.

For convenience of the reviewer a brief summary of the mouse carcinogenicity study as extracted from the monograph is provided below.

Pyraclostrobin, mouse – carcinogenicity study (original dossier BASF DocID 1999/11871)

Pyraclostrobin was administered to groups of 50 male and 50 female B6C3F1 mice at dietary concentrations of 0, 10, 30 and 120 ppm and additionally 180 ppm (females only) for 18 months.
There was no test substance related increase in mortality or clinical signs of toxicity in this study.
After 90 days, body weight and body weight change were significantly reduced at 120 ppm in males only. After one year, this effect was observed at 120 ppm in males and 180 ppm in females.
In the second year, body weight and body weight change were significantly reduced at all dose levels in males on days 455-546 and at 10 ppm, 120 ppm and 180 ppm in females on days 539 and 546. At the high dose levels, body weight was 13% lower than controls in both sexes and body weight development was reduced by 27 and 29% in males and females, respectively. However, the reduced body weights at the end of the treatment period were without any dose-response relationship. Occasionally, food consumption was decreased in the treated groups, in particular in females, without showing any dose-response relationship.
Blood smears did not indicate any substance-related effect. Histopathological investigations did not reveal any test substance related adverse effect. There was no evidence of a carcinogenic response.

Conclusion:
The no observed adverse effect level (NOAEL) in this 18-month carcinogenicity study in mice was 30 ppm (4.1 mg/kg bw/d for males), based on reduction of body weights in males at 120 ppm during the first year of the study.
During the last 6 months of the study, food consumption, body weight and body weight change were significantly reduced at all dose levels. However, these effects at the end of the treatment period were without any dose-response relationship and not regarded as treatment-related. There was no indication of a carcinogenic potential of pyraclostrobin in mice.

In the following the additional carcinogenicity study in mice requested by US-EPA is summarized. This study was previously not submitted.

Report:	CA 5.5/9 [redacted] 2005a BAS 500 F: Terminated carcinogenicity study in female B6C3F1 mice; administration in the diet for 7 months 2005/1026477
Guidelines:	EPA 870.4200, EEC 87/302 B, OECD 451, MAFF Testing Guideline for Toxicology Studies (1985)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

Pyraclostrobin (BAS 500 F) was administered to groups of 50 female B6C3F1 mice at dietary concentration of 360 ppm for a period of 7 months. The study was conducted upon request of US-EPA as the authority considered that the maximum tolerated dose (MTD) was not met in females in the main study. After about 7 months a marked impairment of body weight development was noted at 360 ppm as indicated by a decrease of mean absolute body weights by 31.5% and of cumulative body weight gain by 40.2% when compared to the control. Secondary to the 30% lower (exsanguinated) terminal body weight, a number of absolute organ weights were decreased whereas relative organ weights were increased. The only treatment-related gross necropsy finding was a slight increase in the incidence of glandular stomach erosion/ulceration.
The marked impairment of body weight development indicated that the MTD was exceeded in females administered pyraclostrobin at a dietary concentration of 360 ppm. Thus – after consultation with US EPA - the study was terminated at seven months without histopathological evaluation of the preserved organs.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 F

Description:	Solid / crystalline / yellowish
Lot/Batch #:	LJ 27882/199/b (ToxCharge III/Teil 2)
Purity:	98.7%
Stability of test compound:	Stable until March 28, 2008.

2. Vehicle and/or positive control: Rodent diet

3. Test animals:

Species:	Mouse
Strain:	B6C3F1/Crl
Sex:	Female
Age:	41 ± 1 days
Weight at dosing (Day 0):	mean: 19.5 g
Source:	Charles River, Sulzfeld, Germany
Acclimation period:	6 days
Diet:	Kliba maintenance diet for mouse/rats “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water in bottles, ad libitum
Housing:	Single housing in polycarbonate cages, type M I with wire cover from Becker & Co., Castrop-Rauxel, Germany.
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	not reported
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 07-Sep-2004 to 01-Aug-2005
In life dates: 13-Sep-2004 (start of administration) to 14-Apr-2005 (necropsy)

2. Animal assignment and treatment:

BAS 500 F was administered to groups of 50 female mice at dietary concentrations of 0 and 360 ppm for 7 months. The animals were assigned to the treatment groups by means of computer generated randomization lists based on body weights.

3. Test substance preparation and analysis:

Appropriate amounts of the test item were dissolved in acetone and subsequently srayed on about 3 kg powdered diet in a rotation vaporizer (Büchi, Rotavapor R 153) under partial vacuum. Acetone was removed by heating up to about 40°C for about 1 hour. Thereafter this premix was adjusted to the desired concentration with appropriate amounts of powdered diet and mixed for about 10 minutes in a laboratory mixer. Details of the mixers used are retained with the raw data. The test substance preparation usually was mixed every 5 weeks.
The stability of the test substance in the diet over a period of 43 days at room temperature was proven prior to the conduct of the study.
Homogeneity and concentration analyses of the diet preparations were performed at the beginning of administration, after 3 and 4 months. For this three samples were randomly taken and analyzed for test-item content.
No test-article was determined in control diets. Relative standard deviations of the homogeneity samples in the range of 0.6 to 2.9% indicate the homogenous distribution of pyraclostrobin in the diet preparations. The individual sample test-substance concentrations were in the range of 91.1 to 103.3% of the nominal concentrations with an overall mean of 99.7% ± 4.9 of the nominal concentration. The group average dietary concentrations and % of nominal values are given in the following table.

Analysis of diet preparations for homogeneity and test-item content

Dose level [mg/kg]	Sampling	Analysis	Concentration[#] [mg/kg] Mean ± SD	Mean % of nominal concentration
360	09.09.04	10.09.04	369.7 ± 2.1	102.7 ± 0.6
	24.12.04	13.01.05	338.7 ± 9.7	94.1 ± 2.7
	28.02.05	11.03.05	368.3 ± 2.3	102.3 ± 0.6
average			**358.9 ± 17.5**	**99.7 ± 4.9**

[#] based on mean values of the three individual samples (homogeneity samples)
Values may not calculate exactly due to rounding of figures.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	A comparison of dose group with the control group was performed using the Welch t-test (two sided) for the hypothesis of equal means.

Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality as well as for signs of toxicity twice daily on working days and once daily on weekends and public holidays.
Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this the animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable. The following parameters were examined:

1. abnormal behavior during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. feces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), once weekly for the first 13 weeks, thereafter at 4-week intervals and at the end of the study.

3. Food consumption, food efficiency and compound intake:

Individual food consumption was determined once a week during the first 13 weeks, at 4-week intervals thereafter and prior to the start of necropsy. Food consumption was calculated as mean food consumption in grams per animal and day.

Food efficiency (group means) was calculated based upon individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \text{ x } 100$$

with BW_x and BW_y body weight [g] at day x and day y (last weighing date before day x), $FC_{y\ to\ x}$ as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days from day y to day x.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BW_x as body weight on day x of the study (in g).

4. Water consumption:

Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume.

5. Ophthalmoscopy:

Not performed in this study.

6. Hematology and clinical chemistry:

Due to the termination of the study after 7 months of the test substance administration, no hematology analyses were carried out.

7. Urinalysis:

Not performed in this study.

8. Sacrifice and pathology:

Surviving animals were sacrificed by decapitation under CO_2 anesthesia. The exsanguinated animals were necropsied and assessed by gross pathologically.

The following organs were sampled, weighed and preserved in neutral buffered 4% formaldehyde solution:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H).

C	W	H		C	W	H		C	W	H	
✓	✓	✓	adrenals	✓	✓	✓	liver	✓		✓	stomach (fore- & glandular)
✓		✓	aorta	✓		✓	lung	✓		✓	thymus
✓	✓	✓	brain	✓		✓	lymph nodes[#]	✓		✓	thyroid/parathyroid
✓		✓	bone marrow[§]	✓		✓	mammary gland (♀)	✓		✓	trachea
✓		✓	caecum	✓		✓	muscle, skeletal	✓		✓	urinary bladder
✓		✓	colon	✓		✓	nerve, peripheral (sciatic n.)	✓	✓	✓	uterus
✓		✓	duodenum	✓		✓	nose	✓		✓	vagina
✓		✓	esophagus	✓	✓	✓	ovaries and oviduct**				
✓		✓	eyes (with optic nerve)	✓		✓	pancreas		✓		body (anesthetized animals)
✓		✓	femur (with joint)	✓		✓	pituitary				
✓		✓	gall bladder	✓		✓	pharynx				
✓		✓	gross lesions	✓		✓	rectum				
✓		✓	Harderian gland	✓		✓	salivary glands*				
✓	✓	✓	heart	✓		✓	skin				
✓	✓	✓	kidneys	✓		✓	spinal cord (3 levels)[@]				
✓			lachrymal glands[%]	✓	✓	✓	spleen				
✓		✓	larynx	✓		✓	sternum w. marrow				

[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; [%] extraorbital

No histopathological examinations were performed.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

One female from the dose group showed loss of tail end. This single occurrence was assessed as being incidental and not related to the test substance treatment.

2. Mortality

No animal died prematurely in this study.

3. Ophthalmoscopy

Not performed in this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight in animals treated with 360 ppm of BAS 500 F was statistically significantly decreased from the beginning of the treatment (study day 7, -4%) to the termination of the study (study day 210, -21%). The maximum impairment of body weight was up to 24% below control on study day 147. Body weight change reflected the effects on body weight in the dosed group even more clearly. Body weight change in animals treated with 360 ppm of BAS 500 F was statistically significantly decreased from the beginning of the treatment (study day 7, -177%) to the termination of the study (study day 210, -40%). The maximum impairment of body weight gain, after at least three months of test substance treatment, was up to 52% below control on study day 147. The above-mentioned findings were considered related to the test substance treatment (see Figure 5.5-11 and Table 5.5-27).

Figure 5.5-11: Body weight development of female B6C3F1 mice administered pyraclostrobin for about 7 month

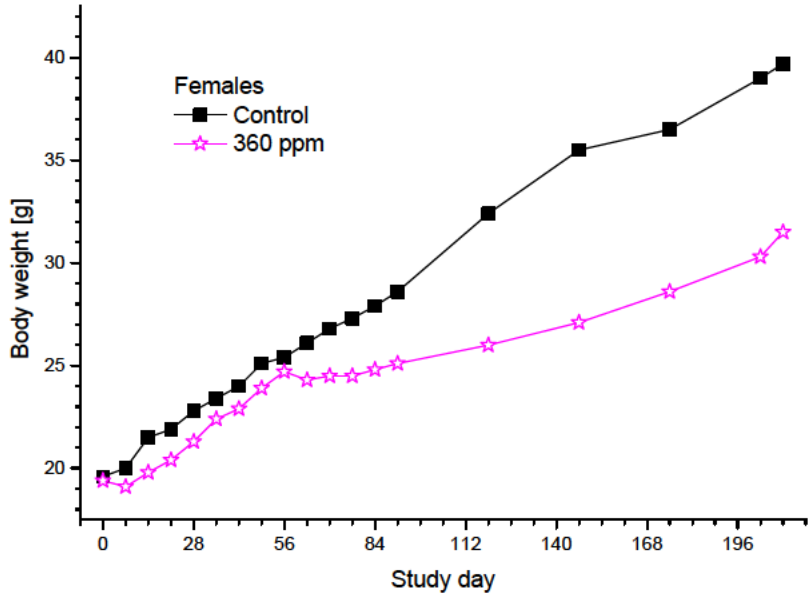


Table 5.5-27: Mean body weights of female mice administered pyraclostrobin about 7 months

Dose level [ppm]	Females 0	Females 360
Body weight [g]		
- Day 0	19.6 ± 0.9	19.4 ± 1.0
- Day 91	28.6 ± 2.4	**25.1 ± 1.3****
- Day 175	36.5 ± 3.9	**28.6 ± 2.1****
- Day 210	39.7 ± 4.0	**31.5 ± 2.4****
Cumulative body weight gain [g]		
- Day 91	9.0 ± 2.5	**5.7 ± 1.3****
Δ% (compared to control)[#]		**-36.6**
- Day 175	16.9 ± 4.0	**9.2 ± 1.9****
Δ% (compared to control)[#]		**-45.7**
- Day 210	20.1 ± 4.0	**12.0 ± 2.3****
Δ% (compared to control)[#]		**-40.2**

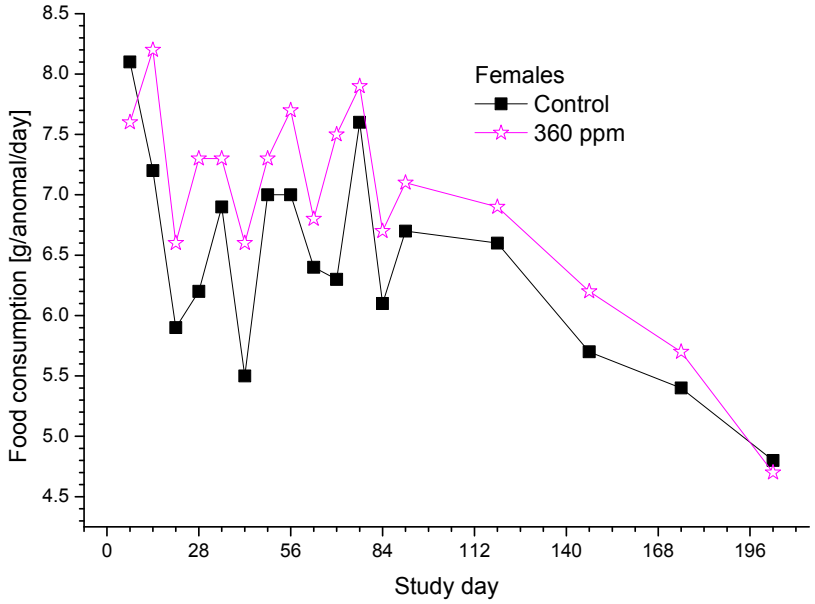
* $p \leq 0.05$; ** $p \leq 0.01$ (Welch t-test,. two sided)
[#] Values may not calculate exactly due to rounding of mean figures

C. FOOD & WATER CONSUMPTION AND COMPOUND INTAKE

1. Food consumption:

During the early phase of the study, statistically significant increases of food consumption (days 14, 21, 28, 42, 56, 70 and 84) were observed (Figure 5.5-12). In view of the significant impairment of body weight development observed in this study period and taking into account the usual food spilling by mice this was assessed as being not biologically relevant.

Figure 5.5-12: Mean daily food consumption of female B6C3F1 mice administered pyraclostrobin for about 7 month



Remark: Food spillage was sporadically observed in all group including controls. The values therefore may not give exact food consumption.

The mean daily test substance intake in 360 ppm females was calculated to be 106.5 mg/kg bw/day. The time weighted test substance intake was 94 mg/kg bw/day.

2. Water consumption:
There were no overt deviations in volume between treated group and control animals.

D. BLOOD ANALYSIS

Due to the termination of the study after 7 months of the test substance administration, no further analyses were carried out.

E. NECROPSY

1. Organ weight

The mean terminal body weights and the absolute weights of the adrenal glands, kidneys and liver were significantly decreased (see Table 5.5-28). Secondary to the decreased terminal body weight relative weights of the adrenal glands, brain, heart, kidneys, liver, ovaries, spleen and uterus were significantly increased (see Table 5.5-28). These significantly increased organ weights are related to the decreased terminal body weights and do not express a direct effect on the single organs.

Table 5.5-28: Selected mean absolute and relative organ weights of mice administered pyraclostrobin for about 7 months

Sex		Females			
Organ weight [mg]	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ%
Terminal weight [g]	0	34.8 ± 4.0		100.0	
	360	**24.6 ± 2.0****	**(-29.3)**	100.0	
Brain [mg]	0	498.5 ± 20.1		1.45 ± 0.20	
	360	500.3 ± 14.7	(0.4)	**2.04 ± 0.17****	**(40.7)**
Heart [mg]	0	140.9 ± 12.9		0.41 ± 0.07	
	360	140.7 ± 15.8	(-0.1)	**0.57 ± 0.07****	**(40.0)**
Adrenal glands [mg]	0	10.1 ± 1.4		0.03 ± 0.01	
	360	**8.8 ± 1.2****	**(-12.7)**	**0.04 ± 0.01****	**(24.1)**
Kidneys [mg]	0	375.5 ± 20.4		1.09 ± 0.13	
	360	**358.2 ± 25.0****	**(-4.6)**	**1.46 ± 0.12****	**(33.9)**
Liver [mg]	0	1189.5 ± 67.4		3.45 ± 0.38	
	360	**1143.5 ± 102.6****	**(-3.9)**	**4.66 ± 0.37****	**(34.8)**
Ovaries [mg]	0	21.2 ± 2.8		0.06 ± 0.01	
	360	22.2 ± 2.4	(4.7)	**0.09 ± 0.01****	**(45.2)**
Spleen [mg]	0	87.0 ± 11.4		0.25 ± 0.05	
	360	81.2 ± 16.5	(-6.7)	**0.37 ± 0.06****	**(46.1)**
Uterus [mg]	0	211.7 ± 56.4		0.62 ± 0.21	
	360	194.6 ± 58.4	(-8.1)	**0.79 ± 0.23****	**(27.4)**

values may not calculate exactly due to rounding of figures
* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)

2. Gross pathology

Four treated females displayed "erosion/ulcer" of the glandular stomach. This was considered to be treatment-related. All other gross necropsy findings occurred in single cases only and were regarded to be of incidental nature (see Table 5.5-29).

Table 5.5-29: Incidence of selected gross pathological findings in mice administered pyraclostrobin for about 7 months

Sex		Female	
Dose [ppm]		0	360
Animals in group		50	50
Glandular stomach	# exam.	50	50
- Erosion/ulcer		-	4
Ovaries	# exam.	50	50
- Cyst		-	1
Pancreas	# exam.	50	50
- Cyst		1	-
Uterus	# exam.	50	50
- Focus		1	-

3. Histopathology

No histopathological examinations were performed in this study.

III. CONCLUSIONS

Dietary administration of pyraclostrobin to mice at a dose level of 360 ppm for 7 months resulted in a significant reduction in body weight and body weight gain. Due to the severity of the body weight effects the study was terminated in agreement with US EPA after about 7 months of treatment.

The following statistical evaluation was done to address questions of US-EPA and Canada PMRA regarding the adequate dosing in the rat and mouse long-term studies.

Report: CA 5.5/10
[redacted]., 2002a
Chronic and oncogenicity studies with BAS 500 F: Further evaluations of body weight, food consumption and food efficiency
2002/5002875
Guidelines: none
GLP: no

Executive Summary

The oncongenic potential of pyraclostrobin (BAS 500 F) was investigated in oncogenicity studies in rats and mice and a chronic toxicity study in rats. The original reports concluded significant decreases in body weight and body weight gain that were considered to have met EPA maximum tolerated dose (MTD) requirements. However, the Joint Review of the data by US EPA and the Canada PMRA concluded that the toxicity observed in the highest dose tested (particularly in females of both species) did not meet MTD requirements. Therefore, this report contains further analyses of body weight and food consumption data from the rodent carcinogenicity and chronic toxicity studies with pyraclostrobin that address the concerns noted in the Joint Review meeting.
The decreased body weights and body weight gains observed at the high doses in both sexes of both species were treatment-related toxic effects that were not due to decreases in food consumption, as it would occur if palatability of the test diets were an issue. Food consumption was only slightly decreased in rats and not affected at all in mice. Food efficiency, however, was decreased along with body weight gain, therefore a toxic effect other than decreased food consumption was the reason for the observed body weight effect. These findings provide clear evidence of treatment-related toxicity. The decrements in body weight and body weight gain reached levels of 10% or greater in both sexes of both species. The effect on body weight and body weight gain became more severe over time indicating a cumulative toxicity. Collectively, these data, specifically the body weight gain decrements of 10 % or more, indicate that the MTD was reached in male and female rats (200 ppm) and in male (120 ppm) and in female mice (180 ppm) that received pyraclostrobin in the diet for a chronic exposure.

I. MATERIAL AND METHODS

A. MATERIALS

This report contains the results of statistical analyses conducted on the rat and mice animal data from the chronic and carcinogenicity studies on BAS 500 F (BASF DocIDs 1999/11672, 1999/11868 and 1999/11871). This analysis also considered the supplemental chronic toxicity and carcinogenicity studies submitted in this supplemental dossier (see BASF DocIDs 2002/1004125, 2002/1004126, 2002/1004123 and 2002/1004124).

B. STUDY DESIGN AND METHODS

1. Dates of work: 04/22/2002 (completion date)

2. Method

Because the chronic toxicity study in rats and the carcinogenicity study in rats were conducted at the same time using similar protocols, the data for these studies were combined for this analysis. For this analysis the data were grouped into intervals. These intervals were used to capture potential treatment-related effects that might occur over time or at certain points during the course of the study. To capture any treatment-related effects and to lessen the effect of minor variations over a weekly period, within study intervals were calculated. For the within study intervals during the growth phase, data was pooled using two week or monthly intervals. In the maintenance phase where the data is only reported monthly, data was pooled using three month intervals. In addition, to see the overall treatment-related effects, data were pooled into across study intervals which started at the beginning of the study. These intervals would tend to smooth out fluctuations at any time period during the interval and show the overall effect from the beginning of the study up to the end of the interval. For these across study intervals, data was pooled over the first two weeks, the first month, and the first 91 days. After the growth period, the next three study intervals increased in length by approximately a month through the first 175 days and the remainder of study intervals increased in length by approximately 90 days each through the end of study.

Therefore, for this analysis, two different types of intervals were used. The across study intervals all start with the first day of the study and include all the information through the end of the interval (e.g., day 0-14, day 0-28, day 0-91, day 0-119, day 0-147, day 0-175, day 0-259, day 0-371, day 0-455, day 0-539 and day 0-728 for rats and day 0-546 for mice). The across study intervals show the overall treatment effect through the last day of the study interval.

Within study intervals are used and each interval starts were the preceding one stops (e.g., day 0-14, day 15-28, day 29-63, day 64-91, day 92-175, day 176-259, day 260-343, day 344-427, day 428-511, and day 512-595, day 596-679 and day 679-728 for rats with day 512-546 for mice). For the within study intervals, the period of time that has the most overall effect on the across study intervals can be seen.

In both the across study intervals and the within study intervals, the five values reported in the tables were calculated on individual animal data. Means, standard deviations and sample sizes for these statistics were calculated and statistical analysis of the data was done.
Since food consumption may be affected by body weight and body weight is affected by food consumption, comparing just food consumption or just body weight across treatments can be misleading. Therefore, two statistics are computed that evaluate the body weight gain relative to the food consumed and the food consumption relative to the overall body weight.
The individual interval values calculated for each animal were then used in an analysis of variance test (ANOVA) to see if the variation between treatment groups was significantly different from the variation within treatment groups. If there was a significant difference in the ANOVA at the $\alpha = 0.05$ level, a two-tailed Dunnett's test was performed to compare the means, standard deviations and sample sizes (N) for each group in each interval. The % value reported is the percent deviation of the treated group mean from the control group mean.
For the rat studies, the ANOVA and pair wise comparison were done in two stages. First, the original control, 25 ppm, 75 ppm, 200 ppm and, for the females only, the 600 ppm groups were analyzed. Then, the concurrent control for the 400 ppm group and the 400 ppm group were analyzed separately.

II. RESULTS AND DISCUSSION

The dose-response patterns observed for the parameters measured in mice and rats administered pyraclostrobin are consistent not only across sexes within a species but across species as well. These findings can be summarized as follows:

Body weight and body weight gain decrements
In female rats, body weight gain in 200 ppm dose group was decreased compared to the control and persisted throughout the study. With continued dosing, significant decreases in body weight gain were noted in the 200 ppm group beginning with a 6% decrease in the 0 to 147 day interval and progressing to 10% after 259 days, 13% after 371 days, 15% after 539 days and 19% by the end of the study at 728 day (see Table 5.5-30). In the 25 and 75 ppm groups, body weight gain was equal to or slightly greater than that in the controls up to the 0 to 175 day interval. Similar trends but with less severity than that with the 200 ppm group were noted for female rats in the 25 and 75 ppm dose groups at later time points. The final body weight gain decrements compared to control were 6, 8, and 19% in the 25, 75, and 200 ppm dose groups, respectively.
A similar pattern was noted in the 400 and 600 ppm female rats dosed for one year. Body weight gain deficit was significant at all time-points but remained about 10% up to the 0 to 175 time-period and then increased to 15 and 25% by the end of day 371. Across all dose groups at day 371, the deficits were 2, 1, 13, 15 and 25% for the 25, 75, 200, 400, and 600 ppm dose groups, respectively.

A pattern similar to the pattern in female rats was observed in male rats. The body weight gain deficits in the 200 ppm male rats progressed reaching 8% at 91 days and 10% after 539 days (see Table 5.5-30). By 728 days, body weight gain in the 200 ppm male rats was 5% less than the controls. However, the control male rats lost weight at the end of the study. Consequently, the magnitude of the difference in body weight gain between the control male rats and the 200 ppm male rats was diminished.
A pattern of progressive decreases in body weight gain was observed in the 400 ppm male rats as well. Body weight gain deficits in the 400 ppm male rats was 9% below the controls in the early portion of the study and was 16% lower than the controls by day 371.
A similar pattern was noted in both male and female mice (see Table 5.5-31). Initially in all dose groups and both sexes, body weight gain in the first two weeks was reduced relative to controls. In female mice, the decrease was 12, 10, 10, and 44% in the 10, 30, 120, 180 ppm groups, while in males the decrease was 3, 16 and 21%, in the 10, 30, and 120 ppm dose groups. Following the initial 14 days, the body weight gain in the lower dose groups was less affected for most of the study, fluctuating between 1 and 10%. However, the effect in high dose animals was greater fluctuating between 15 and 20% until the 0 to 455 interval. After day 455, body weight gains began to be decreased in the lower dosed animals. By the end of the study the body weight gain decrease relative to control was 17, 12, 20 and 26% in the female dose groups and 23, 18 and 28% in the male dose groups. However, the severity of the effect in the lower dose groups is mostly due to an initial decrease during the first 14 days and a decrease compared to controls much later in the study. In contrast the effect on body weight gain on high dose males and females was more severe and more consistent over the course of the study. The only time interval when the body weight gain was less than 15% in the females in the high dose group was in the 0 to 91 day interval. The intervals surrounding that time point (days 0 to 28 and 0 to 119) had decreases of 20 and 15%, respectively, suggesting that the 10% decrease in that time interval may represent variability rather than a true value given the small differences. Body weight gain in high dose males was consistently decreased over the course of the study by at least 15% reaching 28% at the end.
These data strongly suggest that the toxic effects of BAS 500 F are cumulative over time. The animals later in life are less able to handle the toxicity of the chemical. As the animal matures these effects become manifested more strongly than when the animal is younger.

Decreased body weights and body weight gains were not due to decreases in food consumption

There were no consistent and sustained significant decreases in food consumption in either male or female rats dosed up to 200 ppm (see Table 5.5-32). Further, there were no indications of a dose-response either across time or dose-groups found in the food consumption values. In female rats in the 200 ppm group, food consumption was within 2 to 3% of the control group throughout the study. By the end of the study, food consumption was reduced by only 3%, but body weight gain was reduced 19%. In male rats, food consumption was initially significantly reduced below controls values but was only approximately 3 to 4% less than controls up to day 175. After that these differences were no longer significantly different from controls and the differences at the end of the study were 1% less than control.
There were no consistent and sustained significant decreases in food consumption in male and female mice dosed up to 120 and 180 ppm, respectively. Food consumption in the high dose female mouse was consistently higher than the control until day 539 and only 3% lower than control at the end of the study (see Table 5.5-33). In male mice, food consumption was also initially higher than in controls, and remained between 1 to 2% less than controls throughout the study rising only to about 5% less at the end of the study. Food spillage was noted occasionally in the study, but this occurred only in a few animals, did not have a dose-related pattern and when noted, animals with significant food spillage were not included in food consumption calculations for that interval. So, food spillage does not affect the data on food consumption. While there were slight decreases in food consumption, these decreases were not large enough to explain or be responsible for the magnitude of the body weight differences.

Food Efficiency was consistently decreased

Food efficiency in both sexes and species was consistently reduced and these reductions were sustained throughout the study. In female rats, and in male and female mice, the decreases in food efficiency decreased consistently with increased time on treatment. By the end of the study, food efficiency was decreased 17% in female rats (see Table 5.5-32), 23% in female mice and 26% in male mice (see Table 5.5-33). These data indicate that less of the food intake was being converted to body mass in the treated animals than in control, indicative of a toxic effect. In the male rat, food efficiency was lower for the 200 ppm group at all time-points, when compared with the food efficiency for the controls, with many of the time points significantly lower. Food efficiency was decreased by approximately 5% by day 91 and progressed to a 6.5% decrease by day 399. For the entire study period (day 0-728), food efficiency was decreased by approximately 3% for the 200 ppm males, when compared with the controls. However, the control male rats lost weight at the end of the study, which would diminish the difference in food efficiency between the control and 200 ppm male rats.

CONCLUSION

The decreased body weights and body weight gains observed at the high doses in both sexes of both species were treatment-related toxic effects that were not due to decreases in food consumption, as would occur if palatability of the test diets were an issue. Food consumption was only slightly decreased in rats and not affected at all in mice. Food efficiency, however, was decreased along with body weight gain indicating a toxic effect other than decreased food consumption was the cause for the body weight effect. These findings provide clear evidence of treatment-related toxicity. The decrements in body weight gain reached levels of 10% or greater in both sexes of both species. The effect on body weight and body weight gain became more severe over time indicating a cumulative toxicity. Collectively, these data, specifically the body weight gain decrements of 10% or more, indicate that the MTD was reached in male and female rats (200 ppm) and in male (120 ppm) and female mice (180 ppm) that received BAS 500 F in the diet for a chronic exposure.

Table 5.5-30: Rat body weight gained for combined chronic and oncogenicity studies – cumulative from day 0

Body weight gained (g)												
Female rats		Day 0-14	Day 0-28	Day 0-91	Day 0-119	Day 0-147	Day 0-175	Day 0-259	Day 0-371	Day 0-455	Day 0-539	Day 0-728
Control for 25, 75, 200 and 600 ppm	Mean	40	67	125	136	145	152	171	191	213	225	252
25 ppm	Mean	42	70	**133***	143	151	157	172	187	201	213	236
	%	4	4	7	5	4	3	1	-2	-5	-6	-6
75 ppm	Mean	42	69	**132***	143	151	157	171	189	205	216	231
	%	3	3	6	5	4	3	0	-1	-3	-4	-8
200 ppm	Mean	40	63	120	130	**136***	**141****	**154****	**166****	**189****	**192****	**204****
	%	-2	-6	-3	-4	-6	-8	-10	-13	-11	-15	-19
Control for 400 ppm	Mean	43	69	137	146	155	165	179	191			
400 ppm	Mean	**39****	**63****	**125****	**133****	**140****	**147****	**157****	**163****			
	%	-8	-8	-8	-9	-10	-11	-13	-15			
600 ppm	Mean	**36***	**60****	**112****	**122****	**128****	**131****	**141****	**144****			
	%	-10	-12	-10	-10	-12	-14	-17	-25			
Male rats												
Control for 25, 75, and 200 ppm	Mean	101	164	307	334	354	373	420	467	499	521	503
25 ppm	Mean	101	161	303	331	353	372	420	466	493	518	507
	%	0	-1	-1	-1	0	0	0	0	-1	-1	1
75 ppm	Mean	101	162	304	334	355	373	427	471	496	513	517
	%	0	-1	-1	0	0	0	2	1	-1	-2	3
200 ppm	Mean	**95****	**152****	**282****	**310****	**329****	**344****	**387****	**427****	**455****	**471****	478
	%	-6	-7	-8	-7	-7	-8	-8	-9	-9	-10	-5
Control for 400 ppm	Mean	99	162	308	336	357	380	430	471			
400 ppm	Mean	**91****	**147****	**273****	**295****	**311****	**330****	**367****	**396****			
	%	-9	-9	-11	-12	-13	-13	-15	-16			

% - percent difference from control
Statistics: ANOVA with Dunnett's (two-sided): * p<=0.05; ** p<=0.01

Table 5.5-31: Mouse body weight gained for oncogenicity studies – cumulative from day 0

Body weight gained (g)												
Female mice		Day 0-14	Day 0-28	Day 0-91	Day 0-119	Day 0-147	Day 0-175	Day 0-259	Day 0-371	Day 0-455	Day 0-539	Day 0-546
Control	Mean	2	3	7	10	13	14	17	19	18	19	19
10 ppm	Mean	2	3	7	10	12	13	16	18	16	**16***	**16****
	%	-12	2	-5	-8	-7	-3	-9	-3	-13	-16	-17
30 ppm	Mean	2	3	9*	11	13	15	18	20	16	17	17
	%	-10	3	15	8	7	8	3	6	-9	-8	-12
120 ppm	Mean	2	3	7	10	12	13	16	18	**15****	**16***	**15****
	%	-10	-1	-2	-8	-8	-5	-9	-3	-19	-16	-20
180 ppm	Mean	**1***	**2****	7	**9***	**10***	**11***	**14****	**16****	**14****	**14****	**14****
	%	-45	-20	-9	-15	-17	-15	-21	-16	-25	-23	-26
Male mice												
Control	Mean	2	3	10	13	15	16	18	19	19	19	19
10 ppm	Mean	2	4	9	11	14	15	16*	17	**16****	**15****	**15****
	%	-3	5	-9	-9	-7	-6	-11	-9	-14	-19	-23
30 ppm	Mean	2	3	9	12	14	16	17	18	**17****	**16****	**15****
	%	-16	-7	-10	-6	-3	-2	-5	-4	-11	-15	-18
120 ppm	Mean	**2***	**3***	**8****	**11****	**12****	**14****	**15****	**16****	**15****	**14****	**13****
	%	-21	-17	-20	-16	-15	-15	-18	-14	-22	-26	-28

% - percent difference from control
Statistics: ANOVA with Dunnett's (two sided): * p<= 0.05; ** p<= 0.01

Table 5.5-32: Results in rats for the interval 0-728 days

Female Rat	Control	25 ppm		75 ppm		200 ppm	
	Mean	Mean	%	Mean	%	Mean	%
Body Weight	340.63	336.81	-1.12	335.22	-1.59	**316.49****	-7.09
Body Weight gain	251.82	235.86	-6.34	231.37	-8.12	**203.95****	-19.01
Cumulative Food Consumption	19.77	19.66	-0.56	20.03	1.32	19.19	-2.95
Cumulative Food Consumption Relative to Body Weight	58.27	58.55	0.48	**59.97****	2.92	**60.77****	4.30
Cumulative Food Efficiency	1.74	1.64	-5.61	**1.58***	-9.05	**1.45****	-16.68
Male Rat							
Body Weight	615.27	614.2	-0.18	617.88	0.42	**583.44****	-5.17
Body Weight gain	502.87	506.92	0.81	516.76	2.76	478.06	-4.93
Cumulative Food Consumption	25.56	25.95	1.52	26.09	2.09	25.2	-1.38
Cumulative Food Consumption Relative to Body Weight	41.61	42.36	1.81	**42.36***	1.81	**43.1****	3.59
Cumulative Food Efficiency	2.69	2.67	-0.66	2.7	0.39	2.61	-3.04

% - percent difference from control
Statistics: ANOVA with Dunnett's (two sided): * p<= 0.05; ** p<= 0.01

Table 5.5-33: Results in mice for the interval 0-546 days

Female Mouse	Control	10 ppm		30 ppm		120 ppm		180 ppm	
	Mean	Mean	%	Mean	%	Mean	%	Mean	%
Body Weight	34.74	33.43	-3.75	34.7	-0.10	32.91	-5.25	**31.86****	-8.27
Body Weight gain	19.17	**15.9****	-17.07	16.83	-12.23	**15.4****	-19.65	**14.09****	-26.49
Cumulative Food Consumption	5.61	**5.15***	-8.05	**5.17***	-7.74	5.28	-5.85	5.47	-2.51
Cumulative Food Consumption Relative to Body Weight	162.97	154.91	-4.95	**149.96***	-7.99	160.74	-1.37	173.08	6.20
Cumulative Food Efficiency	0.62	0.57	-8.36	0.59	-4.28	0.54	-12.53	**0.48****	-22.75
Male Mouse									
Body Weight	39.67	38.16	-3.80	38.75	-2.33	**36.57****	-7.81		
Body Weight gain	18.84	**14.52****	-22.93	**15.39****	-18.30	**13.47****	-28.49		
Cumulative Food Consumption	5.27	**5.00***	-5.05	5.07	-3.75	5.02	-4.63		
Cumulative Food Consumption Relative to Body Weight	133.37	131.33	-1.53	131.11	-1.69	138.08	3.53		
Cumulative Food Efficiency	0.66	**0.53****	-19.03	**0.56****	-14.90	**0.49****	-25.60		

% - percent difference from control
Statistics: ANOVA with Dunnett's (two sided): * p<= 0.05; ** p<= 0.01

CA 5.6 Reproductive Toxicity

The pyraclostrobin studies evaluated in the draft monograph of the Rapporteur Member State Germany (August 01, 2001) consisted of a two-generation study in rats and developmental toxicity studies in rats and rabbits. Further evaluations of the developmental studies were made in the 2nd and 4th Amendment to the DAR. These studies have been evaluated by European authorities and Germany as Rapporteur Member State (European Commission Peer Review Program) and were considered to be acceptable. For the convenience of the reviewer, these are summarized below as extracted from the monograph.

Table 5.6-1: Summary of reproduction toxicity studies conducted with pyraclostrobin

Study	Dosages (mg/kg bw/d)	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Main adverse effect	Reference (BASF DocID)
2-Generation, oral, feed, Wistar rats (constant diet concentrations: 0, 25, 75 and 300 ppm)	2.7, 8.2 and 32.6 (mean)	Parental toxicity: ca. 8.2 [75 ppm] Reproductive toxicity: ca.8.2 [75 ppm]	Parental toxicity: ca. 32.6 [300 ppm] Repro-ductive toxicity: ca. 32.6 [300 ppm]	Parental toxicity: Reduced food consumption and body weight gain Reproductive toxicity: Reduced pup body weight gain, organ weight changes and a delay in vaginal opening (F_1 females only). No adverse effects on fertility.	1999/11869
Developmental toxicity, gavage (days 6-9), Wistar rats	0, 10, 25 and 50	Maternal toxicity: 10 Developmental toxicity: 25	Maternal toxicity: 25 Develop-mental toxicity:50	Maternal toxicity: Reduced food consumption and body weight gain Developmental toxicity: Increased variations	1999/11511
Developmental toxicity, gavage (days 7-28), Himalayan rabbits (1st study)	0, 5, 10 and 20	Maternal toxicity: <5 Developmental toxicity: 5	Maternal toxicity: 5 Develop-mental toxicity: 10	Maternal toxicity: Reduced food consumption, reduced body weight gain Developmental toxicity: Increased skeletal malformations, at 20 mg/kg bw/d increased resorption and post-implantation losses, reduced number of live fetuses.	1999/11512
Developmental toxicity, gavage (days 7-28), Himalayan rabbits (2nd study with special regard to maternal effects)	0, 1, 3 and 5	Maternal toxicity: 3 Developmental toxicity: 5	Maternal toxicity: 5 Develop-mental toxicity: 10	Maternal toxicity: Reduced food consumption, reduced body weight gain Developmental toxicity: No evidence of developmental toxicity (limited range of parameters investigated).	2001/1003803

In the 2-generation study, the NOAEL for reproductive toxicity was 75 ppm (8.2 mg/kg bw/d), based on impairments in body weight/body weight gain in F_1 and F_2 pups and a slight delay in vaginal opening in F_1 female rats at 300 ppm (32.6 mg/kg bw/d). There were no treatment-related effects on fertility. The NOAEL for parental toxicity was 75 ppm (8.2 mg/kg bw/d) based on the signs of systemic toxicity in male and female animals occurring in both parental generations at 300 ppm (32.6 mg/kg bw/d). Toxicity was characterised by decreased food consumption and impairments in body weight and body weight gain.

In the teratogenicity study done with rats the NOAEL for maternal toxicity was 10 mg/kg bw/d, based on reduced food consumption and reductions in corrected body weight gain.
Under the conditions of this prenatal developmental toxicity study, pyraclostrobin was not teratogenic in rats. The NOAEL for developmental toxicity was 25 mg/kg bw/d, based on increased incidences of several soft tissue and skeletal variations inside the range of the historical control values.

In the teratogenicity study done with rabbits, pyraclostrobin was embryotoxic at dose levels with severe maternal toxicity.
The NOAEL for maternal toxicity was <5 mg/kg bw/d, suggesting a higher susceptibility of this species as compared to the rat at least when the test compound is administered during gestation. The NOAEL for developmental toxicity was 5 mg/kg bw/d, based on increased incidences of skeletal malformations at 10 and 20 mg/kg bw/d, which was just outside the historical control range of this laboratory at the time the study report was written. More complete historical control data including also studies performed at a later time showed that the incidences were within the historical control range. These data were discussed in the 4th Addendum to the DAR. At the highest dose level of 20 mg/kg bw/d, elevated postimplantation losses (mainly due to increased early resorptions) and a subsequent reduction in the mean number of live fetuses/doe were observed. As discussed in the 4th Addendum the "developmental toxicity occurred only at dose levels which were associated with maternal toxicity, and the embryotoxic effects (increased post-implantation losses) were likely to be secondary to the marked nutritional deficit in the dams at a critical time in gestation."
On request of the Rapporteur, a second prenatal developmental toxicity study in rabbits was performed with the main objective to establish a clear NOAEL for maternal toxicity.

In this additional prenatal developmental toxicity study in rabbits, a weak adverse effect on maternal food consumption and body weight gain was observed at a dose level of 5 mg/kg bw/d. The next lower dose of 3 mg/kg bw/d is considered the NOAEL for maternal toxicity since the occasionally reduced food intake at this dose level was not accompanied by an impaired food utilisation or a statistically significantly compromised body weight gain. Even though the applicant argued that the minor effects at the highest dose level (5 mg/kg bw/d) were not related to compound administration the NOAEL of 3 mg/kg bw/d was finally concluded in the EU review.
The gestational parameters were not affected and no evidence of developmental toxicity was obtained up to the highest dose of 5 mg/kg bw/d, however, the range of fetal parameters investigated was rather limited.

Based on the available data, the following endpoints were determined during the last Annex I listing of pyraclostrobin:

Reproductive toxicity (SANCO/1420/2001)

Target / critical effect - Reproduction:	Pup body weight gain reduced in the presence of parental toxicity
Lowest relevant reproductive NOAEL / NOEL:	Rat: 8.2 mg/kg bw/d (75 ppm)
Target / critical effect - Developmental toxicity:	Developmental effects in rats and embryotoxicity in rabbits at maternally toxic doses
Lowest relevant developmental NOAEL / NOEL:	Rabbit: 5 mg/kg bw/day
Lowest relevant maternal NOAEL / NOEL:	Rabbit 3 mg/kg bw/d

There are no new studies available with pyraclostrobin that could affect the overall evaluation for reproduction and developmental toxicity. Thus, the conclusion for relevant endpoints for the current renewal remains as follows:

Reproduction target / critical effect	Reduced pup body weight gain in the presence of parental toxicity No classification required
Lowest relevant reproductive NOAEL / NOEL	Rat: 75 ppm (8.2 mg/kg bw/d)
Developmental target / critical effect	Developmental effects in rats and embryotoxicity (including malformations) in rabbits at maternally toxic doses No classification required
Lowest relevant developmental NOAEL / NOEL	Rabbit: 5 mg/kg bw/d
Lowest relevant maternal NOAEL / NOEL	Rabbit: 3 mg/kg bw/d

The classification and labelling was discussed at ECB in January 2003 (ECBI 52/03 Add.1 Rev. 1) and concluded in September 2004. No classification for reproductive or developmental toxicity was considered necessary. A more detailed discussion can be found below.

For convenience of the reviewer brief summaries of the respective studies as extracted from the monograph are provided under the respective chapters.

Furthermore, a complete study summary of the not yet peer reviewed one-generation range finding reproduction toxicity study can be found below. Based on the results of this study dose levels of 25, 75 and 300 ppm were selected for the 2-generation study.

CA 5.6.1 Generational studies

Report:	CA 5.6.1/1 [redacted] 2002 a BAS 500 F - One generation range-finding reproduction toxicity study in Wistar rats; continuous dietary administration 2002/1004187
Guidelines:	EEC 87/302 B, OECD 415
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

In a one-generation range finding reproduction toxicity study, BAS 500 F (pyraclostrobin; batch: J.-No. 27882/191/c (Tox. III/part 1); purity 97.09%) was administered as a constant homogeneous addition to the diet to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 200, 400 and 600 ppm (corresponding to a mean, 6-week pre-mating period substance intake of approximately 20.5, 39.9, 59.1 and 21.3, 42.5, 60.4 mg/kg bw/day in males and females, respectively).

No treatment-related mortality was observed throughout the study. Signs of systemic toxicity were observed in all treated F_0 parental rats. Food consumption and body weight/body weight gain was impaired at all dose levels. Indication of a hypochromic anemia were noted in males at 400 and 600 ppm in males and at 600 ppm in females. Thickening of the duodenal wall occurred in high dose males.
Signs of developmental toxicity were observed in F_1 pups as indicated by impairment of body weight development. No other pup parameters were affected by treatment.
Treatment had no effects on reproductive performance and fertility up to and including 600 ppm.

Based on the systemic toxicity observed in parental animals and the developmental toxicity in the pups up to the highest dose level, a NOAEL could not be determined in this study. In absence of any effects on fertility and reproductive performance the NOAEL for reproductive performance and fertility was at least 600 ppm (59.1 mg/ kg bw/day).

Based on the results of this study dose levels of 25, 75 and 300 ppm were selected for the 2-generation study.

(BASF DocID 2002/1004187)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 F

Description:	solidified melt / red-brown, clear
Lot/Batch #:	J.-No. 27882/191/c (Tox.III/part 1)
Purity:	97.09%
Stability of test compound:	The test substance was stable over the study period; proven by re-analysis.

2. Vehicle and/or positive control: Rodent diet

3. Test animals:

Species:	Rat, Wistar
Strain:	Chbb = THOM (SPF)
Sex:	Male and female
Age:	27 ± 1 days at delivery; 36 ± 1 days at beginning of treatment
Weight at dosing:	♂: 162.5g (147.5-176.5 g), ♀: 127.3g (113.0-139.1 g)
Source:	Boehringer Ingelheim, Pharma KG, Biberach/Riss, Germany
Acclimation period:	about 9 days
Diet:	Kliba maintenance diet rat/mouse/hamster, Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	drinking water from water bottles, ad libitum
Housing:	individual housing in type DK III stainless steel wire mesh cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area of about 800 cm²), with the following exceptions: • pregnant animals and their litters were housed together from day 18 of gestation until day 14 after birth in Makrolon type M III cages supplied by Becker & Co. pregnant females were provided with nesting material (cellulose wadding) toward the end of gestation bedding: SSNIFF (type 3/4) supplied by SSNIFF Spezialdiaeten GmbH, Soest, Germany
Environmental conditions:	
Temperature:	20 - 24°C (central air-conditioned rooms)
Humidity:	30 - 70% (central air-conditioned rooms)
Air changes:	number of air changes not indicated in report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 24-Feb-1997 to Jun-17-1997
In-life dates: 05-Mar-1997 (start of administration of F_0 parental animals) to 17-Jun-1997 (sacrifice of last parental animals)

2. Animal assignment and treatment:

BAS 500 F was administered in the diet to groups of 10 male and 10 female rats at nominal dose levels of 0, 200, 400, and 600 ppm. The animals used as F_0 parental animals were derived from different litters according to a written statement from the breeder. By this, sibling mating was avoided. The animals were randomly assigned to the test groups by means of a computer generated randomization list based on body weights before the beginning of the administration period (day -1).
After the acclimatization period F_0 parental animals continuously received the test substance throughout the entire study. About 16 hours prior to sacrifice food was withdrawn.
At least 45 days after beginning of treatment, male and female rats of the same dose groups were mated at a ratio of 1:1. Females were allowed to litter and rear their pups (F_1 generation pups) until PND 4 (standardization, see below) or day 21 after parturition. After weaning of F_1 pups the F_0 generation parental animals were sacrificed.
All surviving F_1 pups were sacrificed by means of CO_2 after standardization or weaning.

Mating procedure: Males and females were mated overnight at a 1:1 ratio for a maximum of 3 weeks. Throughout the mating period, each female was paired with a predetermined male from the same dose group. The animals were paired by placing the female in the cage of the male mating partner from about 4.00 pm until 7.00 - 9.00 am of the following morning. Deviations from the specified times were possible on weekends and public holidays, and were reported in the raw data.
A vaginal smear was prepared after each mating and examined for the presence of sperm. If sperm was detected, pairing of the animals was discontinued. The day on which sperm were detected was denoted "gestation day (GD) 0" and the following day "gestation day (GD) 1".

Standardization of litters: On PND 4, the individual litters were standardized in such a way that, where possible, each litter contained 4 male and 4 female pups (always the first 4 pups/sex and litter were taken for further rearing). If individual litters did not have 4 pups/sex it was proceeded in such a way that the most evenly distributed 8 pups per litter were taken for further rearing (e.g., 5 male and 3 female pups). Standardization of litters was not performed in litters with ≤ 8 pups.

3. Test substance preparation and analysis:

The test item was frozen and mechanically crushed and appropriate concentrated solutions of the test item in acetone were prepared. The diets were prepared by spraying these acetonic solutions onto about 3 kg food in a rotation vaporizer under partial vacuum. Acetone was removed by heating up to about 40°C for about 30 minutes. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. Diet preparations were performed at intervals, which guaranteed that the test substance in the diet remained stable throughout the feeding period.

Analyses of the dietary test substance preparations performed with a comparable batch (27882/37/a) prior to the start of the administration period revealed that the test substance was stable in the diet up to 43 days at room temperature.

The above described method for diet preparation was shown to yield in homogenous distribution of the test item in the diet as was demonstrated in the course of the pyraclostrobin 2-year carcinogenicity study in rats (Project No. 82S0494/96086; see BASF DocID 1999/11868). In this study, relative standard deviations of the homogeneity samples in the range of 1.5 to 2.0% indicate the homogenous distribution of pyraclostrobin in the diet preparations at concentrations of 25 and 200ppm.

Concentration control analyses were performed in the course of the 1-generation study. Analyses were carried out at the beginning of the administration period, thereafter in intervals of about 6 weeks during the study, and before study termination.

The actual test article concentrations were in the range of 90.5 to 104.2% of nominal concentration. The means were in the range of 98.0 to 99.4% of nominal concentrations.

Analyses of the test item preparations for stability and test item content (current study: project no. 15R0494/96131)

Dose level [mg/kg] = [ppm]	Sampling	Concentration [mg/kg]	% of Nominal
200	March 5, 1997	206	103.0
	April 16, 1997	181	90.5
	May 21, 1997	201	100.5
Average		**196.0 ± 13.2**	**98.0 ± 6.6**
400	March 5, 1997	415	103.8
	April 16, 1997	365	91.3
	May 21, 1997	408	102.0
Average		**396.0 ± 27.1**	**99.0 ± 6.8**
600	March 5, 1997	625	104.2
	April 16, 1997	557	92.8
	May 21, 1997	607	101.2
Average		**596.3 ± 35.2**	**99.4 ± 5.9**

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to the following tables:

Statistics of clinical examinations

Parameter	Statistical test
Food consumption (parental animals), body weight and body weight change (parental animals and pups; for the pup weights, the litter means were used), number of mating days, duration of gestation, number of pups delivered per litter	Simultaneous comparison of all dose groups with the control group using the DUNNETT-test (two-sided) for the hypothesis of equal means
Male and female mating indices, male and female fertility indices, gestation index, females with liveborn pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, viability index, lactation index, number of litters with affected pups at necropsy	Pairwise comparison of each dose group with the control group using FISHER'S EXACT test for the hypothesis of equal proportions
Proportions of affected pups per litter with necropsy observations	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians

Statistics of pathology and clinical pathology

Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians.
Clinical pathology parameters, except differential blood count	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using MANN-WHITNEY U- test (two-sided) for the equal medians.

C. METHODS

1. Observations:

The animals, i.e. parental animals and pups, were examined for mortality at least once daily. If animals were in a moribund state, they were sacrificed and necropsied. Observations for clinical evident signs of toxicity were performed once daily.
The nesting, littering, and lactation behavior of the dams was generally evaluated in the mornings in combination with the daily clinical inspection of the dams. Only special findings (e.g. disability to deliver) were documented on an individual dam basis.
The littering behavior of the dams was also inspected on weekdays (except holidays) in the afternoons in addition to the evaluations in the mornings.

2. Body weight:

Body weight of **parental** animals was determined once a week at the same time of the day (in the morning). The body weight change of the animals was calculated from these results. The following exceptions are notable for female parental animals:

a. During the mating period the F_0 generation parental females were weighed on the day of positive evidence of sperm (GD 0) and on GD 7, 14, and 20.
b. Females were not weighed during mating until there was a positive evidence of sperm in vaginal smears.
c. Females with litter were weighed on the day after parturition (PND 1) and on PND 4, 7, 14, and 21.
d. Females without litter were not weighed during the lactation phase.

Pup body weights were determined on the day after birth (PND 1) and on PND 4 (before standardization), 7, 14, and 21 at the same time of the day (in the morning) and on PND4 immediately before standardization of the litters. Pup body weight change was calculated from these results.

3. Food consumption, food efficiency and compound intake:

During the premating period of the F_0 generation parental animals, food consumption was determined once a week (for a period of 7 days) and calculated as mean food consumption in grams per animal and day.

Food consumption of the F_0 parental females during pregnancy (animals with evidence of sperm) was determined weekly on GD 0, 7, 14, 20. During the lactation period food consumption of the females was determined on PND 1, 4, 7 and 14.

No food consumption was determined between PND 14 and 21, as required in the test guidelines, since during this time pups will begin to consume considerable amounts of solid food offered, and therefore there was no point in such a measured.

No food consumption was determined after week 6 in F_0 parental males, during the mating period and for females without positive evidence of sperm during the programmed gestation phase and in females without litters during the lactation phase.

The mean daily intake of test substance (group means in mg/kg bw/d) was calculated based upon individual values for body weight and food consumption:

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_y}$$

FC_x = daily food consumption on day x in grams; C = concentration in ppm; BW_y = body weight on day y in grams (last weighing before day x)

4. Ophtalmoscopy:

Not performed in this study

5. Hematology and clinical chemistry:

The following hematological and clinical chemistry parameters were determined for all animals per test group and sex:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Platelet count
✓	Hemoglobin (Hb)	✓	Differential blood count		Prothrombin time
✓	Hematocrit (Hct)				
✓	Mean corp. volume (MCV)				
✓	Mean corp. hemoglobin (MCH)				
✓	Mean corp. Hb. conc. (MCHC)				
✓	Reticulocytes				
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride		Bile acids	✓	Aspartate aminotransferase (AST)
✓	Phosphorus (inorganic)	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Potassium	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Sodium	✓	Creatinine	✓	serum cholinesterase (SCHE)
✓	Magnesium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

Furthermore, differential blood smears and smears for reticulocyte counts were prepared and stained according to Wright without being evaluated.

6. Estrous cycle determination:

Not performed in this study.

7. Male reproduction data

For males, mating and fertility indices were calculated for F_1 litters according to the following equations:

$$\text{Male mating index [\%]} = \frac{\text{number of males with confirmed mating *}}{\text{number of males placed with females}} \times 100$$

* defined by a female with vaginal sperm or with implants in utero

$$\textit{Male fertility index [\%]} = \frac{\textit{number of males proving their fertility *}}{\textit{number of males placed with females}} \times 100$$

** defined by a female giving birth to a litter with pups / fetuses in utero*

8. Sperm parameters

Not performed in this study.

9. Female reproduction and delivery data

For females, mating, fertility and gestation indices were calculated for F_1 litters according to the following equations:

$$\textit{Female mating index [\%]} = \frac{\textit{number of females mated *}}{\textit{number of females placed with males}} \times 100$$

* defined as the number of females with vaginal sperm or that gave birth to a litter or with fetuses in utero

$$\textit{Female fertility index [\%]} = \frac{\textit{number of females pregnant *}}{\textit{number of females mated **}} \times 100$$

* defined as the number of females that gave birth to a litter or with fetuses in utero

*** defined as the number of females with vaginal sperm or with implants in utero*

$$\textit{Female gestation index [\%]} = \frac{\textit{number of females with live pups on the day of birth}}{\textit{number of females pregnant *}} \times 100$$

* defined as the number of females that gave birth to a litter or with fetuses in utero

The total number of pups delivered and the number of liveborn and stillborn pups were noted, and the live birth index was calculated for F_1 litters:

$$\text{Live birth index [\%]} = \frac{\text{number of liveborn pups at birth}}{\text{total number of pups born}} \times 100$$

10. Litter data

All F_1 pups were examined as soon as possible on the day of birth to determine the total number of pups and the number of liveborn and stillborn members of each litter. Pups, which died before the first examination on the day of birth, were designated as stillborn pups.

The number and percentage of dead pups on the day of birth (PND 0) and of pups dying between days 1-4, 5-7, 8-14 and 15-21 of the lactation period were determined.

The number of live pups/litter was calculated on the day after birth, and on lactation days 4, 7, 14, and 21. Furthermore, viability and lactation indices were calculated as follows:

$$\text{Viability index [\%]} = \frac{\text{number of live pups on day 4* after birth}}{\text{number of live pups on the day of birth}} \times 100$$

* before standardization of litters (i.e. before culling)

$$\text{Lactation index [\%]} = \frac{\text{number of live pups on day 21 after birth}}{\text{number of live pups on day 4* after birth}} \times 100$$

* after standardization of litters (i.e. after culling)

On the day of birth (PND 0) the sex of the pups was determined by determination of the anogenital distance. Subsequently the sex of the pups was assessed by the external appearance of the anogenital region and/or the mammary line. The sex of the animals was finally confirmed at necropsy. The sex ratio was calculated at PND 0 and PND 21 after birth using the following equation:

$$\text{Sex ratio [\%]} = \frac{\text{number of live male or female pups on day 0 / 21}}{\text{number of live male and female pups on day 0 / 21}} \times 100$$

11. Sacrifice and pathology:

The **parental animals** were sacrificed by decapitation under CO_2 anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. Animals that were sacrificed in a moribund state were necropsied as soon as possible after death and assessed by gross pathology. The organs were sampled and weighed as indicated in the table below. No histopathological examinations were performed.

Pathology:
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
✓			adrenals	✓			larynx	✓			skin
✓			aorta	✓	✓		liver	✓			spinal cord (3 levels)@
✓			bone marrow§	✓			lung	✓			spleen
✓			brain	✓			lymph nodes#	✓			sternum w. marrow
✓			caecum	✓			mammary gland (♀)	✓			stomach (fore- & glandular)
✓			colon	✓			muscle, skeletal	✓	✓		testes
✓			duodenum	✓			nerve, peripheral (sciatic n.)	✓			thymus
✓	✓		epididymides	✓			nose/nasal cavity‡	✓			thyroid glands
✓			esophagus	✓			ovaries and oviduct	✓			trachea
✓			eyes	✓			pancreas	✓			urinary bladder
✓			femur (with knee joint)	✓			parathyroid glands	✓			uterus
✓			gross lesions				Peyer's patches	✓			vagina
			Harderian gland	✓			pharynx				
✓			heart	✓			pituitary		✓		body (anesthetized animals)
✓			ileum	✓			prostate				
✓			jejunum	✓			rectum				
✓	✓		kidneys	✓			salivary glands				
✓			lacrimal glands%	✓			seminal vesicles~				

§ from femur; # axillary and mesenteric; @ cervical, thoracic, lumbar; *mandibular and sublingual.; % extraorbital, ‡

All **pups** with scheduled sacrifice (i.e. pups, which were culled on PND 4 and pups, which were sacrificed on PND 21 or subsequent days) were killed by means of CO_2.These pups were examined externally and eviscerated, their organs were assessed macroscopically and their duodeni were retained by litter in 4% formaldehyde solution. All stillborn pups and all pups that died up to weaning were examined externally, eviscerated, and their organs assessed macroscopically and their duodeni were retained by litter in 4% formaldehyde solution. All pups without any notable findings or abnormalities were discarded after their macroscopic evaluation.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See section B 3 above.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related clinical observations were noted in the male and female F_0 parental animals in any group during the administration period.

The isolated occurrence and the absence of a dose-response relationship of the following observations indicated a spontaneous origin of these findings:

- During the gestation period one dam (#133) of the high dose group (600 ppm) showed insufficient nesting activity around the expected day of parturition for F_1 litter.
- One low dose (200 ppm) dam (#113) and one mid dose (400 ppm) dam (#125) were sperm-positive but did not deliver any pups.
- During lactation of F_1 one control dam (#105) did not cut all umbilical cords and did not consume all placentae of its litter after delivery.
- One dam (#130) of the mid- dose group (400 ppm) had only one pup which was stillborn or died on the day of birth, thus this dam had no live pups.

2. Mortality

No mortality was observed in any of the male and female F_0 parental animals throughout the study.

C. PARENTAL BODY WEIGHT AND BODY WEIGHT GAIN

Treatment-related effects on body weight and body weight changes were found in mid and high dose F_0 males and females.

Mean body weights of the mid dose (400 ppm) F_0 males were statistically significantly reduced from study week 4 until termination at week 14 (-10%).
Statistically lower mean body weights in the high dose males were already recorded from study week 1 onwards (-14% at week 14). Concomitantly, mean body weight gains of mid and high dose males were statistically significantly lower for weeks 0-14 (-15% and -20%, respectively) [Figure 5.6.1-1].

In high dose (600 ppm) F_0 females mean body weights were slightly but non-significantly lower during premating (-6%), and statistically significantly lower during gestation (GD20: -11%) and lactation (-9% at weaning). As compared with controls body weight gains were impaired in 600 ppm females during premating (-14% for weeks 0-6) and gestation (GD 0-20: -15%), but not during lactation. During lactation days 1 and 14 body weights were decreased in the mid dose (400 ppm) females. Occasionally, body weight gains were impaired in mid dose females during gestation (GD0-20: -18%), but not during lactation [see Figure 5.6.1-1 and Figure 5.6.1-2].

Most of the adverse effects on body weight data were associated with concurrent reductions in food consumption (see below).

Figure 5.6.1-1: Body weight development of F_0 male (left) and female (right) rats during premating

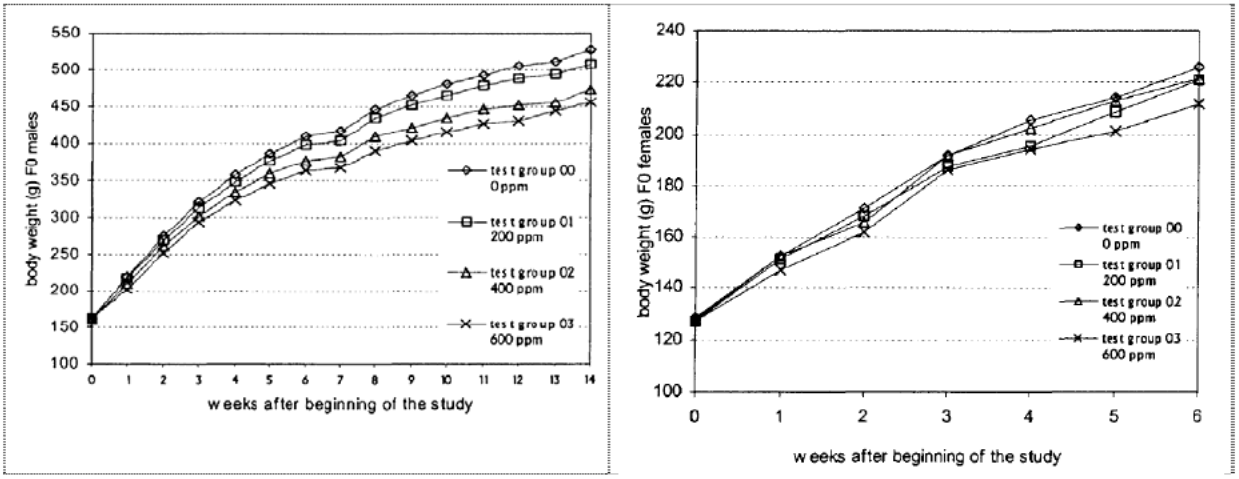
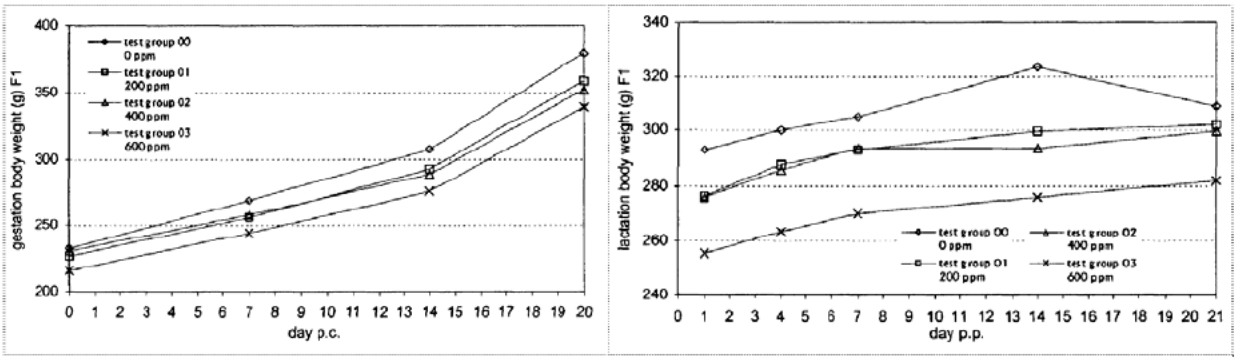


Figure 5.6.1-2: Body weight development of F_0 females during gestation (left) and during lactation (right) of F_1 pups



D. PARENTAL FOOD CONSUMPTION AND COMPOUND INTAKE

During the premating period treatment-related effects on food consumption were seen in mid and high dose (400 and 600 ppm) F_0 parental males and were restricted to high dose females. During gestation and lactation substance-related reduced food consumption was observed in F_0 females at all dose levels, i.e. 200,400, and 600 ppm (gestation) and at the mid and high dose levels (lactation).

A statistically significant reduction of food consumption in F_0 parental males by approx. 8 and 10% in the 400 and 600 ppm dose group, respectively, was observed during the entire premating period (weeks 0-6) [see Figure 5.6.1-3]. Food consumption values of the low dose (200 ppm) males were similar to the concurrent control animals during the pre-mating phase.

A treatment-related decrease in food consumption by up to 10% was observed in high dose F_0 females during the whole premating period, reaching statistical significance for all except one interval (week 5-6). Food consumption values of the low and mid dose group females were not affected by treatment during premating.

During gestation food consumption was statistically significantly lower in all dose groups compared to controls (GD 0-20: -7%, -11%, and -15% at 200, 400, and 600 ppm, respectively, compared to -4%). During lactation days 1-14, the food uptake was reduced by 18% and 23% at 400 and 600 ppm, respectively [see Figure 5.6.1-4].

Figure 5.6.1-3: Food consumption of F_0 male (left) and female (right) rats during premating

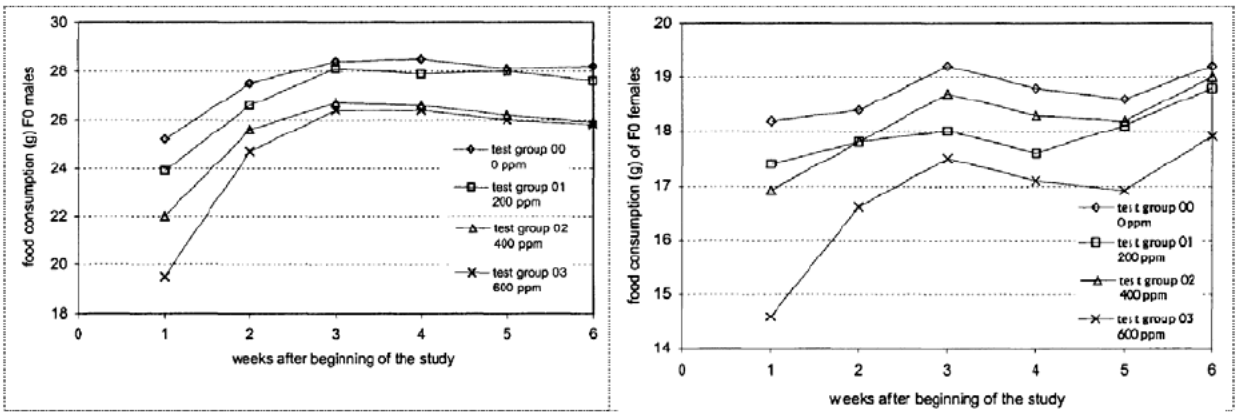
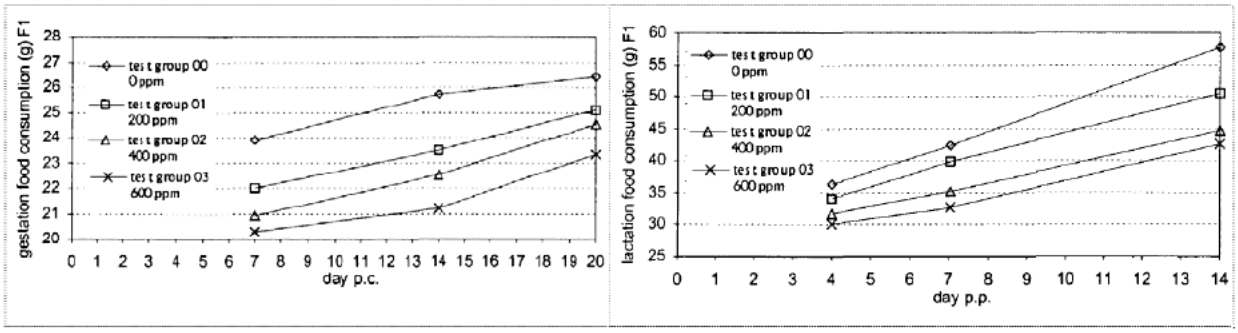


Figure 5.6.1-4: Food consumption of F_0 females during gestation (left) and during lactation (right) of F_1 pups



E. COMPOUND INTAKE

Average compound intakes during the different phases of the 1-generation range-finding study are provided in Table 5.6.1-1. Compound intakes in females tended to be slightly lower during gestation, but were roughly 30% higher during the lactation period when compared to the premating period.

Table 5.6.1-1: Average compound intake in F_0 and F_1 parental animals [mg/kg bw/day]

Parental generation	F_0		
Dose [ppm]	200	400	600
Males	20.5	39.9	59.1
Females			
- premating	21.3	42.5	60.4
- gestation	18.3	35.0	53.2
- lactation (PND 1-14)	29.0	51.9	80.2

F. ESTROUS CYCLE DETERMINATIONS

Not determined in this study.

G. MATING AND GESTATION DATA

1. Male reproductive performance

Male reproductive performance was not affected by treatment [see Table 5.6.1-2]. For all F_0 males which were placed with females to generate F_1 pups, mating was confirmed, thus the male mating index was 100% in all dose groups. For nearly all F_0 parental males, fertility was confirmed within the scheduled mating interval. The male fertility index varied between 90% and 100% without showing a dose-response relationship. Each one male of the low dose (male #13 mated with female #113) and the mid dose group (male #25 mated with female #125) did not generate F_1 progeny. In the absence of a dose-response relationship and gross macroscopic findings these changes in single animals are regarded to be spontaneous in nature and not associated with treatment.

Table 5.6.1-2: Reproduction parameters of male rats treated with BAS 500 F

Parental generation	F_0			
Dose [ppm]	0	200	400	600
Animals per dose	10	10	10	10
Male fertility				
- placed with females	10	10	10	10
- mated [n]	10	10	10	10
- mating index [%]	**100**	**100**	**100**	**100**
- pregnant [n]	10	9	9	10
- Fertility index [%]	**100**	**90**	**90**	**100**

2. Sperm analysis

Not performed in this study.

3. Female reproductive performance

Female reproductive performance was not affected by treatment. The female mating index was calculated to be 100% for all dose groups.

All F_0 females that were mated became pregnant except for one female in the low dose group (female #113) and one female in the mid dose group (female #125). Therefore, the fertility index varied between 90% (at 200 and 400 ppm) and 100% (in the control group and high dose 600 ppm group). Due to the lack of a dose-response relationship and in the absence of gross pathology findings, these changes are assessed to be incidental.

The mean duration until sperm was detected (GD 0; pre coital interval) ranged between 1.4 and 2.6 days, without a relation to treatment.

No treatment related effects on gestational parameters were noted. Gestation length was essentially identical in control and treated groups. The gestation index in F_0 females was 100% for the control, low and high dose groups, indicating that all pregnant females of these groups delivered live F_1 pups. One mid dose dam (#130) had only one dead pup on the day of delivery, leading to a lower gestation index of 89% in this 400 ppm dose group. The single incidence and the lack of a dose-response relationship indicated an incidental occurrence of this finding.
Mean number of F_1 pups delivered per dam, the number of liveborn and stillborn pups and the live birth index were not affected by treatment considering the normal biological variation.

Table 5.6.1-3: Reproduction and gestational parameters of female rats treated with BAS 500 F

Parental generation		F_0			
Dose	[ppm]	0	200	400	600
Animals per dose		10	10	10	10
Female fertility					
- placed with males		10	10	10	10
- mated	[n]	10	10	10	10
- mating index	[%]	100	100	100	100
- pregnant	[n]	10	9	9	10
- **Fertility index**	[%]	100	90	90	100
Pre coital interval	[days]	2.6	2.4	1.7	1.4
Duration of gestation	[days]	21.9	21.8	21.7	21.6
Females with liveborn		10	9	8	10
- **Gestation index**	[%]	100	100	89	100
- with stillborn pups	[n]	1	1	2	3
- with all stillborn	[n]	0	0	1	0
Pups delivered	[n]	144	128	106	137
- per dam	[mean n]	14.4	14.2	11.8	13.7
- liveborn	[n]	140	126	104	129
- stillborn	[n]	4	2	2	8
- **Live birth index**	[%]	97	98	98	94

H. PUP DATA

1. Survival

Survival of F_1 pups was not affected by treatment. The viability index (survival days 0 to 4 pre cull) ranged between 96 and 100% in F_1 offspring [see Table 5.6.1-4]. Pup survival from PND 4-21 (Lactation index) ranged from 97% (in the high dose group) and 100% in all the other dose groups including the control group.

2. Sex ratio

The sex ratios of F_1 pups on day 0 (day of birth) and 21 (weaning) were not affected by treatment [see Table 5.6.1-4].

3. Pup clinical observations

No clinical observations were recorded in **F_1** pups up to weaning which could be attributed to treatment.

Table 5.6.1-4: Pup survival, sex-ratio and body weights

Pup generation		F_1			
Dose	[ppm]	0	200	400	600
Number of litters		10	9	9	10
- with liveborn pups		10	9	8	10
- with stillborn pups		1	1	2	3
- with all stillborn pups		0	0	1	0
Pups liveborn	[n]	140	126	104	129
Pups stillborn	[n]	4	2	2	8
Pups died	[n]	6	3	1	2
Pups cannibalized	[n]	0	2	1	0
Pups culled day 4	[n]	54	50	38	56
Pups day 4 - pre-cull	[n]	134	121	102	129
- Viability index	[%]	96	96	98	100
Pups day 4 - post cull	[n]	80	71	64	73
Pups day 21	[n]	80	71	64	71
- Lactation index	[%	100	100	100	97
Sex ratio	[% live males]				
- Day 0		50.7	51.6	51.9	53.5
- Day 21		47.5	52.1	48.4	50.7
Male pup weight	[g]				
- day 1	[g]	6.6	6.5	6.6	6.1
- day 4 - pre cull	[g]	9.6	9.1	9.2	**8.0****
- day 4 - post cull	[g]	9.6	9.1	9.1	**8.2***
- day 7	[g]	15.3	13.9	13.5	**11.6****
- day 14	[g]	30.3	**25.8****	**25.1****	**20.3****
- day 21	[g]	50.2	**41.9****	**39.7****	**31.4****
Male body weight gain					
- day 4 to 21	[g]	40.6	**32.8****	**30.5****	**23.4****
	[Δ%]		**-9.2**	**-14.9**	**-42.4**
Female pup weight	[g]				
- day 1	[g]	6.4	6.3	6.3	**5.8***
- day 4 - pre cull	[g]	9.4	9.0	9.1	**7.8***
- day 4 - post cull	[g]	9.4	9.1	9.1	**7.7***
- day 7	[g]	14.7	14.0	13.3	**11.3****
- day 14	[g]	29.8	26.4	**24.6****	**20.1****
- day 21	[g]	47.7	**42.0***	**38.9****	**31.1****
Female body weight gain					
- day 4 to 21	[g]	38.4	**33.0***	**29.8****	**23.3******
	[Δ%]		**-14.1**	**-22.4**	**-39.3**

Statistics: *: p≤0.05, **: p≤0.01

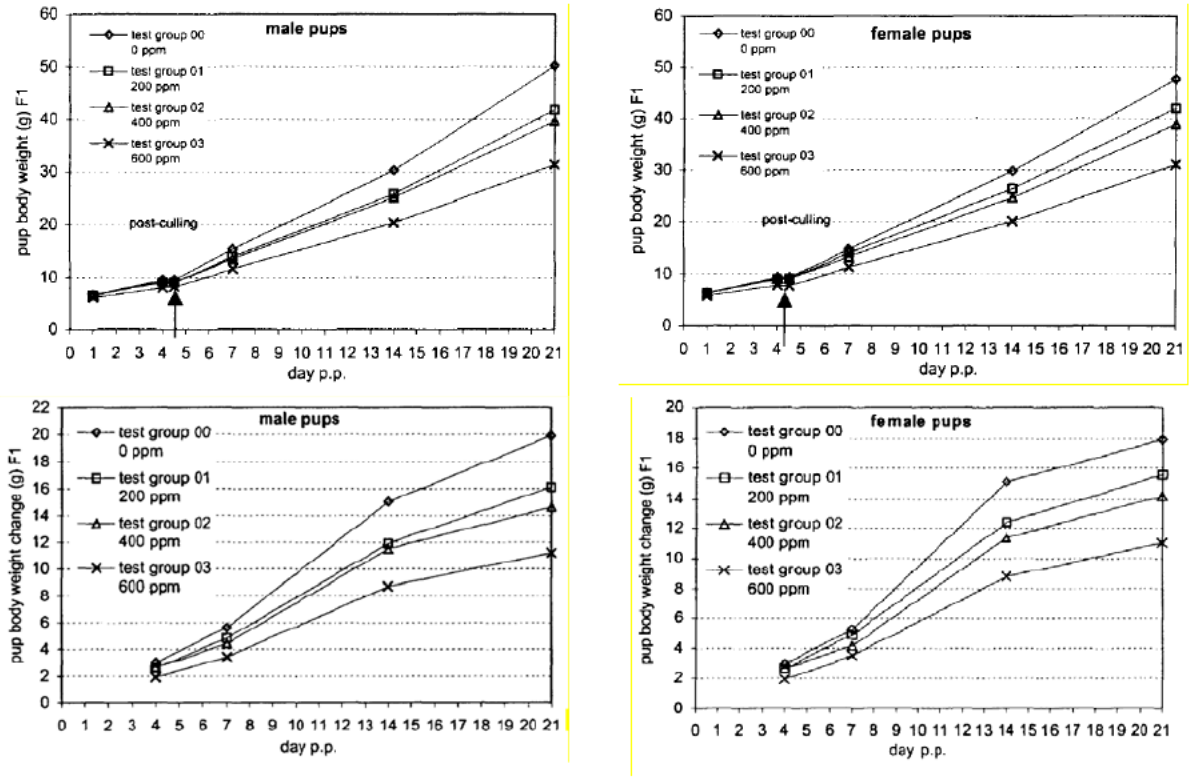
4. Body weight

F_1 male and female pup body development displayed a dose- and time related and statistically significant impairment [see Table 5.6.1-4 and Figure 5.6.1-5].

5. Organ weights

Not performed in this study.

Figure 5.6.1-5: Food consumption of F_0 females during gestation (left) and during lactation (right) of F_1 pups



6. Pup necropsy findings

No treatment-related gross necropsy findings were observed in F_1 pups. The macroscopic examination of stillborn pups, pups that died intercurrently, culled and surplus pups of F_1 litters did not reveal any differences between the test groups neither in the type nor in the number of pup necropsy observations.

Table 5.6.1-5: Incidence of gross necropsy observations in F_1 pups

Dose [ppm]	0	200	400	600
	F_1 pups			
Litters evaluated	10	10	10	10
Pups evaluated	144	125	105	137
- Live	140	123	103	129
- Stillborn	4	2	2	8
Post mortem autolysis	4(4)	0	0	1 (1)
Anasarca	0	1 (1)	0	0
Incisors sloped	0	2 (2)	1 (1)	0
Dilated renal pelvis	1 (1)	0	3 (1)	0
Total pup necropsy observations	5 (5)	3 (3)	4 (2)	1 (1)
- % affected pups/litter	3.4 ± 3.64	2.1 ± 3.24	3.5 ± 7.85	2.0 ± 6.32

( ) values in brackets give litter incidence

A few spontaneous gross necropsy findings were observed in F_1 pups of all groups including the controls. The individual findings, such as anasarca, incisors sloped, dilated renal pelvis, and post mortem autolysis were observed in a low incidence and displayed no dose response [see Table 5.6.1-5].
All findings were therefore considered to be spontaneous and not related to treatment.

7. Sexual maturation

Not performed in this range-finding study.

H. HEMATOLOGY AND CLINICAL CHEMISTRY

At the end of the administration period significantly increased white blood cell (WBC) counts were observed in all treated F_0 male groups and in high dose F_0 females.
In the differential blood counts of the males the increase in leukocytes was associated with an increase in lymphocytes in all treatment groups and with a higher number of polymorphonuclear neutrophils in the mid and high dose animals.
A slight but statistically significant decrease in hemoglobin concentration was found in mid and high dose males.
In parental females white blood cells and polymorphonuclear neutrophils were increased in the high dose animals. Significantly decreased hemoglobin, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) as well as prolonged prothrombin times were found in mid and high dose females [see Table 5.6.1-6].

Table 5.6.1-6: Hematology findings of rats administered BAS 500 F during premating until PND 21

Sex		Males				Females			
Dose	[ppm]	0	200	400	600	0	200	4100	600
WBC	[10^9/L]	6.69	**8.88****	**8.87***	**9.92****	5.97	6.95	6.79	**8.01*****
HGB	[mmol/L]	9.7	9.5	**9.3****	**9.3***	10.1	10.2	9.9	**9.8***
MCH	[mmol]	1.08	1.09	1.09	1.09	1.19	1.17	**1.14*****	**1.11*****
MCHC	[mmol/L]	22.24	22.44	22.40	21.94	22.89	22.64	**22.31****	**21.95*****
HQT	[sec]	28.9	28.1	29.2	29.3	26.5	27.5	**29.3****	**29.6****
Neutrophils	[10^9/L]	0.71	0.82	**1.10**	**1.10**	0.89	0.81	0.93	**1.41**
Lymphocytes	[10^9/L]	5.29	**7.27**	**6.99**	**8.01**	4.32	5.36	5.06	5.69

Statistics: * $p \leq 0.05$; ** $p \leq 0.02$, *** $p \leq 0.002$

Clinical chemistry investigation in F_0 parents revealed statistically significant decreases in alkaline phosphatase (ALP) in all and alanine aminotransferase (ALT) in mid and high dose males. High dose females displayed a significant drop in cholinesterase activity (SCHE).

Blood chemistry investigations revealed a number of dose-dependent and statistically significant changes [see Table 5.6.1-7]. These treatment-related changes were printed in bold typeface. The marginally and not dose dependent decrease of calcium levels in low dose males was not considered to be treatment-related.

Table 5.6.1-7: Clinical chemistry findings of rat administered BAS 500 F during premating until PND 21

Sex		Males				Females			
Dose	[mg/kg]	0	200	400	600	0	200	400	600
		F_0 generation							
ALT	[µkat/L]	1.16	1.00	**0.96****	**0.85*****	0.90	0.82	0.82	0.76
ALP	[µkat/Ll	6.89	**5.89****	**5.20*****	**5.34*****	4.90	4.28	4.40	4.21
SCHE	[µkat/L]	16.58	18.45	18.24	17.03	56.23	61.61	46.38	**42.28*****
Calcium	[mmol/L]	2.93	2.87*	2.87	**2.85****	2.81	2.83	2.83	2.74
Urea	[mmol/L]	7.34	7.48	7.15	**6.63*****	7.58	8.29	7.75	7.29
Creatinine	[µmol/L]	50.4	48.8	48.1	46.5	47.2	48.4	46.9	**42.9****
Glucose	[mmol/L]	7.77	7.82	**7.09****	**7.19****	6.80	7.97	7.29	6.86
Total bilirubin	[µmol/L]	1.58	1.28	1.79	**2.30****	1.42	1.52	1.92	1.73
Total protein	[g/L]	70.15	67.82	66.51	66.70	67.90	67.80	65.60	**61.84****
Albumine	[g/L]	32.74	32.40	32.30	32.95	33.45	33.59	33.09	**31.60****
Globuline	[g/L]	37.42	**35.42***	**34.20****	**33.75*****	34.45	34.20	**32.51***	**30.24*****
Triglyceride	[mmol/L]	4.35	5.29	3.89	4.03	2.93	3.00	2.99	**1.89****
Cholesterol	[mmol/L]	2.26	2.09	**1.84***	**1.76****	2.29	**1.93****	**2.03***	**1.80*****

* $p \leq 0.05$; ** $p \leq 0.02$; *** $p \leq 0.002$

I. Parental terminal investigations

1. Organ weights

A significant and treatment-related decrease of parental terminal body weights was noted in males in the mid and high dose and in females in the low and high dose [see Table 5.6.1-8]. Organ weight determination in F_0 parental animals revealed significant changes of absolute and/or relative organ weights in males but not in females.

The statistically significant decrease of absolute liver and kidney weights as well as the statistically significant increases of relative testes and epididymis weights were not accompanied by respective changes of either relative (liver and kidney) or absolute (testes and epididymis) weights and thus considered secondary to the decrease of terminal body weights in mid and high dose males. The alterations of absolute and/or relative organ weights in parental males were not accompanied by any gross lesions (see below).

Table 5.6.1-8: Organ weights of F_1 male and female parental animals

Sex		F_0 Males				F_0 Females			
	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ%	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ%
Terminal weight [g]	0	491.48				269.11			
	200	471.92	(-4.0)			**251.45****	**(-6.6)**		
	400	**437.1****	**(-11.1)**			253.54	(-5.8)		
	600	**418.57****	**(-14.8)**			**241.58****	**(-10.2)**		
Liver [g]	0	15.971		3.247		8.785		3.263	
	200	15.935	(-0.2)	3.371	(3.8)	8.216	(-6.5)	3.265	(0.1)
	400	13.572**	(-15.0)	3.101	(-4.5)	8.554	(-2.6)	3.372	(3.3)
	600	13.808**	(-13.5)	3.296	(1.5)	8.206	(-6.6)	3.398	(4.1)
Kidney [g]	0	3.088		0.629		2.038		0.758	
	200	3.009	(-2.6)	0.637	(1.3)	2.023	(-0.7)	0.805	(6.2)
	400	2.694**	(-12.8)	0.62	(-1.4)	1.951	(-4.3)	0.770	(1.6)
	600	2.83	(-8.4)	0.679	(7.9)	1.960	(-3.8)	0.813	(7.3)
Testes [g]	0	3.558		0.727					
	200	3.427	(-3.7)	0.727	(0.0)				
	400	3.585	(0.8)	0.822**	(13.1)				
	600	3.668	(3.1)	0.881**	(21.2)				
Epididymides [g]	0	1.355		0.276					
	200	1.339	(-1.2)	0.284	(2.9)				
	400	1.328	(-2.0)	0.304	(10.1)				
	600	1.405	(3.7)	0.336**	(21.7)				

* $p \leq 0.05$, ** $p \leq 0.01$
( ) Percent difference values calculated on means

2. Macroscopic lesions

The only gross pathology finding was a thickening of the wall of the duodenum in all males of the high dose group.

3. Histopathology

Histopathology was not performed.

4. Differential ovarian follicle count

Not performed in this range-finding study.

III. CONCLUSIONS

Administration of BAS 500 F (pyraclostrobin) to rats at dietary dose levels of 0, 200, 400, and 600 ppm resulted in signs of general, systemic toxicity at all dose levels as indicated by impaired body weight development, which was partially accompanied by decreased food consumption. In mid and/or high dose levels a slight anemia was noted as indicated by decreased hemoglobin concentrations and changes of mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. A number of clinical chemistry changes were noted in addition.
Indications of developmental toxicity consisted of impaired body weight development of pups at all dose levels. No effects on fertility and reproductive performance were observed up to the highest dose tested. Therefore, under the conditions of the present range finding study no NOAEL was identified.

2-generation study, rat

Pyraclostrobin (batch No. 27882/199/b; purity: 98.7%) was administered to groups of 25 male and 25 female sexually immature Wistar rats (F0 parental generation) in the diet at concentrations of 0, 25, 75 or 300 ppm. At least 74 days after the beginning of treatment, F0 animals were mated to produce a litter (F1). Groups of 25 males and 25 females selected from F1 pups as the F1 parental generation were offered diets containing 0; 25; 75 or 300 ppm of the test substance post weaning, and the breeding program was repeated to produce an F2 litter. The study was terminated with the sacrifice of the F2 weanlings and F1 adult animals.
There were no overt clinical signs of toxicity in F0 and F1 parental animals. One high dose F0 dam died on the first day of lactation following complete delivery, which is considered to be not treatment-related since it was an isolated case without any gross or histopathological findings. The following effects were observed in animals dosed with 300 ppm pyraclostrobin: Significantly decreased food consumption was observed in the male rats during the first weeks of the premating phase (F0 up to 5%, F1 up to 12%) and in the females during the premating (F0 up to 8%, F1 up to 9%) and gestation period (F1 only, up to 7%). Significantly reduced mean body weights were noted in F0 females during gestation (up to 5%) and lactation (up to 4%) in several weeks. In the F1 generation, a lower body weight than in the control group was determined in the male animals during certain phases of treatment period (weeks 4-11, week 14: up to 7%) and in the females during the first weeks after weaning (up to 9%). A reduction in body weight gain attaining statistical significance was observed during the premating period in high dose F0 females (1st week after initiation of treatment: up to 12%) as well as occasionally in F0 males (weeks 9 –10: about 25%).
Organ weight determination as well as gross or histopathology did not reveal any evidence of compound-related changes.
There were no adverse effects on reproductive performance including sperm and ovarian parameters as well as the estrus cycle of the rats of both generations. A lower mean number of implantation sites in mid and high dose F0 dams, although statistically significant, did not show a clear dose response. Mainly because of a lower postimplantation loss in the high dose group, this difference did not result in a significant decrease in the mean number of pups delivered or the number of live born pups and, thus, did not affect the reproductive success. Furthermore, the number or implantation sites in F0 mid and high dose groups was virtually identical to that in F1 control females (14.5 ± 3.91 and 14.4 ± 2.18 versus 14.5 ± 3.27, respectively).

Finally, in the F1 generation no changes in mean implantation site number were seen. Actually, the mean number of implantations at the high dose level was higher (15.4 ± 2.74) compared to the control. This confirms that the slightly lower number of implantation sites is an expression of biological variability rather than indicative for an effect of compound administration.
Pup birth weight and pup viability were not affected by treatment.

Reduced mean body weights and body weight gains became apparent in F1 and F2 pups from day 4 *post partum* up to weaning at the highest dose levels. At the highest dose also reduced mean absolute weight of thymus (F1 about -18%; F2 about -17%), spleen (F1 about -16%; F2 about -17%) and brain (F2 about -4%) and increased mean relative brain weights (F1 about 13%; F2 about 11%) were observed. These findings were attributed to the reduction in pup body weight (gain). A delay in vaginal opening in the F1 female pups was observed at the highest dose, which was considered to be secondary to a slight delay in physical development due to the observed body weight effects rather than a specific effect of treatment. As a matter of fact, there was no high dose female with vaginal opening later than in the latest control female. Furthermore, statistically significant differences in vaginal opening were only observed for PND 30 to 32, i.e. at an early point in time. This confirms that this effect is rather related to the reduced body weight development than providing evidence of a selective effect of the test substance (for a more detailed discussion see M-CA 5.8.3).

Conclusion:
Fertility was not affected up to the highest dose level of 300 ppm (ca 32.6 mg/kg bw/d). The NOAEL concerning parental toxicity in this study was 75 ppm (approximately 8.2 mg/kg bw/d) for F0 and F1 animals, and this was also the NOAEL for reproductive toxicity in the F1 and F2 litters. Offspring effects were confined to reduced body weight gain and associated changes of organ weights. A single developmental landmark (vaginal opening) was delayed in F1 pups at 300 ppm suggesting a possible (slight) retardation in female pup development due to impaired body weight development.

CA 5.6.2 Developmental toxicity studies

Developmental toxicity, rat

Pyraclostrobin (batch No. CP028719; purity: 98.9%) was tested for its prenatal toxicity in Wistar rats. The test substance was administered as an aqueous suspension to 25 mated female rats/group by stomach tube at dosages of 0, 10, 25 and 50 mg/kg bw on days 6–19 *post coitum* (p.c.).
No mortalities, abortions or premature deliveries occurred during the study. The oral administration of pyraclostrobin elicited overt maternal toxicity at 50 mg/kg bw/d and was still toxic to the dams at 25 mg/kg bw/d. Maternal toxicity was substantiated by reduced food consumption, impairments in body weight gain (high dose only) and reductions in corrected body weight gain.
There was no evidence of an increase in the incidence of malformations up to and including the highest dose level of 50 mg/kg bw/d. However, the incidence of several soft tissue and skeletal variations was increased at 50 mg/kg bw/d. Although all increased incidences were inside the range of the historical control values, a treatment related increase at the highest dose level was not completely excluded in the DAR. At the lowest dose level, the slight increased incidence of dilated ureter, closely related to the finding dilated renal pelvis, appeared without a clear relation to dosing. It should be also noticed that dilatation of the renal pelvis is a very common finding in the offspring of this rat strain.

Conclusion:
In the prenatal toxicity study in rats, developmental toxicity was observed at the highest dose tested (50 mg/kg bw/d), based on increased incidences of several soft tissue and skeletal variations inside the range of the historical control values. This dose level was clearly toxic to the dams, as demonstrated by a 16% reduction in body weight gain. Thus, the NOAEL for maternal toxicity was established at 10 mg/kg bw/d, and the developmental NOAEL was 25 mg/kg bw/d.

Developmental toxicity, rabbit (1st study)

The teratogenic potential and developmental toxicity of pyraclostrobin (batch No. CP028719; purity: 98.9%) was studied in Himalayan rabbits. Groups of 24 or 25 pregnant female rabbits were administered the test substance as an aqueous suspension at dose levels of 0, 5, 10 or 20 mg/kg bw/d by gavage from days 7–28 post insemination (p.i.).
Two accidental deaths occurred in the control and mid dose group, respectively. Furthermore, two mid dose females were not pregnant at termination.
The oral administration of pyraclostrobin to pregnant Himalayan rabbits elicited pronounced maternal toxicity at 20 mg/kg bw/d and was still toxic to the does at 10 and 5 mg/kg bw/d. Maternal toxicity was substantiated by dose-related reduced food consumption (down to 11% of control group) with subsequent impairments in body weight gain at all dose levels and decreased defecation occurring in up to ten does, days 10-14 p.i. and one doe, day 10 p.i. at 20 and 10 mg/kg bw/d, respectively.

The corrected body weight gain (i.e., terminal body weight minus gravid uterus weight) was not affected, but additional adverse clinical findings (blood in bedding, observed in 4 does, days 16-29 p.i. and 2 does, days 16-24 p.i. at 20 and 10 mg/kg bw/d, respectively) and reductions in mean gravid uterus weight were observed, which are well in line with the embryotoxic or fetotoxic effects of the test compound at maternally toxic doses. Necropsy of the does did not reveal indications of a treatment-related effect.
There was a dose-related impact on some gestational parameters reaching statistical significance at the top dose level. Postimplantation losses were elevated mainly due to an increase in early resorptions and (consequently) the mean number of live fetuses/doe was reduced. These are not unusual findings in prenatal developmental toxicity studies in rabbits at dose levels which are clearly toxic to the does.
The incidence of external or soft tissue malformations or variations of any type was not affected by treatment, but there was an apparent but not statistically significant increase in the occurrence of skeletal malformations, consisting mainly of misshapen or absent vertebra. While the absolute number of affected fetuses was increased at the highest dose (6, 4, 5 and 9 at 0, 5, 10 and 20 mg/kg bw/d, respectively), the number of affected fetuses per litter was increased at ≥ 10 mg/kg bw/day (3.8 ± 7.0, 2.2 ± 5.1, 5.3 ± 13.2 and 12.1 ± 24.51). The percentage of affected fetuses was just outside the historical control range of this laboratory and strain. BASF submitted additional historical control data which were discussed in detail in the 4th addendum to the DAR. In essence, the total number of misshapen or absent vertebra was within the historical range.

Conclusion:

In the rabbit developmental toxicity study, maternal toxicity was proven by clear reduction of body weight gain and a lower food consumption at 5 mg/kg bw/d and above. Thus, the NOAEL for maternal toxicity was <5 mg/kg bw/d suggesting a higher susceptibility of this species as compared to the rat at least when the test compound is administered during gestation. Prenatal toxicity was substantiated by embryolethality resulting in elevated postimplantation losses and an according reduction in the mean number of live fetuses/doe at 20 mg/kg bw/d. An increased incidence of vertebral malformations was observed at 10 and 20 mg/kg bw/d, which was just inside the historical control range of this laboratory and this strain. It must be emphasised that developmental toxicity was observed only in the presence of severe maternal toxicity. The NOAEL for developmental toxicity was 5 mg/kg bw/d.
A further rabbit study was required by the Rapporteur to establish a clear NO(A)EL for maternal toxicity.

Developmental toxicity, rabbit (2nd study)

Pyraclostrobin (batch No. CP028719; purity: 98.9%) was tested for its maternal toxicity in Himalayan rabbits. The test substance was administered as an aqueous suspension to 25 inseminated females/group by stomach tube at doses of 0, 1, 3 and 5 mg/kg bw/d on day 7 through day 28 post insemination (p.i.).
There was no unscheduled mortality in this study. No abnormal clinical signs occurred in any of the groups. 24 (low dose group only) or 25 does/group became pregnant.
A dose-related decrease in food consumption was observed at the medium and upper dose levels of 3 and 5 mg/kg bw/d throughout the administration period. As compared to the control group, the difference remained statistically significant up to study day 17 at least in the high dose group, but was apparently more pronounced during the first days of treatment. Food utilisation was impaired at the highest dose level only. The lower food consumption did not result in a significant reduction in body weight. Initially, body weight gain was affected, but this effect reached statistical significance only for the first days of exposure and in the group receiving 5 mg/kg bw/d.
Necropsy of the does did not reveal treatment-related findings.
The gestational parameters were not altered. There was no impact of test substance administration up to the highest dose of 5 mg/kg bw/d on the number or the mean body weight of the fetuses. The marginally lower mean placental weight in the highest dose group (4.2 g as compared 4.6 g in all the other groups) is considered a consequence of the incidentally higher number of live fetuses/doe in this group (7.3 live fetuses/doe at 5 mg/kg bw/d versus 6.8 in the control).

Conclusion:

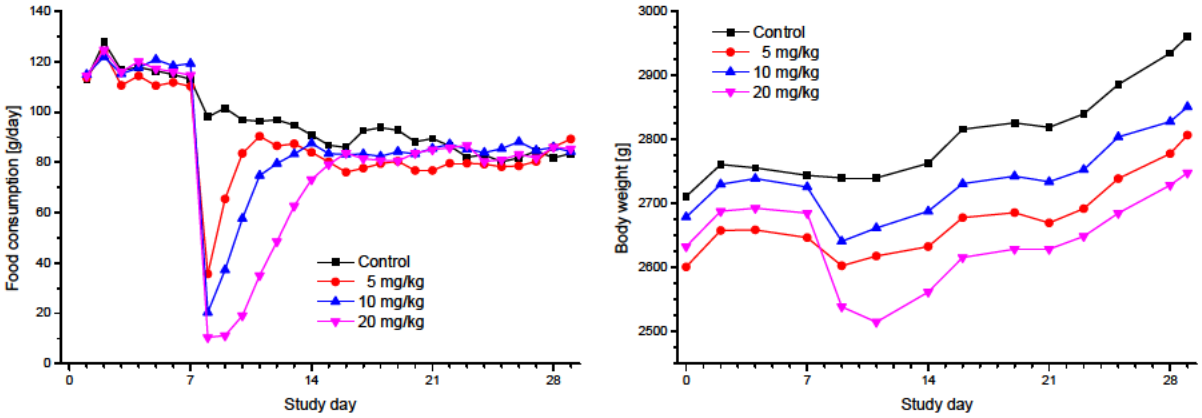
In this second study, a reduction in food consumption at the highest dose of 5 mg/kg bw/d as observed in the 1st experiment was confirmed. Again, body weight gain was reduced at this dose level, however, as compared to the control group, the difference reached statistical significance during the first three days of the exposure period only. In the mid dose group receiving a daily dose of 3 mg/kg bw from gestation days 7 through 28, food consumption was still decreased due to a significant lower food intake on days 7 and 8. In contrast to the top dose level, this effect was not accompanied by an impaired food utilisation and there was no statistically significant impact on the body weight. Thus, 3 mg/kg bw/d is considered the NOAEL for maternal toxicity.
No evidence of developmental toxicity was obtained in this additional study but the range of parameters investigated was rather limited since the foetuses were counted and weighed only.

Overall discussion

Regarding classification for developmental toxicity, the applicant considers a classification of pyraclostrobin not to be justified. For the rat the percentage of affected fetuses per litter for dilated renal pelvis (19%) was well within the historical control range (8.3 to 29.8%). The incidence of dilated ureter displayed no dose response relation-ship (0.0, 2.7. 0.6 and 3.0% at 0, 10, 25 and 50 mg/kg). Likewise, the incidence of incomplete ossified sternebra of 32.7% was within the historical control range (0 – 33.9%). Thus there is no basis for a classification.

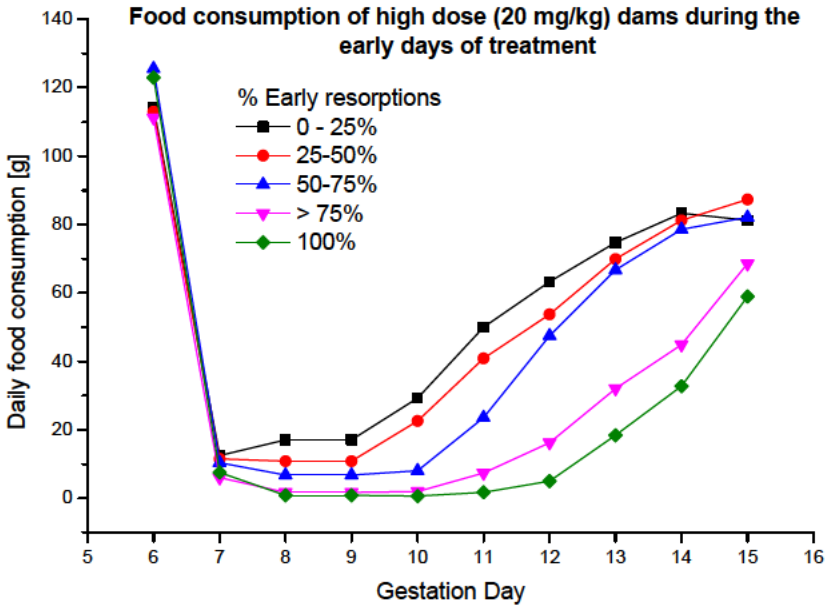
In the rabbit developmental toxicity study severe maternal toxicity as indicated by markedly reduced food consumption, body weight loss (see Figure 5.6.2-1) and clinical signs was observed. The extent and duration of the decreased food consumption was dose dependent. The mean food consumption at 20 mg/kg was decreased down to 11% of the control at days 7 and 8. The decreased food consumption resulted in a substantial body weight loss during the early phase of the pregnancy (-146.3g at 20 mg/kg bw/day vs. -3.8g in the control; days 7 to 11).

Figure 5.6.2-1: Body weight and food consumption of pregnant rabbits administered pyraclostrobin during days 7 to 28 of pregnancy



Secondary to the substantial decrease of food consumption during and shortly after implantation, embryotoxicity was increased at the high dose level (post implantation loss 38.6% ($p \leq 0.01$) at 20 mg/kg bw/day vs. 6.2% in control does). This was mainly due to an increased number of early resorptions. The relation between the extent of food consumption reduction and early resorptions becomes evident when the food consumption of does having 0 – 25%, 25 – 50%, 50 – 75% and > 75% early resorptions is grouped and plotted over time (Figure 5.6.2-2).

Figure 5.6.2-2: Correlation of decreased food consumption during and shortly after implantation and early resoprtions



Does with the highest rate of early resorption had the lowest food consumption over the longest period of time. This is especially evident from does having 100% early resorptions: the average food consumption of the three females affected was 3.2 ± 0.7 g compared to 98.0 ± 27.4 g in controls, i.e. food consumption was reduced to 3.3% of the control value. These animals also displayed a much higher day 7 to 11 body weight loss of 315.7 ± 41.6 g when compared to the high dose group mean (146.3 ± 51.7 g).

There is evidence in the literature that a reduction of food consumption causes hematology and clinical chemistry changes including alterations of progesterone levels (Matsuoka T. et al., J. Toxicol. Sci., 34(1), 129-137, 2009; J. Toxicol. Sci., 37(1), 207-14, 2012). However, the extent of reduction of food consumption in these studies was less pronounced (usually down to 10 to 15% of the control), but for a longer period of time, resulting rather in abortions at ≥ 19 days than early resorptions occurring up to day 12 of pregnancy (Matsuzawa T. et al., Toxicology, 22, 255-259, 1981; Matsuoka T. et al., J. Toxicol. Sci., 31(2), 169-175, 2006).

While all literature studies cited above were conducted in New Zealand White rabbits, the study with pyraclostrobin was conducted in Himalayan (Russian) rabbits. There is evidence from a maternal stress study that Himalayan rabbits respond to stress with an increased number of early resorptions (Schneider S. et al., Abstract 1087v, The Toxicologist, Supplement to Toxicological Sciences, 138(1), 2014, BASF DocID 2014/1177661). In this study groups of 25 pregnant rabbits were either restrained (15 min) or restrained (15 min) and dressed with stretchable bandages for 6 hours during days 6 – 28 of pregnancy. All findings were assessed against an untreated control. While neither 'treatment' resulted in changes to food consumption, maternal body weight development, any clinical signs or any fetal effects (weight, incidence of external, visceral or skeletal malformations or variations), 4 does aborted and a post-implantation loss of 50 ± 40.7% (47.1 ± 42.1% early resorptions) was observed in rabbits exposed to both stressors (restraint and wrapping). No such effects were noted in animals which were only subject to restraint.

Changes to clinical condition and a dramatic drop of food consumption are likely to result in stress in pregnant Himalayan rabbits comparable to the restraint and wrapping treatment in this study. It is likely that the dramatic drop of food consumption probably resulted in perturbations of the homeostasis of the severely affected rabbits, though this was not determined.

Therefore, the increased post-implantation loss is not considered to be a specific substance-related effect but secondary to the severe maternal toxicity. Furthermore, the Himalayan rabbit may be more susceptible than New Zealand White rabbits to perturbations of the normal homeostasis during or shortly after implantation.

The second finding discussed in the DAR and its amendments was an apparent, but not statistically significant increase of skeletal malformations (fetal incidence: 6, 4, 5, and 9; litter incidence: 6, 4, 4, 7; % affected fetuses per litter: 3.8 ± 7.0, 2.2 ± 5.0, 5.3 ± 13.22 and 12.1 ± 24.51 at 0, 5, 10, and 20 mg/kg bw/d, respectively). These malformations mainly pertained to the vertebrae, 'absent/missing lumbar vertebra' was the most common finding. While the total number of vertebral malformations was outside the historical control at the time of reporting, additional historical control data, which included studies performed later, demonstrated that the incidence was actually within the historical control range. These data were discussed in the 4th Addendum to the DAR and are depicted in Figure 5.6.2-3.

As evident from Figure 5.6.2-3, vertebral malformations are relatively common and mostly evenly distributed between controls and treated groups. There are two exceptions – the pyraclostrobin (BAS 500 F) study and study 92082. In both cases the incidence of vertebral malformations at the high dose was considerably higher than in the concurrent controls. However, the incidences are still within the spontaneous occurrence of this finding as indicated by the second of the 2001 studies, which likewise had 7 malformed vertebrae in the control group. Study 92082 is interesting for another reason: In study 92100 the same test-item as in study 92082 was used and the apparent increase of vertebral malformations could not be reproduced even though the rabbits were dosed with 1200 mg/kg bw/day, whereas in study 92082 dose levels of 0, 100, 400 and 1000 mg/kg bw/day were used. This demonstrates that the incidence of vertebral malformations is highly variable and observable in all groups, controls and treated, without obvious relation to treatment.

Figure 5.6.2-3: Incidence for vertebral malformations in 23 studies conducted between 1991 and 2001

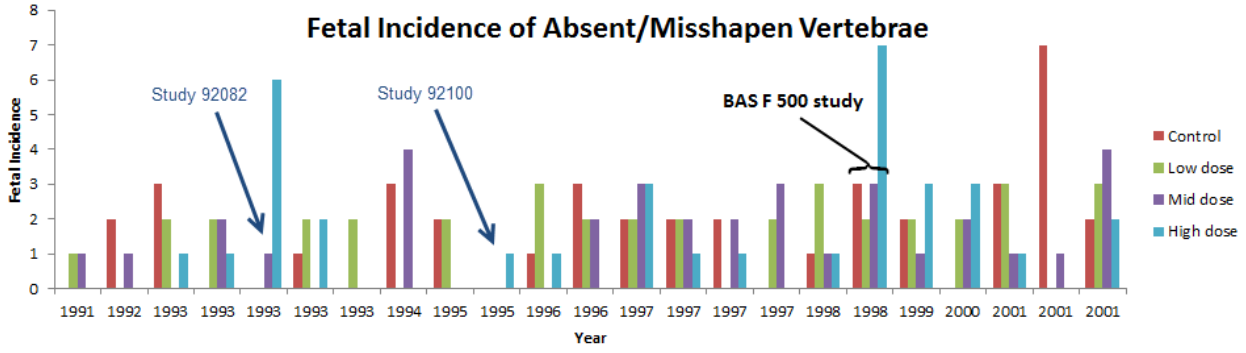
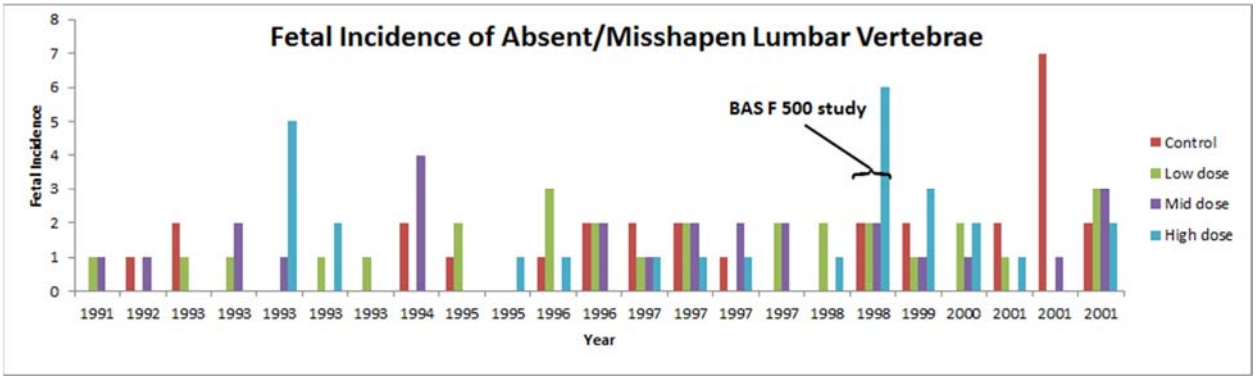


Figure 5.6.2-4: Incidence for lumbar vertebral malformations in 23 studies conducted between 1991 and 2001



The incidences given in Figure 5.6.2-4 depict the incidence lumbar malformations. It is interesting to note that 110 of the 145 cases, i.e. 75.9% of vertebral malformations pertained lumbar vertebrae, i.e. lumbar vertebral malformations are quite common in Himalayan rabbits.

At the time of these studies, the lab assumed 12 thoracic and 7 lumbar vertebrae to be the "typical" pattern of the vertebral column in rabbits. If only 6 lumbar vertebrae were counted without having additional thoracic or sacral vertebrae, this was called a "missing lumbar vertebra" and classified as a malformation. However, there is evidence from the literature that, in contrast to what is observed in rodents, the thoracolumbar border and the number of presacral vertebrae generally is highly variable in rabbits. Variants in the number of vertebrae are quite common in this species. In addition to the historical data presented above, Greenaway et al. (J. Am Anim Hosp Assoc, 37, 27-34, 2001, BASF DocID 2001/1031903) found the "typical" 12/7 lumbosacral vertebral configuration in only 43.8% of the investigated rabbits. Viertel and Trieb (Laboratory Animals, 37, 19–36, 2003; BASF DocID 2003/1034159) showed that "missing lumbar vertebrae" is among the most often seen spontaneous malformations in the Himalayan rabbit breeding line used in the presented studies.
Thus the significance of extra or missing lumbar vertebrae in developmental toxicity is unclear. The lack of concordance of vertebral variation between animal species (i.e. rabbits vs. rodents) suggest that these findings might just reflect species-specific anatomic variation, without indication of a detrimental effect on health.

CA 5.7 Neurotoxicity Studies

Studies evaluated in the draft monograph of Rapporteur Member State Germany of August 01, 2001:

An acute and a short-term neurotoxicity study in rats are available for pyraclostrobin. These studies have been evaluated by European authorities and Germany as Rapporteur Member State (European Commission Peer Review Program) and were considered to be acceptable. For the convenience of the reviewer, these are summarized below as extracted from the monograph.

Table 5.7-1: Summary of neurotoxicity studies with pyraclostrobin

Study Dose levels (Batch / purity)	sex	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Adverse effects at LOAEL	Reference (BASF DocID)
Acute neurotoxicity study Wistar rat, 0, 100, 300 and 1000 mg/kg bw/d	M	1000	not obtained	no signs of neurotoxicity observed	1999/11111
	F	1000	not obtained	no signs of neurotoxicity observed	
Subchronic (3 months) neurotoxicity study Wistar rat, 0, 50, 250 and 750 (males only) and 1500 ppm (females only)	M	49.9 (750 ppm)	not obtained	no signs of neurotoxicity observed	1999/11329
	F	111.9 (1500 ppm)	not obtained	no signs of neurotoxicity observed	

In the acute neurotoxicity study the unspecific signs of toxicity were attributed rather to acute systemic toxicity and/or local effects on the digestive tract than indicative of a specific neurotoxic potential. The NOEL for neurotoxicity under the conditions of this study was 1000 mg/kg bw in both sexes, i.e. the highest dose tested.

Oral administration of pyraclostrobin to rats over three months revealed no adverse neurobehavioral effects and did not show any alterations in neuropathology investigations. The NOELs for neurotoxicity were 1500 ppm (111.9 mg/kg bw/d) in females and 750 ppm (49.9 mg/kg bw/d) in males, the highest dosages applied. As already seen in subchronic studies with pyraclostrobin, the test substance caused signs of general toxicity at 1500 ppm in females and 750 and 250 ppm in males. The no observed effect level (NOEL) for systemic effects was 250 ppm (20.4 mg/kg bw/d) in female rats and 50 ppm (3.5 mg/kg bw/d) in males.

Based on the available data, the following endpoint was determined during the previous Annex I listing process of pyraclostrobin concerning neurotoxicity/delayed neurotoxicity:

Neurotoxicity / Delayed neurotoxicity (SANCO/1420/2001)

	No neurotoxic potential (rat, acute and 13 week studies)

There are no new studies available with pyraclostrobin that could affect the overall evaluation for neurotoxicity and delayed neurotoxicity. Thus, the conclusion for relevant endpoints for the current renewal remains as follows:

Acute neurotoxicity	Not neurotoxic **No classification required**
Subchronic neurotoxicity	Not neurotoxic **No classification required**

For convenience of the reviewer brief summaries of the respective studies as extracted from the monograph of are provided under the respective chapters.

CA 5.7.1 Neurotoxicity studies in rodents

Acute neurotoxicity study, rat

Pyraclostrobin (Batch CP 029053; Purity 99.0%) was administered to groups of 10 male and 10 female Wistar rats as a single oral administration by gavage at dose levels of 0, 100, 300 and 1000 mg/kg bw.
At the high dose level (1000 mg/kg bw), piloerection was observed in 4 females. Impaired body weight change in males on day 7 was also evident. At this dose level, also diarrhoea was observed in 5 males and 4 females. In animals dosed with 300 mg/kg bw, diarrhoea was observed in 2 males and 1 female. Furthermore, soft faeces were observed in 1 male rat of the 1000 mg/kg bw group, 5 males of the 300 mg/kg bw group, and in 3 males and 1 female of the 100 mg/kg bw group. All clinical findings were observed on day 0 (day of administration) only, and were reversible by day 7 post exposure.
Functional observational batteries and motor activity measurements did not reveal evidence of treatment-related differences between the groups. The histological examination of the central and peripheral nervous system did not reveal any substance-dependent changes in the organs examined.

Conclusion:

Pyraclostrobin did not exhibit neurotoxicity following a single oral administration to rats. The unspecific signs of toxicity recorded in the present study were rather due to acute systemic toxicity and/or local effects on the digestive tract than indicative of a specific neurotoxic potential. The NOEL for neurotoxicity under the conditions of this study was 1000 mg/kg bw in both sexes, i.e. the highest dose tested.

Subchronic neurotoxicity study, rat

Pyraclostrobin was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 50, 250 and 750 (males only) or 1500 ppm (females) for 3 months.
Reduced food and water consumption was observed in animals of the high (1500 / 750 ppm) dose level, as well as in males of the mid (250 ppm) dose level. At the high dose level, body weight was reduced in males (11.9%) and females (8.9%). Body weight gain at this dose level was reduced by 17.4% (males) and 18.1% (females).
An impairment of grip strength of forelimbs at the end of the study was noted in high dose animals, but was attributed to the lower body weight. There were no test substance related effects at 50 ppm.
Neurohistopathological investigations did not show any test substance related changes at any dose level.

Conclusion:

Pyraclostrobin caused signs of general toxicity at 1500 ppm in females and 750 and 250 ppm in males. The no observed effect level (NOEL) for systemic effects was 250 ppm (20.4 mg/kg bw/d) in female rats and 50 ppm (3.5 mg/kg bw/d) in males. No signs of selective neurotoxicity were detected under the conditions of this specially designed study. The NOELs for neurotoxicity were 1500 ppm (111.9 mg/kg bw/d) in females and 750 ppm (49.9 mg/kg bw/d) in males, the highest dosages applied.

CA 5.7.2 Delayed polyneuropathy studies

As there was no indication for neurotoxicity and/or neuropathy from any of the studies conducted and as pyraclostrobin does not belong to a chemical class suspected to induce delayed neuropathies, no study is considered to be necessary and thus no further study was conducted.

CA 5.8 Other Toxicological Studies

CA 5.8.1 Toxicity studies of metabolites

Studies evaluated in the draft monograph of rapporteur member state Germany of August 1, 2001: Studies submitted for Annex I listing consisted of 3 Ames tests of water photolysis metabolites BF 500-11 (= Reg.No. 411847 = 500M60), BF 500-13 (= Reg.No. 412785 = 500M62) and BF 500-14 (= Reg.No. 413038 = 500M76). All three tests were negative. For the convenience of the reviewer, these studies are summarized below as extracted from the monograph.

Based on the available data the following assessment was drawn in the Annex I listing of pyraclostrobin:

Other toxicological studies	Three water metabolites (BF500-11, BF500-13, BF500-14) proved negative in the Ames test

Pyraclostrobin is extensively metabolised in all matrices (mammal, plant and soil/water) resulting in numerous metabolites identified. Meanwhile further metabolism studies and studies on metabolites were conducted.

Within the past few years, metabolites in food of plant and animal origin were getting more into focus within the regulatory evaluation process. In 2012, EFSA published a Scientific Opinion on approaches to evaluate the toxicological relevance of metabolites and degradates of pesticides (see: EFSA Journal 2012;10(07): 2799. [187 pp.] doi:10.2903/j.efsa.2012.2799). The opinion identifies the threshold of toxicological concern (TTC) concept as an appropriate screening tool. Pyraclostrobin is intensively metabolized in plants and animals by mainly three key transformation steps:

- N-Desmethoxylation and O-desmethylation of the side chain
- Hydroxylation of the aromatic ring systems
- Cleavage between the ring systems

These reactions followed by subsequent conjugation steps lead to a large number of metabolites. A grouping of plant metabolites into 6 groups was performed according to structure. Details of this grouping can be found mainly in M-CA 5.1 as well as in M-CA 6.7 and M-CA 6.9. An overview of this grouping is given below:

- Group 1: Desmethoxy metabolite 500M07
- **Group 2: Chlorphenyl pyrazole derivatives (500M04 and derivatives)**
- **Group 3: Anthranilic acid derivatives (500M24, 500M49 and 500M51)**
- Group 4: Hydroxylated metabolites (chlorphenyl pyrazole moiety)
- Group 5: Hydroxylated metabolites (tolyl moiety)
- **Group 6: Photo metabolite 500M76**

Due to their presence in significant amounts in the rat metabolism study, the same toxicological endpoints are also applying to the Group 1 metabolite 500M07 (BF 500-3) as well as the Group 4 and the Group 5 metabolites resulting from simple ring hydroxylation reactions (plants: 500M34, 500M54, 500M56, 500M68, 500M70, 500M71; livestock: 500M08, 500M32, 500M39, 500M45, 500M64, 500M66, 500M67). The use of the same ADI value is further corroborated by investigations performed by the Austrian authority AGES (T. Coja et al., presented e.g. at the 10th International Fresenius Conference held in Mainz in February 2012).

Whereas the metabolites in Groups 1, 4 and 5 are covered by the toxicological testing of the parent molecule, the metabolites in **Groups 2, 3 and 6** will be addressed in this section. For details regarding the coverage of metabolites by the toxicological testing of the parent pyraclostrobin see M-CA 5.1 of this dossier. An estimation of the potential and actual exposure through diet is provided in M-CA 6.9.

Additionally, the metabolites **500M02 and 500M106**, which were identified in new metabolism studies (see M-CA 5.1), are addressed.

Finally, the water photolysis metabolites **500M60 and 500M62** are disussed in this section.

Accordingly, the following metabolites have been taken into consideration and their toxicological relevance is addressed in this dossier section.

Table 5.8.1-1: Pyraclostrobin metabolites considered for potential toxicological relevance

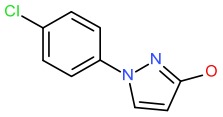
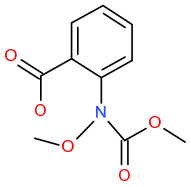
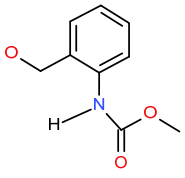
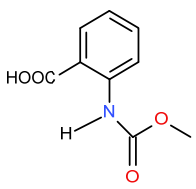
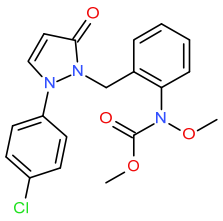
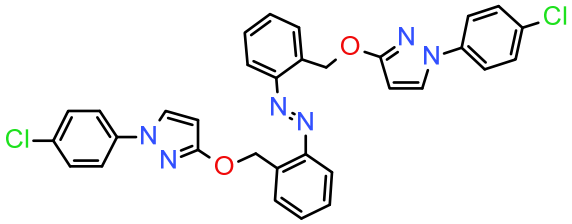
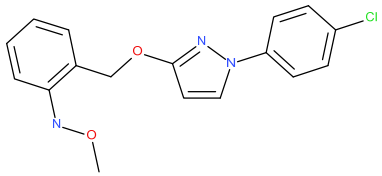
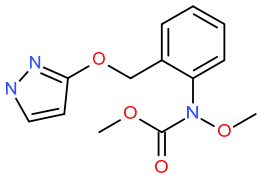
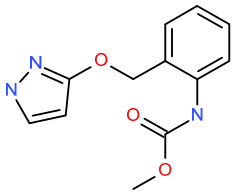
Metabolite	Structure	Reason for relevance assessment
Plant and livestock metabolites		
Group 2: Chlorphenyl pyrazole derivatives		
500M04 Reg.No. 298327		Plant metabolite in edible commodities
	Group 3: Anthranilic acid derivatives	
500M24 Reg.No. 5916421		Plant metabolite in edible commodities
500M49 Reg.No. 5916420		Plant and livestock metabolite in edible commodities
500M51 Reg.No. 78810		Livestock animal (goat) metabolite

Table 5.8.1-1: Pyraclostrobin metabolites considered for potential toxicological relevance

Group 6: Chlorphenyl pyrazole derivatives		
500M76 BF 500-14 Reg.No. 413038		Plant metabolite in feed items / Photolysis metabolite in surface water
Metabolites identified in new metabolism studies		
500M02 BF 500-7 Reg.No. 369315		In vitro metabolite
500M106 Reg.No. 399379		In vitro metabolite (also determined in rat plasma)
Water photolysis metabolites		
500M60 BF 500-11 Reg.No. 411847		Water photolysis metabolite
500M62 BF 500-13 Reg.No. 412785		Water photolysis metabolite

Based on the evaluation performed below, the conclusion for relevant future endpoints was drawn as follows:

Other toxicological studies (SANCO/11802 data point 5.8)

Toxicity studies of metabolites	**Plant and livestock metabolites** **Group 2** **500M04** - LD50 oral > 2000 mg/kg bw - not irritating to skin and eye, not a skin sensitizer - 90-day dietary rat study: NOAEL 102 mg/kg bw/day (males), 316 mg/kg bw/day (females), target organs: red-blood cell system, kidney - no evidence for genotoxicity in vitro and in vivo - ADI: 0.52 mg/kg bw/day (200 SF, 90-day rat study) **Conclusion: not toxicologically relevant** **Group 3** **500M24** - no evidence for genotoxicity in vitro and in vivo - ADI: 0.0015 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) - ARfD: 0.005 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) **Conclusion: not toxicologically relevant** **500M49** - no evidence for genotoxicity in vitro - ADI: 0.0015 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) - ARfD: 0.005 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) **Conclusion: not toxicologically relevant** **500M51** - no evidence for genotoxicity in vitro - ADI: 0.0015 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) - ARfD: 0.005 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) **Conclusion: not toxicologically relevant** **Group 6** **500M76** - no evidence for genotoxicity in vitro and in vivo - ADI: 0.0015 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) - ARfD: 0.005 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) **Conclusion: not toxicologically relevant** **Metabolites (human and rabbit) identified in in vitro comparative metabolism** **500M02** - by weight of evidence no conclusive alert for genotoxicity - not genotoxic in vitro (Ames, Mouse Lymphoma $TK^{+/-}$, micronucleus test) **Conclusion: not toxicologically relevant** **500M106** - 28-day oral gavage study in rats: NOAEL 300 mg/kg bw/day (both sexes) - evidence for genotoxicity in vitro, which was not confirmed in two in vivo genotoxicity studies (in vivo micronucleus test in mice and transgenic rodent assay in Muta™Mice): Overall weight of evidence: not genotoxic

- ADI: 0.5 mg/kg bw/day (600 SF, 28-day rat study)
Conclusion: not toxicologically relevant

Water photolysis metabolites
500M60
- no evidence for genotoxicity in vitro and in vivo
Conclusion: not toxicologically relevant
500M62
- no evidence for genotoxicity in vitro
Conclusion: not toxicologically relevant

The toxicological assessment was performed based on experimental studies and/or QSAR analyses.

QSAR evaluation of metabolites

For all metabolites identified with potential relevance, the presence for potential structural alerts was evaluated with different SAR/QSAR models. Models used, were the OECD toolbox, OASIS TIMES, DEREK (partly) and VEGA. However, the QSAR predictions obtained are limited in reliability as most of the structures evaluated were not in the prediction domain. Thus, given the structural relationship of the metabolites evaluated inter alia and in relation to the parent molecule pyraclostrobin, the predicted alerts were compared to those for the parent and those metabolites were toxicological data were available in order to overcome the limitations of the predictions made.

OECD Toolbox (Profiling module)

The OECD toolbox version 3.2 as downloadable via link of the ECHA webpage [http://www.qsartoolbox.org/download.html] was used for the evaluation. The outcome of the OECD toolbox profiling conducted for the metabolites was exported and collected [see DocID 2014/1172955]. The profiling module provided structural alerts for different endpoints. Of particular interest were the modules dealing with DNA-binding capacity as well as genotoxicity predictions. It should be noted that the profiles just provide structural alerts without consideration on probability that these alerts may become active or inactive due to chemical reactivity and/or sterical hindrance.

OASIS TIMES

OASIS TIMES is a hybrid statistical and knowledge-based model for toxicity prediction. The Tissue Metabolism Simulator (TIMES), developed by LMC (Bourgas University, Bulgaria; http://oasis-lmc.org/) integrates on the same platform a metabolic simulator and QSAR models for predicting toxicity of selected metabolites. The metabolic simulator generates plausible metabolic maps from a comprehensive library of Dettenbiotransformations and abiotic reactions. It allows prioritization of chemicals according to toxicity of their metabolites. Of OASIS TIMES the prediction models for Ames test and in vitro chromosome aberration were considered and therefore predictivity is limited to these test systems only. The reports for the evaluations made are available under DocID 2014/1172952 for Ames mutagenicity and DocID 2014/2014/1172953 for prediction of chromosomal aberration in vitro. Q(SAR) Model Reporting Formats (QMRF) for both endpoints are provided in DocIDs 2013/1414242 and 2013/1414460.

The reactivity model describing interactions of chemicals with DNA is based on an alerting group approach. Only those toxicophores extracted from the training set having clear interpretation for the molecular mechanism causing the ultimate effect are included in the model. The mechanistic interrelation between alerts and related parametric ranges generalizing the effect of the rest of the molecules on the alert, are also considered. The structural component of the model is based on the structural similarity between chemicals in the training set which were correctly predicted by the model. The structural neighborhood of atom-centered fragments is used to determine this similarity. The training set consists of 1514 chemicals for Ames and 808 chemicals for chromosomal aberration.

The derived model is combined with the metabolic simulator TIMES used for predicting metabolic activation of chemicals with the S9 mix. The metabolic simulator is trained to reproduce documented maps for mammalian liver metabolism for 261 chemicals. Parent chemicals and each of the generated metabolites are submitted to a battery of models to screen for a general effect and mutagenicity mechanisms. Thus, chemicals are predicted to be mutagenic as parents only, parents and metabolites, and metabolites only. Mutagenicity could be due to the parent chemical only or as a result of its metabolic activation (i.e., the parent is inactive but it is transformed to a mutagenic metabolite), or both parent structure and metabolites could be mutagenic.

This OASIS QSAR system is also included in the OECD Toolbox (but not in combination with TIMES), in order to make use of (Q)SAR approaches also in the assessment of chemicals under REACH (OECD, 2008). The BASF-internal version has the advantage that it is capable to consider metabolic transformation.

VEGA

Using the VEGA platform, access to a series of QSAR (quantitative structure-activity relationship) models for regulatory purposes was obtained. Of the models offered by VEGA [http://www.vega-qsar.eu/] only the two independent statistical prediction models for mutagenicity (Ames) were selected. The data obtained for pyraclostrobin and its metabolite can be found in DocID 2014/1172954. This report includes both models used (CAESAR and SarPy). CAESAR makes predictions based on the comparison of the structure of interest to the CAESAR database of mutagenicity data of substances in the structure database. A score is provided for the match of the structures, and the mutagenicity data of the closest related substances compared to the structure of interest. Consequently, if a structure is not adequately presented in the database, the prediction is only of very limited validity.

The second algorithm SarPy searches for isolated structural alerts of substructures in the molecule. Again, this is based on the mutagenicity data provided in the structure database. Only predictivity for mutagenicity based on the Ames test was generated.

DEREK

DEREK Nexus (http://www.lhasalimited.org/) is a knowledge-based expert system for the prediction of toxicological hazard. It is designed to assist chemists and toxicologists in predicting toxicological hazards based on an analysis of chemical structure. The DEREK Nexus knowledge base is written and maintained by experts. A collaborative group of major agrochemical, pharmaceutical, chemical and regulatory organisations oversees the development of the DEREK Nexus system and knowledge base.

DEREK Nexus uses a knowledge base, which contains alerts describing structure-toxicity relationships, with an emphasis on the understanding of mechanisms of toxicity and metabolism.

DEREK Nexus identifies any toxophores or substructures associated with toxicity, and highlights these to the user with a brief statement about the hazard it represents. The user can access additional information concerning the structure-toxicity relationship including literature references and supporting examples.

The knowledge base covers a wide variety of important toxicological end points, which include mutagenicity and neurotoxicity.

DEREK analysis for mutagenicity and neurotoxicity were performed for pyraclostrobin and a number of metabolites. The investigated species were given in the report as ‘bacterium, Escherichia coli, Salmonella typhimurium, all mammalien species’ [see BASF DocID 2011/1022602].

CAVEAT on reliability of QSAR modules implied

With regard to the QSAR evaluations as implied in the OECD toolbox, in OECD TIMES and in VEGA it should be noted that for nearly all analysis the algorithm reported an out of structural domain error. Each of this QSAR models is build on a set of chemicals that forms its chemical domain, space or applicability domain. That means that the prediction is best if a structure of interest is represented in the original baseline dataset. Substances outside of the dataset are evaluated in comparison to the chemical space, and only in case that the chemical space adequately covers all structural elements or the querried structure, the prediction is considered to be adequately covered by experimental data. Predictions outside of the applicability domain have far lower predictability. In addition, all mentioned QSAR models check for structural alerts, like those identified by the Benigni-Bossa rules that have been implicated in mutagenic actions.

As a consequence, the predictivity is solely based on the proposed DNA-interaction via the structural alert, not (OECD toolbox and VEGA) or not appropriately (OASIS TIMES) taking into account possible functional group interaction and stereochemical hindrance. It is well established that structure elements have to be evaluated within the context of a structure.

To evaluate the predictivity of each QSAR model, we also tested pyraclostrobin and all metabolites for which experimental data were available. The genotoxic alerts identified in these structures, that did not result in positive experimental genotox results are considered to be false positive.
In general, the predictivity of various QSAR models for genotoxicity equilvalent to the Ames test has been considered to be reasonable accurate. Predictivity rates expressed as accuracy and specificity are usually >80%. This is in particular true, if information from more than one QSAR model is combined.

QSAR models for chromosome aberration however are far less well established. One of the underlying limitations is that typical in vitro assays for chromosome aberration, like chromosome aberration in V79 cells or the in vitro micronucleus assay have false discovery rates of approximately 30%. This means that three out of ten molecules are falsely categorized. In addition, a high number of in vitro positive substances are negative in adequate in vivo assays. The later is often a function of the underlying mode of action and the kinetic behavior of a substance. Both are not adequately covered by QSAR predictions.

Predictions for chromosome damage were performed with OASIS Times and OECD Toolbox. The prediction model used in the OECD toolbox is similar to that of OASIS Times, with the major difference being the different underlying database of reference compounds and the combination with the metabolism generator in OASIS Times.

BASF has tested the valididy of the the OASIS Times predictions for chromosome aberration. For this > 100 chemicals with in vitro experimental data on chromosome aberration were evaluated with OASIS Times. It is important to note that all chemicals from this subset were within the applicability domain of the algorithm. The concordance between the predicted values and the experimental values was below 50%. This was even worse for a second set of chemicals, which was outside of the applicability domain of the QSAR model.

The above is exemplified with the data provided in this chapter (M-CA 5.8.1). Table 5.8.1-2 gives a short summary of the OECD Toolbox predictions and the actual results of the mutagenicity testing of pyraclostrobin and its tested metabolites. There were 4 predictions of a positive (mutagenic) outcome of the Ames test. Three of the positive outcomes were contradicted by negative test results. The fourth prediction (500M106) was not confirmed in the Ames test under the conditions of the actual test, though the in vitro gene mutation test in mammalian cells was positive. However, two in vivo genotoxicity assays (micronucleus assay in mice and a transgenic rodent assay) were clearly negative. Thus, the weight of evidence for 500M106 shows that there is no in vivo genotoxicity potential. In 8 cases the negative Ames test was confirmed by a mutagenicity assay in mammalian cells.

Table 5.8.1-2: Comparison of genotoxicity test results with QSAR predictions made by the OECD Toolbox

Identifier	Ames QSAR	Ames Test	Mam. gene mutation test	CA in vitro QSAR	CA in vitro Test	CA in vivo Test
Pyraclostrobin	negative	negative	negative	positive	negative	negative
500M60 (RegNo 411847)	negative	negative	negative	negative	positive	negative
500M62 (RegNo 412785)	positive	negative		positive	negative	
500M76 (RegNo 413038)	negative	negative	negative	negative	positive	negative
500M49 (RegNo 5916420)	positive	negative	negative	positive	negative	
500M51 (RegNo 78810)	negative	negative	negative	negative	negative	
500M24 (RegNo 5916421)	negative	negative	negative	negative	positive	negative
500M04 (RegNo 298327)	negative	negative	negative	negative	positive	negative
500M106 (RegNo 399379)	positive	negative	positive	positive	negative	negative
500M02 (RegNo 369315)	positive	negative	negative	positive	negative	

In the case of in vitro chromosome aberration 5 out of 10 predictions were positive. Only one of the predictions was actually correct (the one for 500M76 that was predicted and tested to be negative), 5 were false positive and 4 were false negative. It is important to note that in all cases of a positive in vitro CA result, the respective in vivo higher tier study was negative. As a conclusion, in the case of in vitro CA the QSAR predictions are not better than one could expect from tossing a coin.

Table 5.8.1-3: Comparison of genotoxicity test results with QSAR predictions made by OASIS TIMES

Identifier	Ames QSAR	Ames Test	Mam. gene mutation test	CA in vitro QSAR	CA in vitro Test	CA in vivo Test
Pyraclostrobin	negative	negative	negative	positive	negative	negative
500M60 (RegNo 411847)	negative	negative	negative	positive	positive	negative
500M62 (RegNo 412785)	positive	negative		positive	negative	
500M76 (RegNo 413038)	negative	negative	negative	positive	positive	negative
500M49 (RegNo 5916420)	positive	negative	negative	positive	negative	
500M51 (RegNo 78810)	negative	negative	negative	positive	negative	
500M24 (RegNo 5916421)	negative	negative	negative	negative	positive	negative
500M04 (RegNo 298327)	negative	negative	negative	negative	positive	negative
500M106 (RegNo 399379)	positive	negative	positive	positive	negative	negative
500M02 (RegNo 369315)	positive	negative	negative	positive	negative	

Due to the similarity of the algorithms behind OASIS TIMES and the OECD Toolbox, the predictions of OASIS TIMES were comparable to a great extent. The predictions for the Ames test were identical, whereas for the in vitro CA three of the OECD Toolbox negative predicted outcomes were predicted positive by OASIS TIMES [see Table 5.8.1-3]. This time the prediction for in vitro CA was correct in 2 cases, 6 were false positive and 2 were false negative. This is at the end not much better than the prediction of the OEDC Toolbox.

The VEGA system - with the CAESAR and SarPy modules used - only predicts bacterial mutagenicity. CAESAR had 5 positive and 5 negative predictions, i.e. was wrong in at least 4 cases. The fifth positive (500M106) was negative under the actual conditions of the Ames test, but positive in the mammalian cell mutagenicity test (mouse lymphoma assay). However, two in vivo genotoxicity assays (micronucleus assay in mice and a transgenic rodent assay) were clearly negative. Thus, the weight of evidence for 500M106 shows that there is no in vivo genotoxicity potential. The prediction of the SarPy system was positive in all cases, i.e. 90% of the predictions were definitively wrong.

The best prediction was finally obtained from DEREK. For none of the 6 evaluated molecules a genotoxic potential was predicted, which was 100% correct considering the in vivo micronucleus assay as higher tier study to the positive in vitro CA study, leading to the weight of evidence conclusion that the respective molecules are not genotoxic.

The applicant therefore believe that predictions made by the applied QSAR models have to be considered with substantial care and need to be evaluated based on a read across to similar structures (pyraclostrobin parent and metabolites with experimental data) that contain the respective structural elements. At least for pyraclostrobin and its metabolites the chemical space is not sufficiently represented in the databases used by the various QSAR models.

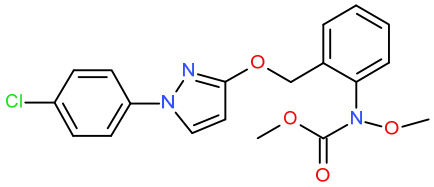
Threshold of toxicological concern concept

A further approach to assess whether chemical structures are of concern for which no or only limited information on the toxicological profile is available, is to consider whether the predicted exposure is above or below a threshold of toxicological concern. The threshold of toxicological concern (TTC) [Cramer et al. 1978a, DocID 1978/1001324; Kroes et al. 2004a, DocID 2004/1036074; Munro et al 1996a, DocID 1996/1005180] has meanwhile been considered in the EU e.g. for the evaluation of chemicals under the REACH regulation [*ECHA (2012) Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health*] and has been employed or considered for the evaluation of food flavourings [*EFSA, 2010e. Guidance on the data required for the risk assessment of flavourings to be used in or on foods. European Food Safety Authority. The EFSA Journal 8(6): 1623. Available at: http://www.efsa.europa.eu/en/efsajournal/doc/1623.pdf*] or pesticidal degradation products by EFSA [*EFSA, 2012b. Scientific Opinion: Exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). European Food Safety Authority (EFSA) Scientific Committee, Parma, Italy; EFSA Journal, 10(7), 2750 and EFSA, 2012a. Scientific opinion on evaluation of the toxicological relevance of pesticide metabolites for dietary risk assessment. EFSA Panel on Plant Protection Products and their Residues (PPR), European Food Safety Authority (EFSA), Parma, Italy; EFSA Journal, 10(07), 2799*].

The proposed threshold levels are 0.0025 µg/kg bw/day for potentially genotoxic compounds and 1.5 µg/kg bw/day for non-genotoxic Cramer Class III compounds.

The available toxicological database for metabolites will be discussed in the following paragraphs. This starts with an assessment of the QSAR predictions of the parent pyraclostrobin and is followed by the metabolites indicated above.

Pyraclostrobin (other denominators: BAS 500 F, 500M00, Reg.No. 304428, CAS-No. 175013-18-0)



Available experimental genotoxicity information (see M-CA 5.4):

Ames:	negative
In vitro chromosome aberration	negative
In vitro mammalian gene mutation	negative
In vivo chromosome aberration (MNT)	negative

QSAR predictions:

OECD Toolbox (Version: 3.2) [see DocID 2014/1172955]

In the OECD toolbox no DNA alerts for genotoxicity (Ames, MN, CA) and no alerts for DNA or protein binding were observed.

OASIS TIMES (MIX V.2.27.13 TB; Mutagenicity S-9 activated v08.08) [see molecule 1 of report DocID 2014/1172952 and 2014/1172953]

There were no Ames mutagenicity alerts for pyraclostrobin or in-silico generated metabolites. In all cases the structures were out of domain. For in vitro chromosome aberration the prediction for pyraclostrobin was negative (out of domain). Four out of 10 in-silico metabolites were predicted positive (in domain) with the alert info 'phenols'. The remaining in-silico metabolites were negative (out of domain).

Discussion: The in vivo and in vitro data indicates no chromosome damaging potential of pyraclostrobin. Therefore, the positive prediction for CA is not reflecting the toxicological database and is rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.9) [see molecule 1 of report DocID 2014/1172954; for both VEGA models]

Pyraclostrobin is out of the model applicability domain. The prediction is 'mutagen' with no specific structural alerts. Three of the six most similar molecules had positive experimental data. The other three had the prediction non-mutagen, which was experimentally confirmed. The concordance of the total underlying database was low (0.341) and thus is not very robust. Neither the three positive (similarity 0.593 to 0.614) nor the three negative molecules (similarity 0.583 to 0.611) has a reasonably similar structure to pyraclostrobin. Therefore, the chemical space does not adequately cover the structure of pyraclostrobin.

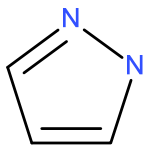
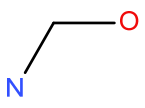
Discussion: Pyraclostrobin did not show genotoxic activity in bacterial or mammalian in vitro assay. Thus, it is apparent, that the positive prediction for genotoxicity does not reflect the experimental evidence. Therefore, the positive prediction is rejected.

VEGA: Mutagenicity model (SarPy; version 1.0.4-BETA)

Pyraclostrobin is out of the model applicability domain. The prediction is 'mutagen' with the structural alerts SA 96 and SA 98. The prediction for the entire molecule is based on the same 6 molecules, 3 of them positive as for the CAESAR model.

The SA 96 alert (structure see below) is based on 3 molecules in the training data set, two of them were experimentally tested positive. The structure similarity for the three compounds is 0.541, 0.52 and 0.47. Therefore, the compounds are considered to be structurally dissimilar from pyraclostrobin. Pyraclostrobin did not show genotoxic activity in bacterial or mammalian in vitro assay. Thus, it is apparent, that the positive prediction for genotoxicity does not reflect the experimental evidence. Therefore, the positive prediction is rejected.

The same structural element was seen in a number of metabolites for which in vitro bacterial genotoxicity data is available, namely 500M60 (Reg.No. 411847), 500M76 (Reg.No. 413038), 500M62 (Reg.No. 412785), 500M04 (Reg.No. 298327) and 500M02 (Reg.No. 369315). None of them was mutagenic. Together with the data generated for pyraclostrobin, this clearly demonstrates that this chemical structure class has no intrinsic genotoxic potential. Therefore, the positive prediction is rejected.

Structure Alert SA 96	Structure Alert SA 98
	

The SA 98 alert (structure see above) is based on 3 molecules in the training data set, with only one of them experimentally tested positive. The lack of concordance indicates that even for the model's database SA98 is not predictive. The structure similarity for the three compounds is 0.577, 0.571 and 0.57. Therefore, the compounds are considered to be structurally dissimilar from pyraclostrobin. Pyraclostrobin did not show genotoxic activity in bacterial or mammalian in vitro assay. Thus, it is apparent, that the positive prediction for genotoxicity does not reflect the experimental evidence. Therefore, the positive prediction is rejected.
The same structural element was seen in a number of metabolites for which in vitro bacterial genotoxicity data is available, namely 500M60 (Reg.No. 411847), 500M76 (Reg.No. 413038), 500M24 (Reg.No. 4916421), 500M49 (Reg.No. 5916420), 500M51 (Reg.No. 78810), 500M62 (Reg.No. 412785). None of them was mutagenic. Together with the data generated for pyraclostrobin parent, this clearly demonstrates that this chemical structure class has no intrinsic genotoxic potential. Therefore, the positive prediction is rejected.

DEREK (Version 2.0.0.201011301322; Knowledge base version: 11_20_05_2010)
The prediction for genotoxicity and neurotoxicity was negative.

Summary and discussion:

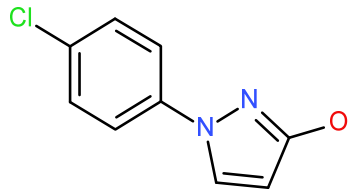
Pyraclostrobin does not induce mutagenic effects in in vitro and in vivo studies (see M-CA 5.4). All QSAR analysis models were out of domain and are thus of low predictivity. Analysis by the OECD Toolbox was negative. OASIS Times predicted no mutagenicity in bacteria (Ames test). The alert for chromosome aberration is not reflected by the available experimental data for pyraclostrobin and metabolites of similar structures and therefore is rejected. The positive prediction for genotoxicity by CAESAR is based on structures with low structural similarity and the concordance of the experimental data in the CAESAR database is only 0.341 and thus considered not to be predictive. The structural alerts SA98 and SA96 were identified via SarPy. Like for the CAESAR model the concordance and similarity was low. Based on the negative experimental results for pyraclostrobin and a number of metabolites containing the same structure elements, predictions based on SA96 or SA98 are rejected. In the following descriptions of the database for the respective metabolites, the structural alerts SA96 and SA98 identified by SarPy will therefore automatically be rejected. Prediction for genotoxicity with DEREK was negative.

This summary demonstrates that the positive association to mutagenicity or chromosome damage identified by the various QSAR algorithms are not reflected by the experimental database of pyraclostrobin.

Plant Metabolites

Group 2: Chlorphenyl pyrazole derivatives

500M04 (other denominators: Reg.No. 298327, BF 500-5, 'Pyrazolon')



500M04 (Reg.No. 298327) is a metabolite found in all compartments, i.e. soil, water, plants, livestock, fish, rat as well as in comparative in vitro metabolism studies using human, rat, rabbit and dog hepatocytes. In addition, a chlor-phenyl hydroxylated and a number of conjugated derivatives of 500M04 (Reg.No. 298327) have been identified.

QSAR Predicitions on 500M04 (Reg.No. 298327, BF 500-5, 'Pyrazolon')

OECD Toolbox (Version: 3.2) [see DocID 2014/1172955]

For 500M04 (Reg.No. 298327) the OECD toolbox did not indicate DNA alerts for genotoxicity (Ames, MN, CA) or alerts for protein binding.

Nonetheless, there is a DNA-binding alert: '*Radical|Radical >> Radical mechanism via ROS formation (indirect)|Radical >> Radical mechanism via ROS formation (indirect) >> Hydrazine Derivatives*'

Discussion: In view of the overall negative mutagenicity data package (as shown under paragraph E: genotoxicity studies) the DNA binding alert is rejected.

OASIS TIMES (MIX V.2.27.13 TB; Mutagenicity S-9 activated v08.08) [see molecule 8 of report DocID 2014/1172952 and 2014/1172953]

There were no Ames mutagenicity alerts for 500M04 (Reg.No. 298327) or in-silico generated metabolites. In all cases the structures were out of domain. The same holds true for the in vitro chromosome aberration.

VEGA: Mutagenicity model (CAESAR, version 2.1.9) [see molecule 8 of report DocID 2014/1172954; for both VEGA models]

500M04 (Reg.No. 298327) is out of model applicability domain. The prediction is 'mutagen' with no specific structural alerts. The prediction is based on 6 molecules, of which only one was a predicted and actual mutagen. The similarity to 500M04 (Reg.No. 298327) of the molecules in the data set is low as indicated by similarity factors of 0.583 to 0.628. Based on the negative Ames test (see CA 5.8.1/9) the positive prediction for genotoxicity does not reflect the experimental evidence. Therefore, the positive prediction is rejected.

VEGA: Mutagenicity model (SarPy; version 1.0.4-BETA)

500M04 (Reg.No. 298327) is out of model applicability domain. The prediction is 'mutagen' with the structural alert SA 96. As discussed above, this alert was also obtained for pyraclostrobin and a number of pyraclostrobin metabolites, which like 500M04 (Reg.No. 298327) were actually tested negative. The predicition is therefore rejected.

DEREK (Version 2.0.0.201011301322; Knowledge base version: 11_20_05_2010)

The prediction for genotoxicity and neurotoxicity is negative.

Acute toxicity studies on 500M04 (Reg.No. 298327, BF 500-5, 'Pyrazolon')

Report:	CA 5.8.1/11 [redacted] 1997a Study on the acute oral toxicity of Pyrazolon in rats 1997/10963
Guidelines:	EEC 92/69, Official Journal of the European Communities L383A (1992)
GLP:	yes (certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Executive Summary

In an acute oral toxicity study 6 Wistar rats (3/sex) were given a single oral dose via gavage of 'Pyrazolon' (synonym to Reg.No. 298327, 500M04, BF 500-5; batch 27967/95) suspended aqueous solution of 0.5% tylose CB 30.000 at a dose level of 200 and 2000 mg/kg bw.

No mortality occurred in both dose groups. Accordingly, the oral LD_{50} was found to be greater than 2000 mg/kg bw for rats.

Rat, oral: LD_{50} > 2,000 mg/kg bw

Signs of toxicity were restricted to male animals of the 2000 mg/kg bw dose group and consisted of impaired or poor general state, dyspnea, apathy and staggering. These symptoms are considered to be unspecific toxicity symptoms. The animals appeared normal two days after application. The expected body weight gain was observed in the course of the study, with the exception of one female animal of the 200 mg/kg bw dose group which showed weight reduction. No abnormalities were noted at necropsy of animals sacrificed at the end of the study.

(BASF DocID 1997/10963)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: 1-(4-chlorphenyl)-1,2-dihydro-3H-pyrazol-3-on (Pyrazolon)

Description:	crystalline powder, light-beige
Lot/Batch #:	27967/95
Purity:	100.5 g/100 g
Stability of test compound:	The stability of the test substance in 0.5% tylose CB 30.000 in aqua bidest. For a time period of 4 hours was confirmed by analysis.
CAS-No.	76205-19-1

2. Vehicle: 0.5% tylose CB 30000 (cleaned natriumcarboxy-methylcellulose in aqua bidest

3. Test animals:

Species:	Rat
Strain:	Wistar (CHBB: THOM (SPF))
Sex:	males and females
Age:	young adults
Weight at dosing (mean):	mean female: 173 g; mean male: 179 g
Source:	Dr. K. Thomae GmbH, Biberach, Germany
Acclimation period:	at least one week
Diet:	Kliba-Labordiaet 343, Klingentalmuehle AG, Kaiseraugust, Switzerland, ad libitum except 16 hours before administration (fasting period)
Water:	tap water ad libitum
Housing:	Single housing in stainless steel wire mesh cages, type DK-III (Becker & Co, Castrop-Rauxel, Germany)
Environmental conditions:	
Temperature:	20 – 24°C
Humidity:	30 – 70%
Air changes:	no data available
Photo period:	12-hour light-dark cycle (06:00 – 18:00, 18:00 – 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work:

30-May-1996 to 09-Jul-1996 (day of first administration and day of last administration + 14 days observation period)

2. Animal assignment and treatment:

6 rats (3 per sex per group) received a single dose of each 200 or 2000 mg/kg bw of test substance in 0.5% aqueous suspension of tylose CB 30.000 by oral gavage. Test substance was given to the animals at a volume of 10 mL/kg bw. The observation period lasted 14 days. Individual body weights were recorded shortly before application (day 0) and weekly thereafter and at the end of the study. Clinical signs and symptoms were recorded several times on the day of administration, at least once each workday for the individual animals. After the observation period a necropsy of all animals with grosspathology followed.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred after oral administration of 200 and 2000 mg/kg bw of the test substance.

B. CLINICAL OBSERVATIONS

At a dose of 2000 mg/kg bw p.o. male rats showed an impaired or poor general state, dyspnea, apathy, staggering and staggering on the day of administration whereas female rats did not show any symtpoms. Likewise, no symptoms were noted for males and females at the dose of 200 mg/kg bw.

C. BODY WEIGHT

Normal body weight gain was observed during the 14 days of the observation period with exception of a 200 mg/kg female, which displayed a body weight loss during the second week of observation.

D. NECROPSY

No macroscopic anomaly was noted in the organs examined after oral treatment.

III. CONCLUSION

Under the experimental conditions of this study the oral LD_{50} of 'pyrazolon' (synonym to Reg.No. 298327, 500M04, BF 500-5) in rats was determined to be greater than 2000 mg/kg bw for males and females.

Report: CA 5.8.1/12
[redacted] 1997b
Study on the acute dermal irritation/corrosion of Pyrazolon in the rabbit
1997/10964
Guidelines: OECD 404, EEC 92/69 B 4
GLP: yes
(certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Executive Summary

In a primary dermal irritation study the skin irritation/corrosion potential of pyrazolon (synonym to Reg.No. 298327, 500M04, BF 500-5, Batch No. 27967/95) was tested. The clipped intact skin of three New Zealand White rabbits was exposed to 0.5 g of the test-substance for four hours under semiocclusive dressing. Observation of local reactions was performed 1, 24, 48 and 72 hours after removal of the patch. No erythema or edema of intact skin was noted after 24 to 72 hours in the 3 rabbits treated with pyrazolon. Based on the findings of this study pyrazolon does not show a skin irritation potential.

(BASF DocID 1997/10964)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: 1-(4-chlorphenyl)-1,2-dihydro-3H-pyrazol-3-on (Pyrazolon)

Description:	crystalline powder, light-beige
Lot/Batch #:	27967/95
Purity:	100.5 g/100 g
Stability of test compound:	The stability of the test substance over the study period has been proven by reanalysis.

2. Vehicle: test substance was used unchanged

3. Test animals:

Species:	Rabbit
Strain:	White New Zealand (SPF)
Sex:	2 males and 1 females
Age:	young adults
Weight at dosing (mean):	female: 3.87 kg; mean male: 3.37 kg
Source:	Dr. K. Thomae GmbH, Biberach, Germany
Acclimation period:	at least one week
Diet:	Kliba-Labordiaet 341, Klingentalmuehle AG, Kaiseraugust, Switzerland, about 130 g/day
Water:	about 250 mL/day tap water
Housing:	Single housing in stainless steel wire mesh cages with grating, floor area: 3000 cm²
Environmental conditions:	
Temperature:	20 – 24°C
Humidity:	30 – 70%
Air changes:	no data available
Photo period:	12-hour light-dark cycle (06:00 – 18:00, 18:00 – 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 15-Jul-1996 (day of application)

2. In vitro pre-test: No in vitro pre-test was conducted.

3. Animal assignment and treatment:

The potential of pyrazolon to cause acute dermal irritation or corrosion was assessed by a single topical application of the unchanged test substance (moistened with water) to the clipped intact skin of three New Zealand White rabbits. The test patch (2.5 cm x 2.5 cm) was covered with a dose of 0.5 g of moistened unchanged test substance and applied to the skin (upper third of the back or flank). The patch was secured in position with a semiocclusive dressing (Idealbinde, Pfaelzische Verbandstoff-Fabrik, Kaiserslautern and Fixomull® stretch (adhesive fleece), Beiersdorf AG) for 4 hours. The test substance was removed at the end of the exposure period with Lutrol® (=PEG 400) and Lutrol ®/Water (1:1). Observation of local reactions was performed 1, 24, 48 and 72 hours after removal of the patch. At the beginning of the study the body weights were recored. A check for any dead or moribund animals was made twice each workday and one on Saturdays, Sundays and on public holidays.

II. RESULTS AND DISCUSSION

No erythema or edema of intact skin was noted after 24 to 72 hours in the 3 rabbits treated with pyrazolon Table 5.8.1-4. All animals showed after one hour after removal of the test patch an erythema with a score of 1 which was fully reversible until the next reading time point (24 hours).

Table 5.8.1-4: Skin irritation study of 'Pyrazolon' in New Zealand White rabbits

Readings	Animal	Erythema	Edema	Additional findings
	01 (♂)	1	0	
1 h	02 (♂)	1	0	
	03 (♀)	1	0	
	01	0	0	
24 h	02	0	0	
	03	0	0	
	01	0	0	
48 h	02	0	0	
	03	0	0	
	01	0	0	
72 h	02	0	0	
	03	0	0	
Individual means 24 – 72 h	01	0.0	0.0	
	02	0.0	0.0	
	03	0.0	0.0	
24-72 h mean	all	0.0	0.0	

S: Scaling

III. CONCLUSION

Based on the findings of this study 'pyrazolon' (synonym to Reg.No. 298327, 500M04, BF 500-5) does not show a skin irritation potential under the test conditions chosen.

Report: CA 5.8.1/13
[redacted] 1997c
Study on the acute eye irritation of Pyrazolon in the rabbit
1997/10965
Guidelines: OECD 405, EEC 92/69 B 5
GLP: yes
(certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Executive Summary

In an eye irritation study, the eye irritation/corrosion potential of pyrazolon (synonym to Reg.No. 298327, 500M04, BF 500-5; batch 27967/95) was determined by instillation of 0.1 mL bulk volume (about 72 mg) of the test substance into the conjunctival sac of the right eye of three New Zealand White rabbits.

The ocular reactions were assessed approximately 1, 24, 48 and 72 hours after the administration of the test substance.

No reaction concerning the corneal opacity, iritis and conjunctival chemosis was observed during the observation period. Slight conjunctival redness was observed in two animals during the course of the study. No additional findings were noted in the animals during the observation period. The ocular reactions were fully reversible within 72 hours.

Eye irritation scores (24 to 72 hours) for each animal were 0.0 for corneal opacity, iris and conjuctival discharge and 0.3, 0.7 and 0.0 for conjunctival redness. Consequently, mean average scores for irritation were calculated to be 0.0 for corneal opacity, iris and chemosis and 0.3 for conjunctival redness.

(BASF DocID 1997/10965)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: 1-(4-chlorphenyl)-1,2-dihydro-3H-pyrazol-3-on (Pyrazolon)

Description:	crystalline powder, light-beige
Lot/Batch #:	27967/95
Purity:	100.5 g/100 g
Stability of test compound:	The stability of the test substance over the study period has been proven by reanalysis.

2. Vehicle: test substance was used unchanged

3. Test animals:

Species:	Rabbit
Strain:	White New Zealand (SPF) and HSDIF: NZW (SPF)
Sex:	1 male and 2 females
Age:	young adults
Weight at dosing (mean):	mean female: 3.67 kg; male: 3.17 kg
Source:	Dr. K. Thomae GmbH, Biberach, Germany
Acclimation period:	at least one week
Diet:	Kliba-Labordiaet 341, Klingentalmuehle AG, Kaiseraugust, Switzerland, about 130 g/day
Water:	about 250 mL/day tap water
Housing:	Single housing in stainless steel wire mesh cages with grating, floor area: 3000 cm²
Environmental conditions :	
Temperature :	20 – 24°C
Humidity :	30 – 70%
Air changes:	no data available
Photo period:	12-hour light-dark cycle (06:00 – 18:00, 18:00 – 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 22-Jul-1996 and 29-Jul-1996 (days of application)

2. In vitro pre-test: No in vitro pre-test was conducted.

3. Animal assignment and treatment:

The potential of pyrazolon to cause acute eye irritation/corrosion was assessed by instillation of 0.1 mL bulk volume (about 72 mg) of the undiluted test substance into the conjunctival sac of the right eye. The left eye, which remained untreated, served as the negative control. About 24 hours after application of the test substance, the treated eye was washed out with tap water.

The application of the test substance was performed in a stepwise procedure starting with one animal and – after no severe eye irritation was observed – completed with the treatment of two additional animals.

The ocular reactions were assessed approximately 1, 24, 48 and 72 hours after the administration of the test substance.

Body weights were measured shortly prior to application. The animals were checked for mortality and morbidity twice on working days and once daily at weekends and on public holidays.

II. RESULTS AND DISCUSSION

No ocular reaction on the cornea and iris were observed. Slight to moderate conjunctival redness and chemosis was observed in all animals at the 1 hour reading point [see Table 5.8.1-5]. Discharge was observed only in one animal. At the 72-hour reading point all effects were fully reversed.

III. CONCLUSION

Based on the findings of this study 'pyrazolon' (synonym to Reg.No. 298327, 500M04, BF 500-5) does not have an eye irritation potential under the test conditions chosen.

Table 5.8.1-5: Individual and mean eye irritation scores after ocular application of pyrazolon

Readings	Animal	Cornea		Iris	Conjunctiva			Additional findings
		Opacity	Area involved		Redness	Chemosis	Discharge	
1 h	01 (♀)	0	0	0	2	1	1	
	02 (♀)	0	0	0	1	1	0	
	03(♂)	0	0	0	1	1	0	
24 h	01	0	0	0	1	0	0	
	02	0	0	0	1	0	0	
	03	0	0	0	0	0	0	
48 h	01	0	0	0	0	0	0	
	02	0	0	0	1	0	0	
	03	0	0	0	0	0	0	
72 h	01	0	0	0	0	0	0	
	02	0	0	0	0	0	0	
	03	0	0	0	0	0	0	
Mean 24 – 72 h	01	0.0		0.0	0.3	0.0		
	02	0.0		0.0	0.7	0.0		
	03	0.0		0.0	0.0	0.0		
Mean		0.0		0.0	0.3	0.0		

Report: CA 5.8.1/14
[redacted] 1997a
Pyrazolon – Maximization test in guinea pigs
1997/10968
Guidelines: EEC 92/69, OECD 406
GLP: yes
(certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Executive Summary

For the determination of potential sensitizing properties of 'pyrazolon' (synonym to Reg.No. 298327, 500M04, BF 500-5; Batch 27967/95) a Maximization test based on the method of Magnusson and Kligman was conducted using a control and a treated group of 5 and 10 female Pirbright White Dunkin Hartley guinea pigs, respectively.

The test-substance concentrations for the main test were selected based on the results of the pretests and the result of the first challenge. The intradermal induction was performed with a 5% test-substance preparation in 1% aqueous tylose CB 30.000 solution or in Freund's complete adjuvant / 0.9% aqueous NaCl-solution (1:1). The epicutaneous induction was conducted with a 25% test-substance preparation in 1% aqueous tylose CB 30.000 solution. Two challenges were performed 14 and 21 days after percutaneous induction.

After the first challenge with a 10% test substance preparation very slight to well defined skin reactions were observed in three test group animals. The second challenge with a 10% substance preparation did not cause any skin reactions.

Based on the results of this study and applying the evaluation criteria, it was concluded that pyrazolon does not have a sensitizing effect on the skin of the guinea pig in the Maximization Test under the test conditions chosen.

(DocID 1997/10968)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: 1-(4-chlorphenyl)-1,2-dihydro-3H-pyrazol-3-on ('Pyrazolon')

Description:	crystalline powder, light-beige
Lot/Batch #:	27967/95
Purity:	100.5 g/100 g
Stability of test compound:	The stability of the test substance in 0.5% tylose CB 30.000 in aqua bidest. For a time period of 4 hours was confirmed by analysis.

2. Vehicle: 0.5% tylose CB 30000 (cleaned natriumcarboxy-methylcellulose in aqua bidest.)

3. Test animals:

Species:	Guinea pigs
Strain:	Pribright White, Dunkin Hartley, Crl:(HA)BR (SPF)
Sex:	female
Age:	young adult animals
Weight on day 0:	344 to 400 g
Source:	Charles River GmbH – Wiga, Kisslegg, Germany
Acclimation period:	7 days
Diet:	Kliba-Labordiät 341 (Kaninchen & Meerschweinchenhaltungdiaet), Klingentalmuehle AG, Kaiseraugst, Switzerland, ad libitum
Water:	tap water ad libitum (about 2 g ascorbic acid per 10 L was added twice per week)
Housing:	groups of 5 animals housed in Macrolon cages type
Environmental conditions :	
Temperature :	20 – 24°C
Humidity :	30 – 70%
Air changes:	not indicated in the report
Photo period:	12 h light / 12 h dark (6:00 a.m. – 6:00 p.m. / 6:00 p.m. – 6:00 a.m.)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 16-Jul-1996 to 16-Aug-1996

2. Animal assignment and treatment:

The skin sensitizing potential of pyrazolon was assessed using the Guinea Pig Maximization Test. This test consists of a pretest for the determination of suitable induction and challenge concentrations and the Maximization test itself.

For this, female Guinea pigs were allocated to groups according to the randomization instructions of „Nijenhuis, A. and Wilkf, H.S..: Cominatorial Algorithms, Academic Press, New York, San Francisco, London, 1978, pp. 62-64“.

For the intradermal and epicutaneous application, animal fur was clipped at least 2 hours before each test-substance application at the appropriate treatment sites. If necessary, additional hair clipping was performed 2 hours prior to the evaluation of skin reactions.

Evaluations of the skin reactions were performed according to Draize, J.H. (1959): Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Association of Food and Drug Officials of the United States Austin, Texas.

Individual body weights were determined on study day 0 and on the last day of observation. Mortality was checked twice daily on working days and once on weekends and public holidays.

A: Preliminary tests

Two animals per test substance concentration received six intradermal injections (see below) in groups of two at the neck region in order to determine a test substance concentration that is well-tolerated locally and systemically for the intradermal induction treatment in the main test. The evaluation of the skin reactions was performed 24 h after application.

Intradermal injections:

(i) front row: 2 injections each of 0.1 mL Freund’s adjuvant without test substance emulsified with 0.9% aqueous NaCl-solution in a ratio of 1:1

(ii) middle row: 2 injections each of 0.1 mL of the test substance formulation

(iii) back row: 2 injections each of 0.1 mL Freund’s adjuvant / 0.9% aqueous NaCl-solution (1:1) with test substance

Additionally, for the dermal induction treatment the highest concentration of the test substance that causes slight to moderate irritation and for the challenge the maximum non-irritant concentration was determined. The test-design took into account the possible influence of a previous intradermal treatment with Freund’s complete adjuvant on irritating effects. For this animals receiving dermal injections of 0.1 mL Freund’s complete adjuvant / 0.9% aqueous NaCl-solution at a ratio of 1:1 (w/w) three weeks prior to the epidermal pretest were used.

Four animals per test substance concentration received the test substance percutaneous at the flank in order to determine the skin reactions. The filter paper strips were coated with an approximately 0.5 mm thick layer of the test substance formulation. The dressing was occlusive and consisted of rubberized linen patches (4 x 4 cm), patches of Idealbinde (5 x 5 cm) and Fixomull® stretch (adhesive fleece). The test substance was applied 2 times for 24 hours within a period of 96 hours in order to detect nonspecific phenomena that are not caused by a sensitization reaction but could possibly be attributed to a shift in the irritation threshold. 24 and 48 hours after removal of the patch evaluations of the skin reactions were performed.

B: Main study – Induction

The intradermal induction consisted of 6 intradermal injections in groups of two per animal into the neck of the animals according to the scheme given above.

Control animals received the same injections (A, B, C) but without test substance, only with the formulated agent.

Skin reactions were assessed 24 hours after injection. The treated and control groups consisted of 10 and 5 females, respectively.

Epidermal induction was performed one week after intradermal induction. For this a 2 x 4 cm filter paper strip containing the test substance formulation were applied to the skin of the shoulder (same area as in the case of the previous intradermal application) under an occlusive dressing. The filter pater strip was soaked in the test substance formulation. The dressing consisted of rubberized linen patches (4 x 6 cm) and Fixomull® stretch (adhesive fleece). The exposure lasted 48 hours and after removal of the patch evaluations of the skin reactions were performed.

C: Challenge

The 1st challenge was carried out 14 days after epidermal induction. A 2nd challenge was performed one week after the first. For this 2 x 2 cm filter paper strip containing the test substance formulation were applied to the skin of the flank under an occlusive dressing. The filter pater strip was soaked in the test substance formulation. The dressing consisted of rubberized linen patches (4 x 4 cm), patches of Idealbinde (5 x 5 cm) and Fixomull® stretch (adhesive fleece). Exposure duration was 24 hours. Skin reactions were determined 24 and 48 hours after removal of the patches.

D: Positive controls:

A positive control (reliability check) with a known sensitizer was not performed in this study. However, positive control studies are performed twice a year in the laboratory. The positive control with alpha-hexylcinnamaldehyde techn. 85% showed that the test system was able to detect sensitizing compounds under the laboratory conditions chosen.

E: Evaluation of results:

The number of animals with skin findings at 24 and/or 48 hours after the removal of the patch was taken into account for the determination of the sensitization rate.

The evaluation "sensitizing" results if at least 30% of the test animals exhibit skin reactions in this adjuvant test.

The evaluation is based on the criteria of the Commission Directive 67/548/EEC and the OECD Harmonized Integrated Classification System that were in place on the date of report signature.

3. Analysis of treatment solutions:

The stability of the test substance in 0.5% tylose CB 30.000 in aqua bidest. For a time period of 4 hours was confirmed by analysis. The homogeneity of the test substance preparations was provided by stirring.

4. Statistics:

Not performed in this study.

II. RESULTS AND DISCUSSION

A. Pre-Test

A first intradermal injection of 5% test substance in 1% aqueous tylose CB 30000 caused moderate erythema and edema [see Table 5.8.1-6].

Table 5.8.1-6: Skin irritation scores 24 hours after intradermal injection – Preliminary test

preliminary intradermal test				
Animal #	Application site	A) Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1)	B) 5% test substance preparation in 1% aqueous tylose CB 30.000-solution	C) 5% test substance in A)
99	left	2/2	2/2	2/2
	right	2/2	2/2	2/2
100	left	2/2	2/2	2/2
	right	2/2	2/2	2/2

x/y erythema/edema
2 well defined erythema / slight edema (edges of area well defined by definite raising)

The dermal irritation scores observed 24 and 48 hours after a 48-hour epidermal exposure of 5%, 10%, 25%, 50% dilutions in 1% aqueous tylose CB 30.000 are given in Table 5.8.1-7. Signs of dermal irriation were observed at ≥ 25%.

Based on the data from the pre-test a 5% dilution of pyrazolon in 1% aqueous tylose CB 30.000-solution or in Freund's complete adjuvant / 0.9% aqueous NaCl-solution (1:1) was used for intradermal induction whereas a 25% dilution in 1% aqueous tylose CB 30.000 was used for epidermal induction. As no skin findings were observed 24 and 48 hours after dermal administration of a 10% and 5% aqueous dilution, a 10% dilution was used for the 1st and 2nd challenge.

Table 5.8.1-7: Skin irritation scores after epidermal application – Preliminary test

Animal #	Readings 24 hours after application				Readings 48 hours after application			
Application site	373tf l. Ant.	Lft fl. Ant.	Lft. Fl. Post.	Rt. Fl. Post.	373tf l. Ant.	Lft fl. Ant.	Lft. Fl. Post.	Rt. Fl. Post.
1st percutaneous application (conducted 24-Jun-1996)								
test substance preparation in 1% aqueous tylose CB 30.000-solution	50%	25%	10%	5%	50%	25%	10%	5%
29	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
30	2/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
31	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
32	2/0	2/0	0/0	0/0	1/0	0/0	0/0	0/0
2nd percutaneous application (conducted 26-Jun-1996)								
test substance preparation in 1% aqueous tylose CB 30.000-solution	50%	25%	10%	5%	50%	25%	10%	5%
29	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
30	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
31	1/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0
32	2/0	2/0	0/0	0/0	1/0	0/0	0/0	0/0

rt. Fl. = right flank
lft. Fl. = left flank
ant. = anterior
post. = posterior
x/y = erythema/edema
Grading: 0: no erythema/edema
1: very slight erythema (barely perceptible) / very slight edema (barely perceptible)
2: well-defined erythema / slight edema (edges of area well defined by definite raising)

B. Induction reactions

Intradermal induction of Freund's adjuvant/0.9% aqueous NaCl-solution (1:1) with and without 5% pyrazolon resulted in well-defined erythema and slight edema in all animals. Injections of 5% test substance preparations in tylose CB 30.000, 1% in aqua bidest. Or in Freund's adjuvant/0.9% aqueous NaCl-solution (1:1) caused well-defined erythema and slight edema in all test group animals, whereas injection of the vehicle in control group animals did not show any skin reaction [see Table 5.8.1-8].

After the percutaneous induction with a 25% test substance preparation incrustations, partially open (caused by the intradermal induction), were observed in addition to well-defined erythema and slight edema in all test group animals. Likewise, control groups 1 and 2 animals displayed the same skin reactions when tylose CB 30.000, 1% in aqua bidest, was applied.

Table 5.8.1-8: Skin irritation scores 24 hours after intradermal injection – Main test

Control Animals				
Animal #	Application site	A) Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1)	B) 1% aqueous tylose CB 30.000-solution	C) Freund's complete adjuvant / 0.9% aqueous NaCl-solution (1 : 1)
all animals	left	2/2	0/0	2/2
	right	2/2	0/0	2/2
Treated Animals				
Animal #	Application site	A) Freund's adjuvant / 0.9% aqueous NaCl-solution (1 : 1)	B) 5% pyrazolon preparation in 1% aqueous tylose CB 30.000	C) 5% pyrazolon in A)
all animals	left	2/2	2/2	2/2
	right	2/2	2/2	2/2

Grading: 2: well-defined erythema / slight edema (edges of area well defined by definite raising)

C. Challenge

After the first challenge with a 10% test substance preparation 3 out of 10 test group animals showed skin reactions 24 and 48 hours after patch removal [see Table 5.8.1-9]. Two out of these animals additionally showed scaling. Control group 1 animals did not show any skin reaction. Tylose CB 30.000, 1% in aqua bidest., which was applied as a vehicle control to all animals, did not cause any skin reaction. After the 2nd challenge with a 10% test substance preparation no skin reaction was observed in all animals of the test group and control groups 1 and 2 24 and 48 hours after removal of the patches. Tylose CB 30.000, 1% in aqua bidest, which was applied as a vehicle control to all animals, did not cause any skin reations [see Table 5.8.1-9].

Table 5.8.1-9: Challenge skin reaction scores 24 hours after intradermal injection – Main test

	1st challenge		2nd challenge	
	Test substance 10% in 1% aqueous tylose CB 30.000		Test substance 10% in 1% aqueous tylose CB 30.000	
	24 h	48 h	24 h	48 h
Control group 1				
- Grade 1	0/4	0/4	0/4	0/4
- Grade 2	0/4	0/4	0/4	0/4
Control group 2				
- Grade 1	0/4	0/4	0/4	0/4
- Grade 2	0/4	0/4	0/4	0/4
Test group				
- Grade 1	1/10	3/10	0/10	0/10
- Grade 2	2/10	0/10	0/10	0/10

x/y: number of positive reactions/number of animals tested (reading at 24 h and/or 48 h after the removal of the patch)

D. Observations/Mortality

One animal of control group 1 and one animal of control group 2 died 10 days after the beginning of the study. Macroscopic examination revealed that the animals suffered from pneumonia. The cause of death was not related to the test substance treatment.

E. Body weights

The expected body weight gain was generally observed in the course of the study.

F. Positive control

The sensitivity of Pirbright White, Dunklin Hartely Guinea pigs (Crl℗HA)BR (SPF), Charles River GmbH- Wiga, Kisslegg, Germany) and the reliability of experimental techniques is assessed regularly using a known sensitizer as recommended by the test guidelines.

The study procedures were based on the methods of Magnusson and Kligmann (1969) (Maximization Test) and Buehler, E.V. (1965) (Buehler Test) and test conditions are summarized below:

Name of test substance:	alpha-Hexylcinnamaldehyde, techn. 85%
CAS number:	101-86-0
Substance number:	98/0288-3
Purity:	98.01%
Maximization Test:	
Intradermal induction:	Test substance 5% in olive oil DAB 10 Test substance 5% in Freund's complete adjuvant / 0.9% aqueous NaCl solution (1:1)
Epicutaneous Induction:	Test substance 75% in olive oil DAB 10
Challenge:	Test substance 50% in oil DAB 10

As evident from Table 5.8.1-10 the test system and the used procedures were capable to detect the sensitizing properties of alpha-hexylcinnamaldehyde.

Table 5.8.1-10: Positive control studies – Number of animals with skin findings after the challenge in control studies with the test-substance alpha-hexylcinnamaldehyde (techn. 85%)

				Challenge					
				Test substance in the vehicle			Vehicle		
Project No.	Study type	Date of performance	Group	24 h	48 h	Total	24 h	48 h	Total
30H0387/952180	Maximization Test	Jun / Jul 1996	Control group	1/7	0/7	1/7	1/7	0/7	1/7
			Test group	16/17	11/17	16/17	0/17	0/17	0/17

x/y: number of positive reactions/number of animals tested (reading at 24 h and/or 48 h after the removal of the patch)

III. CONCLUSION

Based on the evaluation criteria the results of this study show that 'pyrazolon' (synonym to Reg.No. 298327, 500M04, BF 500-5) does not have a sensitizing effect on the skin of guinea pig in the Maximization Test under the chosen test conditions.

Short-term toxicity of 500M04 (Reg.No. 298327, BF 500-5, 'Pyrazolon')

Report:	CA 5.8.1/15 [redacted] 2013a Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Repeated dose 90-day oral toxicity study in Wistar rats - Administration via the diet 2013/1042164
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.7 No. L 142, OECD 408, EPA 870.3100, JMAFF No 12 Nosan No 8147
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Administration of Reg.No. 298327 (metabolite of BAS 500 F, pyraclostrobin; batches L84-174; Purity: 99.6%) to Wistar rats target dose levels of 0, 100, 300 and 1000 mg/kg bw/day for 3 months resulted in adverse signs of systemic toxicity at target dose levels ≥ 300 mg/kg bw/day in male and at 1000 mg/kg bw/day in female rats.

Treatment with Reg.No. 298327 did not affect the survival, body weight development or food consumption of rats. High dose (1000 mg/kg bw/day) animals displayed an increased consumption of drinking water, which resulted in an increased discharge of less concentrated urine. In mid and high dose males a massive increase of unknown crystals was noted in the urine sediment. The above changes were in line with the histopathological findings observed in the kidneys (eosinophilic material in the renal pelvis, slightly increased medullar mineralization and chronic progressive nephropathy) and ureter urothelial hyperplasia) at this dose level. However, there was no indication of renal dysfunction as indicated by the absence of respective clinical chemistry alterations.

In high dose males a slight regenerative normochromic-macrocytic anemia was noted. This was accompanied by an adaptive increase of extramedullary hematopoiesis in the spleen of high dose males. Finally, high dose males displayed some changes of clinical chemistry parameters, i.e. decreased total protein and globulin as well as increased triglyceride levels.

Under the conditions of the present study the no observed adverse effect level (NOAEL) in males was 103 mg/kg bw/d (target dose level of 100 mg/kg bw/d) and 316 mg/kg bw/d (target dose level of 300 mg/kg bw/d) in female Wistar rats.

(BASF DocID 2013/1042164)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 298327 (Metabolite of BAS 500 F, Pyraclostrobin)

Description:	solid / yellow
Lot/Batch #:	L84-174
Purity:	99.6% (tolerance ± 1.0%)
Stability of test compound:	The test substance was stable over the study period until 01 Apr 2020.

2. Vehicle and/or positive control: Rodent diet

3. Test animals:

Species:	Rat
Strain:	Crl:WI (Han)
Sex:	Male and female
Age:	35 ± 1 day at delivery
Weight at dosing:	means: males: 158 g, females: 129 g
Source:	Charles River Laboratories, Sulzfeld, Germany
Acclimation period:	7 days
Diet:	Kliba maintenance diet mouse/rats "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Drinking water from water bottles, ad libitum
Housing:	Group housing (5 animals/cage) housed in H-Temp polysulfonate cages (TECNIPLAST, Hohenpeißenberg, Germany) floor area about 2065°cm²; bedding: wooden dust free bedding; enrichment: wooden gnawing blocks type NGM E-022 (Abedd® Lab. And Vet. Service GmbH, Austria

Environmental conditions:

Temperature:	20 - 24°C (central air-conditioning)
Humidity:	30 - 70% (central air-conditioning)
Air changes:	15 per hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

4. Dates of experimental work: 15-May-2012 to 25-Jan-2013
Inlife dates: 22-May-2012 (start of administration) to 23-Aug-2012 (necropsy of last female animals)

5. Animal assignment and treatment:

Reg.No. 298327 was administered to groups of 10 male and 10 female Wistar rats at target concentrations of 0, 100, 300 and 1000 mg/kg bw/day for three months by the dietary route.

The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights. At the end of the administration period the animals were sacrificed after a fasting period of at least 16 hours.

6. Test substance preparation and analysis:

The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. In order to meet the target dose levels, the dietary concentrations of the test-item for each test group and sex were adjusted weekly, based on body weight and food consumption measurements from the previous week. For the first week of administration the dietary concentrations were based on historical control data. Accordingly, e.g. nominal dietary concentrations of low dose (100 mg/kg) females varied between 908 and 1583 ppm whereas for example the nominal dietary concentrations in high dose (1000 mg/kg) males varied between 9421 and 17753 ppm.

The stability of Reg.No. 298327 in the diet at room temperature for a period of 33 days was demonstrated before the start of the study (BASF project No. 01Y0615/10Y057; copy of the report included in Volume III of the report).

The homogenous distribution of Reg.No. 298327 in the diet was verified for the highest and lowest concentration (see below) using the first diet preparation. Additionally, the test-item concentrations were determined in all preparations not analyzed for homogeneity. Concentration control analyses were also performed at the end of the administration period with the last diet batches produced.

Analysis of diet preparations for homogeneity and test-item content

Nominal concentration [ppm	Sampling	fed to	Concentration measured [ppm] Mean ± SD#	Relative standard deviation [%]	Mean % of nominal concentration
942	18.05.2012	males	991.1 ± 24.5	2.5	106.1
986	18.05.2012	females	1049		106.5
2826	18.05.2012	males	2682.9		94.9
2957	18.05.2012	females	2897.7		98.0
9421	18.05.2012	males	9786.4		103.9
9857	18.05.2012	females	9296.2 ±457.9	4.9	94.3
1719	22.08.2012§	males	1576.7		102.2
1210	22.08.2012	females	1301.1		107.5
5351	22.08.2012R	males	5703.7		106.6
4051	22.08.2012R	females	4010.2		99.0
16741	22.08.2012	males	15352.6		91.7
12801	22.08.2012	females	11679.4		91.2

R Reserve samples; § Samples prepared 14.08.2012
based on mean values of the three individual samples (homogeneity determinations)
Values may not calculate exactly due to rounding of values

Relative standard deviations of the homogeneity samples in the range of 2.5 to 4.9% indicate the homogenous distribution of Reg.No. 298327 in the diet preparations. The concentration of the test-item in the diet mixtures was in the range of 90.1 to 107.5% and thus in the acceptable range.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
Body weight, body weight change	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
Feces, rearing, grip strength forelimbs, grip strength hindlimbs, footsplay test, motor activity	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON test (two-sided) for the equal medians

Statistics of clinical pathology

Parameter	Statistical test
Blood parameters	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians For parameters with unidirectional changes: Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) with Bonferroni-Holm adjustment for the hypothesis of equal medians
Urinalysis parameters (apart from pH, volume, specific gravity, color and turbidity)	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians
Urine pH, volume, specific gravity, color and turbidity	Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians. Urine color and turbidity are not evaluated statistically.

Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test (two-sided) for the equal medians

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. If animals were in a moribund state, they were sacrificed and necropsied.

Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this, the animals were transferred to a standard arena (50 x 37.5 cm; sides 25 cm high). The findings were ranked according to the degree of severity, if applicable. The following parameters were examined:

1. abnormal behavior during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. feces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0) and at weekly intervals thereafter. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.

3. Food consumption and compound intake:

Food consumption was determined weekly over a period of 1 day and calculated as mean food consumption in grams per animal and day.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and mean food consumption per cage.

$$D = \frac{FC_x \times PPM_x}{BW_x}$$

with FC_x as the mean daily food consumption (in g) on day x and BW_x as body weight on day x of the study (g) and PPM_x as dietary concentration of the test item at day x.

4. Water consumption:

Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume. According to a personal communication of the study director decreased water consumption was first observed after 5 weeks (35 days) In order to quantify this visual observation water consumption was monitored weekly from day 49 onwards by determining water consumption over a period of 4 days. Water consumption was calculated as mean water consumption in grams per animal and day.

5. Ophthalmoscopy:

The eyes of all animals were examined prior to the start of the administration period. At the end of the administration period, i.e. study day 91, the eyes of animals in test groups 0 (control) and 3 (1000 mg/kg bw/d) were examined for any changes using an ophthalmoscope ophthalmoscope (HEINE OPTOTECHNIK, Herrsching, Germany) after application of a mydriatic (Mydrum, Chauvin ankerpharm GmbH, Rudolstadt, Germany).

6. Functional observation battery (FOB):

A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation special attention was paid to posture, tremors and convulsions, abnormal movements gait abnormalities.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements
5. nose discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces (number of fecal pellets/appearance/ consistency) within two minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1.	approach response	8.	behavior during "handling"
2.	touch response	9.	vocalization
3.	vision ("visual placing response")	10.	pain perception ("tail pinch")
4.	pupillary reflex	11.	grip strength of forelimbs
5.	pinna reflex	12.	grip strength of hind limbs
6.	audition ("startle response")	13.	landing foot-splay test
7.	coordination of movements ("righting response")	14.	other findings

7. Motor activity measurement:

Motor activity was measured at the same day when the FOB was performed in a randomized order. The measurement was performed in the dark using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 beams per cage. During the measurement the animals were kept in clean Polycarbonate cages with absorbent material. Motor activity measurements were from 2:00 p.m onwards. The number of beam interrupts was counted over twelve 5 minute intervals. Measurement started when the first beam was interrupted by pushing the cage into the rack (staggered start). Measurements ended exactly 60 minutes thereafter. During the measurements the animals received no food and no water.

8. Hematology and clinical chemistry:

Blood was taken in the morning from fasted, isoflurane anesthetized animals from the retro-orbital venous plexus. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.

The following hematological and clinical chemistry parameters were determined for 10 animals per test group and sex:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Platelet count
✓	Hemoglobin (Hb)	✓	Differential blood count	✓	Prothrombin time
✓	Hematocrit (Hct)				
✓	Mean corp. volume (MCV)				
✓	Mean corp. hemoglobin (MCH)				
✓	Mean corp. Hb. conc. (MCHC)				
✓	Reticulocytes				
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
✓	Phosphorus (inorganic)	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Potassium	✓	Creatinine	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:

The dry chemical reactions on test strips (Combur-10-test M, Roche, Mannheim, Germany) used to determine urine constituents semiquantitatively were evaluated with a reflection photometer (Miditron M; Roche, Mannheim, Germany). The following parameters were determined in all animals:

Urinalysis					
	Quantitative parameters:		*Semiquantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilinogen
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

10. Sacrifice and pathology:

All animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. Special attention was given to the gastrointestinal tract (duodenum). The organs were sampled, weighed, and examined histopathologically as indicated in the table below.

Pathology:
The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓		#	larynx	✓		#	skin
✓		#	aorta	✓	✓	✓	liver	✓		#	spinal cord (3 levels)[@]
✓		#	bone marrow[§]	✓		#	lung	✓	✓	✓	spleen[+]
✓	✓	#	brain	✓		#	lymph nodes[#]	✓		#	sternum w. marrow
✓		#	caecum	✓		#	mammary gland (♀)	✓		#	stomach (fore- & glandular)
✓		#	colon	✓		#	muscle, skeletal	✓	✓	#	testes
✓		#	duodenum	✓		#	nerve, peripheral (sciatic n.)	✓	✓	#	thymus
✓	✓	#	epididymides	✓		#	nose/nasal cavity[‡]	✓	✓	#	thyroid glands
✓		#	esophagus	✓	✓	#	ovaries and oviduct**	✓		#	trachea
✓		#	eyes (with optic nerve)	✓		#	pancreas	✓		#	urinary bladder
✓		#	femur (with knee joint)	✓		#	parathyroid glands	✓	✓	#	uterus with cervix
✓		✓	gross lesions			#	Peyer's patches	✓		✓	vagina
✓		#	Harderian gland	✓		#	pharynx				
✓	✓	#	heart	✓		#	pituitary		✓		body (anesthetized animals)
✓		#	ileum	✓	✓	#	prostate				
✓		#	jejunum (w. Payer's plaque)	✓		#	rectum				
✓	✓	✓	kidneys	✓		#	salivary glands*				
✓			lacrimal glands[%]	✓	✓	#	seminal vesicles~				

[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; [%] extraorbital, [‡] histopathology at level III, ~ with coagulation glands; [+] histopathology in all males only for low and mid dose group

The organs or tissues were fixed in 4% formaldehyde solution or in modified Davidson's solution. The hematoxylin-eosin (HE) stained slides were examined and assessed by light microscopy.

Besides standard HE staining, the following special stains were performed in the kidneys of selected animals:

- Periodic Acid Schiff (PAS) for glycoproteins,
- Mallory-Heidenhain's stain for proteins
- Perl's stain for the detection of ferric iron (Fe^{+++})

The following animals were selected for the special stain:

- Control animals: one male (No. 4) and one female (No. 43)
- Test group 3 (1000 mg/kg bw/d): two males (No. 32 and 34) and two females (No. 71 and 75)

II. RESULTS AND DISCUSSION

A. Observations

1. Clinical signs of toxicity

No test substance-related effects were observed.

2. Mortality

No animal died prematurely in the present study.

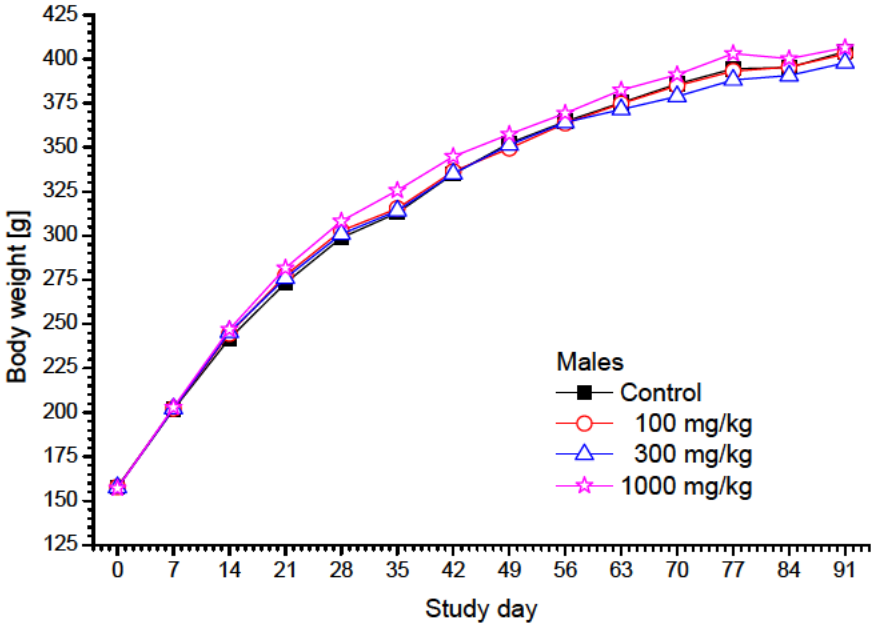
3. Ophthalmoscopy

No test substance-related effects were observed. The incidence and distribution of findings was either identical (corneal stiplings) or decreased to lower incidences without any relation to dose (remainders of the pupillary membrane) when comparing the observations at day -1 and day 91.

B. Body weight and body weight gain

No test stubstance-related changes in body weight parameters of both sexes were observed in treated animals when compared to controls. All values represent the normal biological variability typical for animals of this strain and age [see Figure 5.8.1-1 and Table 5.8.1-11].

Figure 5.8.1-1: Body weight development of rats administered Reg.No. 298327 for 91 days



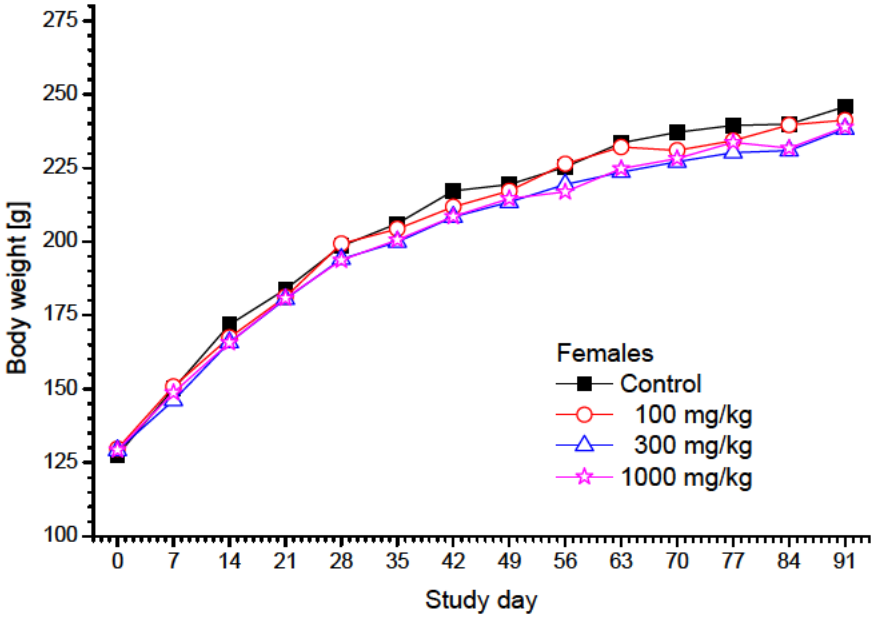


Table 5.8.1-11: Body weight development of rats administered Reg.No. 298327 for 91 days

Dose level [mg/kg]	0	100	300	1000
Males				
Body weight [g]				
- Day 0	157.6 ± 8.8	157.8 ± 6.8	157.6 ± 6.7	157.1 ± 7.5
- Day 91	404.1 ± 44.3	402.8 ± 39.7	397.8 ± 43.3	406.4 ± 28.9
Δ% (compared to control)#		-0.3	-1.6	1.2
Overall body weight gain [g]	246.5 ± 38.5	245.0 ± 34.5	240.2 ± 39.7	249.2 ± 29.1
Δ% (compared to control)#		-0.6	-2.6	1.1
Females				
Body weight [g]				
- Day 0	127.5 ± 8.8	129.7 ± 6.0	129.2 ± 4.9	129.3 ± 8.0
- Day 91	245.8 ± 15.5	241.2 ± 14.9	238.2 ± 13.2	238.9 ± 18.2
Δ% (compared to control) #		-1.9	-3.1	-2.8
Overall body weight gain [g]	118.3 ± 11.2	111.5 ± 10.6	108.9 ± 12.7	109.5 ± 12.0
Δ% (compared to control) #		-5.8	-8	-7.4

D. Food and water consumption and compound intake

Food consumption was highly variable in all groups including control groups throughout the study [see Figure 5.8.1-2].There was no treatment-related trend when the average daily food consumption was considered [see Table 5.8.1-12].

Table 5.8.1-12: Average food consumption of rats administered Reg.No. 298327 for 91 days

	Males				Females			
Dose level [mg/kg bw/day]	0	100	300	1000	0	100	300	1000
Mean food consumption [g/animal]								
Day 0 to 91#	22.3	22.3	21.5	23.3	16.6	16.9	16.5	17.6
Δ% (compared to control)#		-0.1	-3.5	4.7		1.5	-0.6	5.9

Values were calculated based on weekly mean individual daily food consumption values. Values may not calculate exactly due to rounding of mean values; No statistics was performed because n=2

Figure 5.8.1-2: Mean daily food consumption in rats administered Reg.No 298327 for 91 days

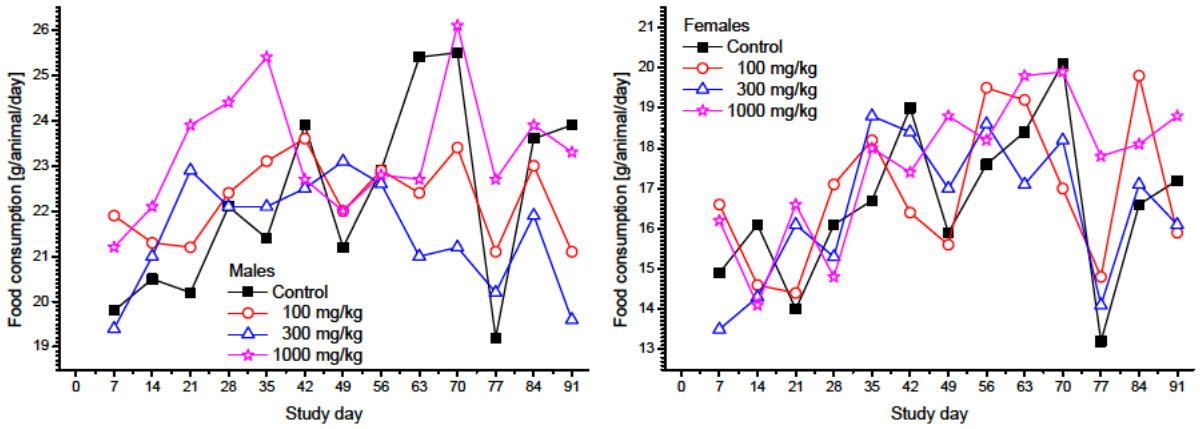
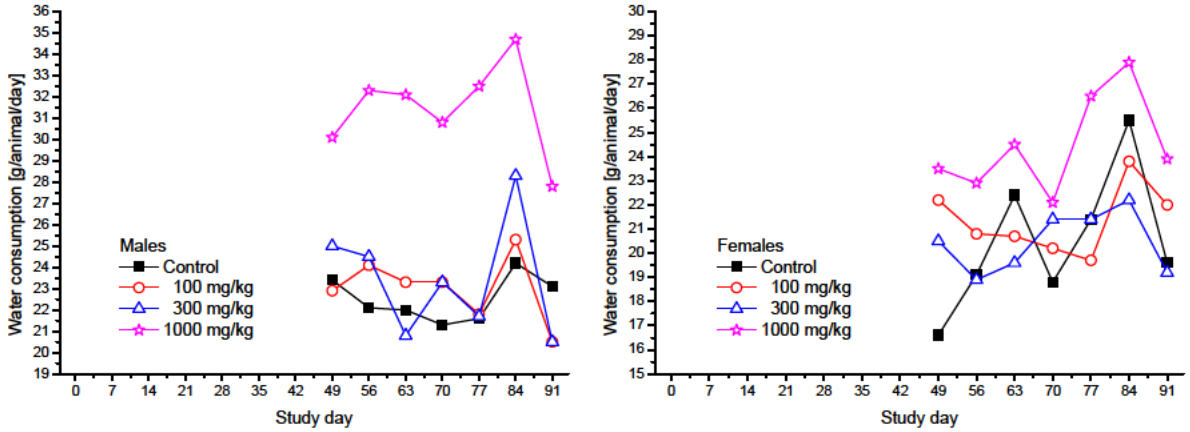


Figure 5.8.1-3: Mean daily water consumption in rats administered Reg.No 298327 for 91 days



According to a personal communication of the study director decreased water consumption was first observed after 5 weeks (35 days) of treatment as assessed by visual inspection of the water volume in drinking bottles. In order to quantify this visual observation water consumption was monitored from day 49 onwards. Daily drinking water consumption was consistently increased in both sexes at the high dose level (1000 mg/kg bw/d) throughout the observation period [see Figure 5.8.1-3], with males more severely affected than females. The increased water consumption is also evident if the average water consumption between day 49 and 91 is calculated [see Table 5.8.1-13].

The effect on water consumption in mid and low dose animals was neither consistent over time nor dose-dependent and difference of the overall mean water consumption [see Table 5.8.1-13] to the controls was marginal. Thus, only the effect at 1000 mg/kg was considered to be treatment related.

Table 5.8.1-13: Average water consumption of rats administered Reg.No. 298327 for 91 days

	Males				Females			
Dose level [mg/kg bw/day]	0	100	300	1000	0	100	300	1000
Average daily water consumption [g/animal]								
Day 49 to 91[#]	22.5	23.0	23.4	31.5	20.5	21.3	20.5	24.5
Δ% (compared to control)[#]		2.2	4.1	**39.7**		4.2	-0.1	**19.5**

[#] Values were calculated based on mean individual daily food. Values may not calculate exactly due to rounding of mean values; No statistics was performed because n=2

The mean daily test substance intake was calculated as 103, 302, and 1017 mg/kg bw/day in males and 106, 316, and 1066 mg/kg bw/day in females at dietary dose levels of 100, 300 and 1000 mg/gk bw/d, respectively.

E. Functional observation battery and motor activity

1. Functional observational battery

No treatment-related effects were observed during the FOB examinations. There were a few findings, which however were either equally distributed between treated groups and controls, were without a dose response relationship or occurred in single rats only. These observations were considered to be of incidental nature rather than related to treatment.

At the home cage observations and sensorimotor tests/reflexes the animals showed no test substance-related effects. Likewise, the determination of quantitative parameters (feces, rearing, grip strength of fore- and hindlimbs and foot splay test) did not reveal significant changes. During the open field observation period low dose (100 mg/kg) male #13 did not walk. Due to the single occurrence and the lack of a dose response-relationship this finding was considered as incidental and not test substance-related.

2. Motor activity measurement

No test substance-related effects were observed.

F. Blood analysis

1. Hematological findings

Treatment-related hematology changes were restricted to high dose males and consisted of a slight regenerative normochromic-macrocytic anemia indicated by decreased erythrocyte counts (RBC) and hemoglobin values (HGB) and increased mean corpuscular volume (MCV), mean hemoglobin content (MCH) as well as increased relative reticulocyte counts [see Table 5.8.1-14].

Table 5.8.1-14: Selected hematology findings in rats administered Reg.No 298327 for 91 days (Group means ± SD)

	Study day	RBC [10^{12}/L]	HGB [mmol/L]	MCV [fL]	MCH [fmol]	RET [%]	EOSA [10^{12}/L]
Males							
Control	Day 92	8.82 ± 0.48	9.1 ± 0.3	49.7 ± 2.5	1.04 ± 0.06	2.0 ± 0.5	0.10 ± 0.03
100 mg/kg bw/day	Day 92	8.80 ± 0.30	9.1 ± 0.2	50.1 ± 1.9	1.04 ± 0.05	1.9 ± 0.4	0.11 ± 0.04
300 mg/kg bw/day	Day 92	8.66 ± 0.30	9.1 ± 0.3	50.4 ± 1.0	1.05 ± 0.02	2.2 ± 0.3	0.10 ± 0.04
1000 mg/kg bw/day	Day 92	**8.02 ± 0.28****	**8.8 ± 0.2***	**53.5 ± 2.2****	**1.10 ± 0.05***	**2.9 ± 0.4***	0.09 ± 0.03
Females							
Control	Day 93	7.53 ± 0.31	8.4 ± 0.3	52.4 ± 1.4	1.12 ± 0.03	2.6 ± 0.6	0.08 ± 0.03
100 mg/kg bw/day	Day 93	7.41 ± 0.29	8.3 ± 0.2	52.6 ± 1.5	1.12 ± 0.04	2.9 ± 0.5	0.05 ±0.01*
300 mg/kg bw/day	Day 93	7.58 ± 0.34	8.5 ± 0.4	53.2 ± 1.2	1.12 ± 0.03	2.9 ± 0.5	0.08 ± 0.01
1000 mg/kg bw/day	Day 93	7.54 ± 0.21	8.4 ± 0.2	53.1 ± 1.2	1.12 ± 0.03	2.6 ± 0.5	0.08 ± 0.02

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-tests two sided)

The only statistically significant difference observed in females was a lower mean absolute eosinophil count at the low dose of 100 mg/kg bw/d. In absence of a dose-response relationship this alteration was regarded as incidental and not treatment-related.

2. Clinical chemistry findings

Treatment-related clinical chemistry findings were restricted to high dose males and consisted of decreased total protein and globulin values as well as increased triglyceride levels [see Table 5.8.1-15].

Table 5.8.1-15: Selected clinical chemistry findings in rats administered Reg.No. 298327 for 91 days (Group means ± SD)

Dose [mg/kg bw/d]		0	100	300	1000
Males					
ALP	[µkat/L]	1.19 ± 0.18	1.04* ± 0.10	1.24 ± 0.21	1.05 ± 0.21
Protein, total	[g/L]	62.99 ± 2.76	62.03 ± 2.04	61.77 ± 2.03	**59.01 ± 1.75****
Globulin	[g/L]	24.16 ± 1.94	23.85 ± 1.80	23.30 ± 1.31	**21.41 ± 1.46****
Triglycerides	[mmol/L]	1.16 ± 0.48	1.18 ± 0.61	1.44 ± 0.49	**1.86 ± 0.57****
Glucose	[mmol/L]	6.32 ± 0.89	6.20 ± 0.52	6.32 ± 0.57	6.33 ± 0.51
Females					
ALP	[µkat/L]	0.46 ± 0.12	0.47 ± 0.10	0.41 ± 0.07	0.41 ± 0.11
Protein. total	[g/L]	64.11 ± 3.71	64.98 ± 2.87	65.03 ± 3.52	66.65 ± 3.20
Globulin	[g/L]	22.83 ± 1.77	22.51 ± 1.36	22.63 ± 1.76	23.69 ± 1.09
Triglycerides	[mmol/L]	0.64 ± 0.20	0.49 ± 0.12	0.58 ± 0.25	0.97 ± 0.57
Glucose	[mmol/L]	4.81 ± 0.37	5.84 ± 0.61**	5.13 ± 0.34	5.09 ± 0.39

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test. two sided)

The decreased alkaline phosphatase (ALP) levels in low dose males and the increased glucose levels in low dose females were not dose-dependent and thus considered not to be related to treatment.

3. Urinalysis

Urinalyis revealed increased mean urine volumes which were accompanied by lower specific gravity of the urine in both sexes at 1000 mg/kg [see Table 5.8.1-16]. This is consistent with the higher water consumption noted at this dose level. In addition, more crystals of unknown origin were found in the urine sediment of males at ≥ 300 mg/kg. The aforementioned findings were considered to be treatment-related.

Table 5.8.1-16: Selected urinalysis parameters in rats administered Reg.No. 298327 for 91 days (Group means ± SD)

Dose [mg/kg bw/d]	0	100	300	1000
Males				
Urine volume [ml]	3.2 ± 1.4	3.9 ± 1.6	3.5 ± 0.7	**6.5 ± 1.8****
Specific gravity [g/L]	1060 ± 14	1055 ± 19	1062 ± 13	**1040 ± 5****
Crystals[#]	2	2	3*	3**
Females				
Urine volume [ml]	2.4 ± 1.4	2.7 ± 0.9	3.0 ± 0.9	**3.9 ± 0.7****
Specific gravity [g/L]	1071 ± 28	10621 ± 21	1051 ± 15	**1045 ± 8****
Crystals[#]	2	2	2	2

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test. two sided)
[#] semi-quantitative parameter: Grade 0 = none, Grade 1 = few, Grade 2 = many, Grade 3 = masses

G. Necropsy

1. Organ weight

No statistically significant absolute or relative organ weight differences were observed.

2. Gross and histopathology

There were no treatment-related gross necropsy findings. All findings were observed in single incidences only.

Treatment-related histopathological findings were observed in the kidney of mid dose males and high dose males and females as well as in the spleen of high dose males [see Table 5.8.1-17].

In the kidney the eosinophilic material was localized in the lumen of the renal pelvis. This material had a heterogeneous structure, characterized by granulated and smooth components. Occasionally, remnants of lytic superficial urothelium (exfoliated cells and slightly mineralized superficial tissue) were admixed in this material. Special stains (PAS, Mallory-Heidenhain, Perl's stain) failed to better characterize the eosinophilic material. The urothelial hyperplasia most probably represented a reactive response to the presence of the eosinophilic material in the renal pelvis. Both findings were considered to be adverse.

The increased mineralization was seen in the inner medulla of males, whereas in females the mineralization was localized at the transition of the outer to the inner medulla. The chronic progressive nephropathy, which showed a slightly increased incidence and severity in both sexes at the high dose level, was characterized by cortical areas with increased tubular basophilia and nuclear crowding associated with minimally thickened basement membranes, dilation of tubules, some of them containing hyaline casts and minimal interstitial fibrosis and mononuclear cellular infiltrates in-between the tubules. Both findings described above are frequently observed in control animals of this age. The slight increase in incidence and/or severity at the high dose levels was considered as a treatment-related exacerbation of spontaneously occurring kidney changes. In absence of changes of clinical chemistry parameters of renaly dysfunction, these observations were considered to be non-adverse.

The papilar ulceration observed in a high dose male was considered to represent an incidental finding due to its single occurrence.

Table 5.8.1-17: Selected histopathological changes in rats administered Reg.No. 298327 for 91 days

Dose [mg/kg bw/d]	0	100	300	1000	0	100	300	1000
Kidney [organs examined]	10	10	10	10	10	10	10	10
Eosinophilic material, renal pelvis	0	0	**1** ***[2.0]***	**6** ***[2.8]***	0	0	0	**1** ***[2.0]***
Hyperplasia, urothelial	0	0	**1** ***[1.0]***	**7** ***[1.4]***	0	0	0	**3** ***[2.0]***
Mineralization, medulla - Grade 1	2 *[1.0]*	4 *[1.0]*	0	**7** ***[1.0]***	8 *[1.3]*	9 *[1.3]*	10 *[1.6]*	**10** ***[2.3]***
Nephropathy, chronic	4 *[1.0]*	7 *[1.0]*	5 *[1.0]*	**9** ***[1.3]***	6 *[1.0]*	1 *[1.0]*	1 *[1.0]*	**9** ***[1.3]***
Ulceration, pappilar				1 *[2.0]*				
Spleen [organs examined]	10	10	10	10	10	-	-	10
Hematopoiesis, extramedullary	6 *[1.0]*	10 *[1.4]*	10 *[1.5]*	10 *[2.0]*	8 *[1.2]*			9 *[1.2]*

[ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

The increase in the severity of the extramedullary hematopoiesis in the spleen of high dose males was regarded as treatment-related. The extramedullary hematopoiesis was of erythroid type and, therefore, characterized by foci of cells with markedly basophilic nuclei in the red pulp. The histopathologic finding correlates well to the observed regenerative normochromic-macrocytic anemia in high dose males.

In this study special attention was given to the gastrointestinal tract. No treatment-related findings were observed in the duodenum of high dose male and female animals as a hyperplasia of the duodenal mucosa was observed with the parent molecule pyraclostrobin. However, no treatment-related findings were noted.

There were a number of other histopathological findings which occurred either individually or were biologically equally distributed over control and treatment groups. They were therefore considered to be incidental or spontaneous in origin and without any relation to treatment.

III. CONCLUSIONS

The dietary administration of 500M04 (Reg.No. 298327, metabolite of pyraclostrobin) to male and female Wistar rats for 91-days caused adverse histopathological effects in the kidney of males at ≥ 300 mg/kg bw/day and females at 1000 mg/kg bw/day as well as on the hematological system of high dose males. The effects in the kidney were accompanied by higher water consumption and increased urinary discharge, whereas the anemia in males was accompanied by an increased severity of extramedullary hematopoiesis in the spleen. Additionally, lower total protein and globulin and higher triglyceride levels were observed in high dose males.

Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 103 mg/kg bw/d (target dose level of 100 mg/kg bw/d) in male and 316 mg/kg bw/d (target dose level of 300 mg/kg bw/d) in female Wistar rats, respectively.
Genotoxicity studies of 500M04 (Reg.No. 298327, BF 500-5, 'Pyrazolon')

Report:	CA 5.8.1/16 Woitkowiak C., 2012a Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2012/1220416
Guidelines:	OECD 471 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium (strains TA98, TA 100, TA 1535 and TA 1537) and E. coli strain WP2 uvrA were exposed to Reg.No. 298327 (Metabolite of BAS 500 F, pyraclostrobin); batch: L83-44-2, purity: 97.6%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.

The test substance was tested in concentrations between 10 and 5200 µg/plate. A bacteriotoxic effect was observed depending on the strain and test conditions from about 1000 µg/plate onwards. Precipitation of the test substance was found from 2600 µg/plate with and without S9 mix.

A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.

The test substance Reg.No. 298327 (Metabolite of BAS 500 F, pyraclostrobin) is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2012/1220416)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Reg.No. 298327 (Metabolite of BAS 500 F, pyraclostrobin)

Description: Solid, beige
Lot/Batch #: L83-44-2
Purity: 97.6%
Stability of test compound: The test substance was stable over the study period under the storage conditions and guaranteed until 01 Jan 2013. The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 h was verified analytically.
The homogeneity of the test substance was ensured by mixing prior to preparation of test substance solutions.
Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).
Vehicle control: The vehicle control with and without S9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60 µg/plate

To demonstrate the efficacy of the S9 mix in this assay, the S9 batch was further characterized with benoz(a)pyrene in the strains TA 100 and TA 98 as required in OECD 471.

3. Activation:

S9 was produced from the livers of induced male Wistar rats. The rats received on three consecutive days intraperitoneal injections of 80 mg phenobarbital and 80 mg β-naphthoflavone orally per kg body weight. 24 hours after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9 mix, was kept on ice until used.

The S9 mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay:	In the first experiment triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2600 and 5200 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without S9) for all tester strains indicated above.
Pre-incubation assay:	In the second experiment the test article / vehicle / positive control substance, bacterial and S9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 10, 33, 100, 333, 1000 and 2600 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains.

B. TEST PERFORMANCE:

7. Dates of experimental work: 21-Mar-2012 to 30-Mar-2012

8. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates). In the experiments with E. coli the warm soft agar contains 0.5 mM tryptophan instead of histidine + biotin.

9. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37°C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

10. Titer determination:

In order to assess bacteriotoxic effects, the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37°C the number of bacterial colonies was determined.

11. Statistics:

No special statistical tests were performed.

12. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two at least two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A bacteriotoxic effect (decrease in the number of his^+ and trp^+ revertants, reduction in the titer) was observed in the standard plate test depending on the strain and test conditions from about 1000 µg/plate onwards.

In the preincubation assay bacteriotoxicity (reduced his^+ or trp^+ background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed depending on the strain and test conditions from about 1000 µg/plate onwards.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiment with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested [see Table 5.8.1-18]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Test substance precipitation was found from 2600 µg/plate onwards with and without S9 mix.

Table 5.8.1-18: Bacterial gene mutation assay with Reg.No. 298327 - Mean number of revertants

Experiment 1: Plate incorporation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	31	17	87	74	13	11	8	7	43	36
Reg.No. 298327										
33 µg/plate	32	17	86	73	12	12	8	7	43	37
100 µg/plate	36	17	93	77	13	12	8	7	40	35
333 µg/plate	27	19	84	76	12	11	8	8	44	33
1000 µg/plate	25	17	63	68	9	7	6	5	39	30
2600 µg/plate	9[P]	5[P]	4[P]	7[P]	3[P]	1[P]	2[P]	2[P]	34[P]	17[P]
5200 µg/plate	2[P]	1[P]	1[P]	0[P]	1[P]	0[P]	1[P]	1[P]	15[P]	9[P]
Pos. control[§]	816	622	1165	582	153	623	133	390	257	723
Experiment 2: Preincubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	28	18	87	75	12	11	7	6	44	38
Reg.No. 298327										
10 µg/plate	27	18	84	72	13	11	7	6	47	39
33 µg/plate	3	19	90	74	12	12	8	8	44	39
100 µg/plate	26	18	95	76	12	11	7	7	43	36
333 µg/plate	27	17	80	70	13	10	8	6	40	37
1000 µg/plate	5	7	26	24	6	5	3	1	35	17
2600 µg/plate	2[B/P]	1[B/P]	12[B/P]	10[P]	0[B/P]	0[B/P]	0[B/P]	0[B/P]	12[B/P]	10[B/P]
Pos. control[§]	583	547	895	765	155	628	130	385	250	622

[§] = Compound and concentrations see Material and Methods (I.A.2.) above
[B] = reduced background growth
[P] = Precipitation

III. CONCLUSION

According to the results of the present study the test substance 500M04 (Reg.No. 298327, metabolite of pyraclostrobin) is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/17 Schulz M., Landsiedel R., 2012b Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in CHO cells (HPRT locus assay) 2012/1272482
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300, OECD 476 (1997)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg. No. 298327 (Metabolite of BAS 500 F, pyraclostrobin; batch: L84-174, purity: 99.6%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells. Two independent experiments were conducted in the presence and absence of metabolic activation with concentrations of up to 1000 µg/mL. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h for both experiments. Ethylmethanesulfonate (EMS) and 7,12-Dimethylbenz[a]anthracene (DMBA) served as positive controls in the experiments without and with metabolic activation, respectively.

The test substance did not cause any relevant increase in the mutant frequencies either without S9 mix or after the addition of a metabolizing system in two experiments performed independently of each other.
Based on the results of the study it is concluded that under the conditions of the test Reg.No. 298327 (Metabolite of BAS 500 F, pyraclostrobin) does not induce forward mutations in mammalian cells in vitro.

(BASF DocID 2012/1272482)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

	Reg.No. 298327 (Metabolite of BAS 500 F, pyraclostrobin)
Description:	Solid, yellow
Lot/Batch #:	L84-174
Purity:	99.6% (tolerance ± 1.0%)
Stability of test compound:	The stability of the test substance under storage conditions was guaranteed until 01-Apr-2020 as indicated by the sponsor. The homogeneity of the test substance was guaranteed on account of the high purity and ensured by mixing prior to preparation of test substance solutions. The stability of a comparable batch (L83-44-2) at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	1% (v/v) DMSO in culture medium
Positive control -S9:	Ethyl methanesulfonate (EMS) 300 µg/mL
Positive control +S9:	7.12-Dimethylbenz[a]anthracene (DMBA) 1.25 µg/mL

3. Activation:

S9 was produced from the livers of at least 5 induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organism:	Chinese hamster CHO cells. Stocks of the CHO cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).
5. Culture media:	
Culture medium:	Ham's F12 medium with L-glutamine and hypoxanthine supplemented with 10% (v/v) fetal calf serum (FCS).
Pretreatment medium:	("HAT" medium): FCS-supplemented Ham's F12 medium with L-glutamine containing per mL 13.6 µg hypoxanthine, 0.18 µg aminopterin and 3.88 µg thymidine.
Selection medium:	("TG" medium): L-Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL All media were supplemented with 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL) and 1% (v/v) amphotericine B (250 µg/mL) During pulse exposure (4 hours) to the test substance, Ham‘s F12 medium was used without FCS supplementation. In the case of continuous treatment (24 hours) Ham‘s F12 medium with FCS supplementation was used.
6. Locus examined:	hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)
7. Test concentrations:	
a) Preliminary toxicity assay:	Nine concentrations ranging from 7.8 to 2000 µg/mL
b) Mutation assay:	
1st experiment:	10.9, 21.9, 43.8, 87.5, 175.0 and 350.0 µg/mL with and 21.9, 43.8, 87.5, 175.0, 350.0 and 700.0 µg/mL without metabolic activation
2nd experiment:	31.3, 62.5, 125.0 and 250.0 µg/mL with and 31.3, 62.5, 125.0, 250.0, 500.0, 1000.0 µg/mL without metabolic activation

B. TEST PERFORMANCE

1. Dates of experimental work: 06-Sep-2012 to 2-Nov-2012

2. Preliminary cytotoxicity assay:

Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25-cm² flasks with 9 test substance concentrations in serum-free Ham's F12 medium for about 4 hours (with and without metabolic activation) or 24 hours (only without metabolic activation; serum-supplemented medium) after an attachment period of 20-24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS) or PBS, covered with Ham's F12 and incubated for a further 5 to 8 days. After this incubation period, colonies were fixed, stained and counted. In addition to the cloning efficiency the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

3. Mutation Assay:

Pretreatment of Cells: Cells with a passage number ≥ 2 after thawing from the frozen cells stock were seeded into 75 cm^2-flasks and incubated for 3-4 days with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants. Afterwards, a passage into culture medium followed and the cells were incubated for further 3-4 days.

Cell treatment: For each test group, about $1x10^6$ cells per flask were seeded into 175 cm^2 flasks containing about 20 mL Ham's F12 medium supplemented with 10% FCS and incubated for about 20 - 24 hours with 5% CO_2 at 37°C and ≥ 90% humidity for cell attachment. Two flasks were used for each test group.

After the cell attachment period the medium was replaced by the treatment medium. In case of experiments without metabolic activation the treatment medium consisted of 18 mL Ham's F12 medium without FCS plus 2 mL positive control substance. For the vehicle control and the test groups, 20 mL of the treatment medium was supplemented with 0.2 mL of the vehicle or test substance preparation. In case of metabolic activation, the treatment medium consisted of 16 mL Ham's F12 medium without FCS, 0.2 mL positive control and 4 mL S9-mix. Analogously, for the vehicle control and test groups, 14 mL of medium was supplemented with 0.2 mL vehicle or test substance preparation and 4 mL S9-mix.

	Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix (or for 24 hours without S9-mix in the second experiment) at 5% CO_2, 37°C and ≥ 90% humidity.
Expression:	After incubation for 4 or 24 hours, respectively, the treatment medium was replaced by at least 20 mL Ham's F12 medium with 10% FCS after having been rinsed several times with Hanks' balanced salt solution (HBSS). The following 1st passage was carried out after an incubation period of about 3 days following the 4 hour exposure or 2 days following the 24 hour exposure period. After an entire expression period of 7 - 9 days the cells were transferred into the selection medium (2nd passage).
Selection:	For the mutant selection, six 75-cm^2 flasks each were seeded with $3x10^5$ cells from each treatment group in selection medium (TG medium) and incubated for about 6 to 7 days. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.
Determination of Cytotoxicity:	Cloning efficiency 1 (survival): The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25 cm^2 flasks using 5 mL Ham`s F12 medium with 10% FCS. After a 20-24 hour attachment period the cells were incubated with vehicle, test substance or the positive control for 4 or 24 hours as described above. Following exposure, cells were rinsed several times with HBSS. Finally, cells were cultured in 5 mL Ham's F12 medium incl. 10% (v/v) FCS.
Cloning efficiency 2 (viability):	The viability (cloning efficiency 2; CE_2) was determined after the expression period. About 200 cells were separated during the transfer into selection medium and seeded in two flasks (25 cm^2) containing 5 mL Ham's F12 medium incl. 10% (v/v) FCS each. After seeding of the cells, the flasks were incubated for 5 - 8 days to form colonies. These colonies were fixed, stained and counted.

Calculations:

Mutant frequency: Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{\text{total number of mutant colonies}}{\text{number of seeded cells}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\,absolute}} \times 100$$

Cloning efficiency (CE, %) absolute:

$$CE_{absolute} = \frac{total\ number\ of\ colonies\ in\ the\ test\ group}{total\ number\ of\ seeded\ cells\ in\ the\ test\ group} x100$$

relative, in comparison to control:

$$CE_{relative} = \frac{CE_{absolute}\ of\ the\ test\ group}{CE_{absolute}\ of\ the\ vehicle\ control} x100$$

4. Statistics:

An appropriate statistical trend test was performed to assess a dose-related increase of mutant frequencies. The number of mutant colonies obtained for the test substance treated groups was compared with that of the respective vehicle control groups. A trend is judged as statistically significant whenever the p-value (probability value) is below 0.10 and the slope is greater than 0. Both, biological and statistical significance will be considered together.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies ($MF_{corr.}$) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose-response relationship.

Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose-response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

A test substance is generally considered negative in this test system if:

- The corrected mutation frequency ($MF_{corr.}$) in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance under storage conditions was guaranteed until 20 Apr 2020 as indicated by the Sponsor. The stability of a comparable batch (L83-44-2) at room temperature in the vehicle DMSO and in water over a period of 4 hours was verified analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY

After 4 hours treatment in the absence of S9 mix cytotoxicity indicated by reduced relative cloning efficiency of about or below 20% relative survival was observed at ≥ 500 µg/mL, while no cytotoxicity was observed up to 2000 µg/ml in the presence of metabolic activation. In addition, after 24 hours treatment in the absence of S9 mix strongly reduced relative cloning efficiency of below 20% relative survival was observed at 500 and 1000 µg/mL.

Precipitation of the test substance was macroscopically observed at ≥ 125 µg/mL irrespective of metabolic activation. Microscopically, precipitation wat down to 31.3 µg/ml at the end of the treatment period. No marked effect on osmolarity and pH value was observed.

Based on these data the highest concentration tested in the mutagenicity experiments was up to 1000 µg/mL without and up to 350 µg/mL with metabolic activation.

C. MUTAGENICITY ASSAYS

Cytotoxic effects indicated by clearly reduced cloning efficiencies of about or below 20% of control values were observed in both experiments in the absence of S9 mix at least at the highest applied concentrations [see Table 5.8.1-19 and Table 5.8.1-20]. In contrast, in the experimental parts with S9 mix neither a decrease in the number of colonies nor of the cell densities was observed.

No relevant increase in the number of mutant colonies was observed with or without S9 mix. In both experiments after 4 and 24 hours treatment with the test substance the values for the corrected mutation frequencies (MFcorr.: 0.75 - 6.78 per 106 cells) were close to the respective vehicle control values (MFcorr.: 0.42 - 3.28 per 106 cells) and clearly within the range of historical negative control data (MFcorr.: 0.00 - 16.43 per 106 cells).

In the 2nd experiment with metabolic activation a statistically significant dose-related increase in the mutant frequency was found after 4 hours treatment (MFcorr.: 1.55 - 4.22 per 106 cells) in the trend test. However, the values obtained for the corrected mutation frequency of this experimental part were well within historical negative control data range (MFcorr.: 0.00 - 16.43 per 106 cells). Therefore, this finding has to be regarded as biologically irrelevant (see Table 5.8.1-19 and Table 5.8.1-20).

Treatment with the positive controls EMS and DMBA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

The pH and osmolarity of the tested concentrations were not altered at the concentrations tested. In the 1st experiment in the absence and presence of S9 mix, test substance precipitation was observed in culture medium 4 hours after start of treatment at 87.5 µg/mL and above. In the 2nd experiment in the absence of S9 mix, test substance precipitation was observed at 250.0 µg/mL and above after 24 hours exposure. Besides in the presence of S9 mix precipitates in culture medium occurred at 125.0 µg/mL and above at the end of 4 hours exposure period.

Table 5.8.1-19: Gene mutation in mammalian cells - 1st experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	11	3.06	3.28	78.4	100.0	92.9	100.0
Reg.No. 298327							
21.9 µg/mL	12	3.33	3.66	77.5	98.9	92.5	99.6
43.8 µg/mL	11	3.06	3.28	73.4	93.6	92.5	99.6
87.5 µg/mL	13	3.61	3.50	78.4	100.0	82.8	89.1
175.0 µg/mL	10	2.78	2.90	77.0	98.2	94.1	101.3
350.0 µg/mL	n.c.[2]	n.c.[2]	n.c.[2]	0.0	0.0	n.c.[2]	n.c.[2]
700.0 µg/mL	n.c.[2]	n.c.[2]	n.c.[2]	0.0	0.0	n.c.[2]	n.c.[2]
Positive control EMS							
300.0 µg/mL	191	53.06	66.16	78.4	100.0	80.4	86.5
With metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	8	2.22	2.43	81.6	100.0	93.3	100.0
Reg.No. 298327							
10.9 µg/mL	n.c.[1]	n.c.[1]	n.c.[1]	81.8	100.2	n.c.[1]	n.c.[1]
21.9 µg/mL	6	1.67	1.80	85.0	104.1	91.3	97.9
43.8 µg/mL	13	3.61	4.12	78.3	95.9	94.0	100.8
87.5 µg/mL	6	1.67	1.92	85.4	104.6	83.6	89.7
175.0 µg/mL	4	1.11	1.31	81.8	100.2	85.6	91.8
350.0 µg/mL	5	1.39	1.43	87.4	107.0	90.8	97.3
Positive control DMBA							
1.25 µg/mL	497	138.06	191.38	76.4	93.6	72.1	77.3

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
n.c.[1] culture was not continued since a minimum of only four analyzable concentrations is required
n.c.[2] culture was not continued due to strong cytotoxicity

Table 5.8.1-20: Gene mutation in mammalian cells - 2nd experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	Relative
Without metabolic activation; 24-hour exposure period							
Vehicle (DMSO)	7	1.94	2.51	70.6	100.0	77.1	100.0
Reg.No. 298327							
31.3 µg/mL	n.c.[1]	n.c.[1]	n.c.[1]	81.0	114.7	n.c.[1]	n.c.[1]
62.5 µg/mL	3	0.83	1.01	74.8	105.8	82.0	106.3
125.0 µg/mL	16	4.72	6.78	41.5	58.8	68.0	88.2
250.0 µg/mL	2	0.56	0.75	34.0	48.1	74.1	96.1
500.0 µg/mL	17	4.72	6.43	29.9	42.3	72.9	94.5
1000.0 µg/mL	6	1.67	2.14	17.8	25.1	77.3	100.2
Positive control EMS							
300 µg/mL	489	138.89	287.30	44.1	62.5	48.3	62.6
With metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	1	0.28	0.42	77.3	100.0	70.5	100.0
Reg.No. 298327							
31.3 µg/mL	4	1.11	1.55	83.9	108.6	74.0	105.0
62.5 µg/mL	7	1.94	2.45	81.3	105.2	77.9	110.5
125.0 µg/mL	7	1.94	2.55	84.9	109.9	76.8	108.9
250.0 µg/mL	11	3.06	4.22	81.4	105.3	72.5	102.8
Positive control DMBA							
1.25 µg/mL	400	111.11	185.03	48.5	62.8	60.0	85.1

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
n.c.[1] culture was not continued since a minimum of only four analyzable concentrations is required
n.c.[2] culture was not continued due to strong cytotoxicity

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test 500M04 (Reg.No. 298327, metabolite of pyraclostrobin) does not induce forward mutations in the HPRT locus in CHO cells in vitro.

Report:	CA 5.8.1/18 Schulz M., Landsiedel R., 2012a Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro chromosome aberration assay in V79 cells 2012/1185707
Guidelines:	OECD 473, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.10, EPA 870.5375
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 298327 (metabolite of BAS 500 F, pyraclostrobin; batch: L83-44-2, purity: 97.6) was tested in vitro for the ability to induce chromosome and numerical aberrations in Chinese Hamster V79 cells in two independent experiments in the presence and absence of metabolic activation. Concentrations of 31.3 µg/mL to 2000 µg/mL were tested for clastogenic effects with and without metabolic activation in experiments with a pulse treatment of 4 hours. The cells were prepared 18 h post treatment-begin. Vehicle (DMSO) and positive controls (cyclophosphamide (CPP) and ethylmethanesulfonate (EMS) for the experiment with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system.

The test substance caused a statistically significant and biologically relevant increase in the number of structurally aberrant metaphases only after adding a metabolizing system. This result was seen in two experiments performed independently of each other. No increase in the frequency of cells containing numerical chromosome aberrations was observed.

Based on the results of this study, Reg.No. 298327 is considered to have a chromosome damaging (clastogenic) effect under in vitro conditions in Chinese hamster V79 cells in the presence of metabolic activation.

(BASF DocID 2012/1185707)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 298327 (Metabolite of BAS 500 F, pyraclostrobin)

Description: Solid, beige
Lot/Batch #: L83-44-2
Purity: 97.6%
Stability of test compound: The stability of the test substance under storage conditions was guaranteed until 01 Jan 2013 by the sponsor. The stability of the test substance at room temperature dissolved in the vehicle DMSO over a period of 4 hours was verified analytically.
The homogeneity of the test substance was ensured by mixing prior to preparation of test substance formulations and on account of the high purity.
Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: A negative control was not employed in this study.
Solvent control: DMSO
Positive control, -S9: Ethylmethanesulfonate (EMS) 500 µg/mL
Positive control, +S9: Cyclophosphamide (CPP) 0.5 µg/mL

3. Activation: S9 was produced from the livers of 5 induced male Wistar Sprague-Dawley rats. The rats received on three consecutive days intraperitoneal injections of 80 mg phenobarbital and 80 mg β-naphthoflavone orally per kg body weight. 24 hours after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so called S9 mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms: Chinese hamster V79 cells

5. Culture medium: MEM medium with glutamine supplemented with
- 10% (v/v) fetal calf serum (FCS)
- 1% (v/v) penicillin/streptomycin (10000 IU/10000 µg/mL)
- 1% (v/v) amphotericine B (250 µg/mL)
During exposure to the test substance (4-hour treatment), MEM medium was used without FCS supplementation.

6. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 15.6 to 2000 µg/mL were used in pretests for dose selection for the main experiments. V79 cells were prepared at a sampling time of 18 hours after 4 and 18 hours exposure time without metabolic activation and after 4 hours exposure time with metabolic activation.

b) Mutation assay:
1st experiment: 31.3, 62.5, 125, 250, 500, 1000 and 2000 µg/mL with and without metabolic activation (18 h preparation interval)
2nd experiment: 50, 100, 150, 200 and 250 µg/mL with metabolic activation (18 h preparation interval)

B. TEST PERFORMANCE:

1. Dates of experimental work: 13-Mar-2012 to 15-Oct-2012

2. Preliminary cytotoxicity assay: A range-finding cytotoxicity test was conducted with V79 cultures exposed for 4 and 18 hours to test substance concentrations of 15.6 - 2000 µg/mL both with and without metabolic activation. At the end of the exposure period, cell count, cell attachment, mitotic index and the quality of metaphases were determined in order to derive appropriate test substance concentrations for the main test.

3. Cytogenicity Assay:

Cell treatment:	Cells were exposed to the test substance, solvent or positive control in pulse treatment experiments for 4 hours with or without metabolic activation. The cells were incubated in Quadriperm® dishes at 37°C, 5% CO_2 and ≥ 90% humidity. Two chambers of a Quadriperm dish were used for each concentration (=duplicate cultures). The preparation interval was 18 h post treatment-begin. For determination of cytotoxicity, additional cell cultures (using 25 cm² plastic flasks) were treated in the same way as in the main experiment. Growth inhibition was estimated by comparing the cell number in the treated groups with the concurrent control.
Spindle inhibition:	100 µL colcemide (stock: 10 µg/mL phosphate buffered saline) was added to the cultures 2 - 3 hours prior to harvesting.
Cell harvest:	At the end of the incubation time the culture medium was completely removed. For hypotonic treatment, 5 mL of a 0.4% KCl solution (37°C) was added for about 20 minutes. The cells were fixed by addition of 5 mL methanol/glacial acetic acid (3:1 v/v). The fixative was changed twice.
Slide preparation:	The slides were removed from the Quadriperm chambers, briefly allowed to drip off and passed through a Bunsen burner flame. After drying, the cells were stained with Giemsa and Titrisol. After rinsing and clarifying in Xylene the cover slips were mounted in Corbit-Balsam.
Metaphase analysis:	Slides were coded prior to analysis. As a rule, the first 100 consecutive well-spread metaphases of each culture were counted for all test groups, and if cells had 20 - 22 chromosomes, they were analyzed for structural chromosome aberrations. Numerical chromosome aberrations were also recorded. If there is a clear increase in chromosomally damaged cells, the number of metaphases to be analyzed is reduced to at least 50 mitoses/ test group. A mitotic index based on 1000 cells per culture was determined for all evaluated test groups in both experiments.

4. Statistics:

The proportion of metaphases with aberrations was calculated for each group.

A comparison of each dose group with the vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test was Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A statistically significant, dose-related and reproducible increase in the number of cells with structural chromosome aberrations (excl. gaps).
- The number of aberrant cells (excl. gaps) exceeds both the concurrent negative/vehicle control value and the historical negative control data range.

A test substance is generally considered as “negative” if the following criteria are met:

- The number of cells with structural aberrations (excl. gaps) in the dose groups is not statistically significant increased above the concurrent negative/vehicle control value and is within the historical negative control data range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY:

A preliminary cytotoxicity assay was performed to determine appropriate test substance concentrations for the main test. Dose selection was based on the cell count, cell attachment, mitotic index and the quality of metaphases. 2000 µg/mL with and without metabolic activation was selected as the top dose for the 1st experiment of the main test. 250 µg/mL with metabolic activation was selected as the top doses for the 2nd experiment of the main test.

C. CYTOGENICITY ASSAYS:

In the first experiment after a treatment time of 4 hours no relevant increase in the number of chromosomally damaged cells was observed without S9 mix [see Table 5.8.1-21]. The aberration rates after test substance treatment were within the historical negative control data range (0.0% - 5.5% aberrant metaphases, excl. gaps). In the presence of S9 mix a single, statistically significant increase of structural chromosome aberrations (9% aberrant metaphases, excl. gaps) was observed at the intermediate dose of 125 µg/mL [see Table 5.8.1-22]. This concentration was at the border of test substance solubility in culture medium. To confirm the observation an increased sample of 400 metaphase cells was scored. The aberration rate clearly exceeded historical negative control data range (0.0% - 5.5% aberrant metaphases, excl. gaps). The other two scored dose groups (62.5 and 250 µg/mL) showed aberration rates (5.0 and 5.5% exclusive gaps) being within historical negative control data range.

In the second experiment, a statistically significant increase in the number of chromosomally damaged cells was observed after 4 hours exposure in the presence of a metabolizing system. At 100, 200 and 250 µg/mL the number of aberrant metaphases (9.0, 6.5 and 9.5%, respectively) exceeded the historical negative control data range. The aberration rates at 100 and 250 µg/mL were statistical significanct different from the control [see Table 5.8.1-23].

In both experiments, no indication for an aneugenic effect was observed.

No suppression of the mitotic activity was observed under any of the experimental conditions in both experiments. No growth inhibition was observed under any of the experimental conditions, except in the 2nd experiment at 250 µg/mL (49.1% of control).

The osmolarity and pH values were not influenced by the test substance treatment. Test substance precipitation in culture medium at the end of exposure period was macroscopically observed from 250 µg/mL onwards in both experimental parts in the 1st experiment and from 200 µg/mL in the 2nd experiment.

Vehicle and positive controls were all in a range to ensure the validity of the test.

III. CONCLUSION

Based on the results of the study it is concluded that 500M04 (Reg.No. 298327, metabolite of pyraclostrobin) is a chromosome damaging (clastogenic) substance under in vitro conditions using V79 cells in the presence of metabolic activation. The relevance of this in vitro result was evaluated in a higher tier in vivo study (see below) according to Commission Regulation (EU) No 283/2013, Section 5.4.2.

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Table 5.8.1-21: Chromosome aberration test with Reg.No. 298327 without metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	7.9	6	6.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	B	100	9.2	4	4.0	4	4.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**8.6**	**10**	**5.0**	**6**	**3.0**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**
Reg.No. 298327																			
62.5 µg/mL	A	100	4.3	10	10.0	7	7.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	7.7	5	5.0	4	4.0	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**6.0**	**15**	**7.5**	**11**	**5.5**	**5**	**2.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
125 µg/mL	A	100	6.5	3	3.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	7.9	4	4.0	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**7.2**	**7**	**3.5**	**5**	**2.5**	**3**	**1.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
250 µg/mL	A	100	6.3	3	3.0	2	2.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	8.6	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**7.5**	**6**	**3.0**	**3**	**1.5**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Positive control EMS																			
500 µg/mL	A	50	8.4	9	18.0	9	18.0	6	12.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	7.8	12	24.0	10	20.0	8	16.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100#**	**8.1**	**21**	**21.0****	**19**	**19.0****	**14**	**14.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Table 5.8.1-22: Chromosome aberration test with Reg.No. 298327 with metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	11.8	3	3.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	11.3	6	6.0	4	4.0	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0
	A + B	**200**	**11.6**	**9**	**4.5**	**7**	**3.5**	**4**	**2.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**
Reg.No. 298327																			
62.5 µg/mL	A	100	6.8	8	8.0	5	5.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	6.8	8	8.0	5	5.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**6.8**	**16**	**8.0**	**10**	**5.0**	**8**	**4.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
125 µg/mL	A	100	8.0	12	12.0	9	9.0	5	5.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	5.2	8	8.0	7	7.0	6	6.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	C	100	0.0	17	17.0	13	13.0	7	7.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	D	100	0.0	15	15.0	7	7.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**400**	**6.6**	**52**	**13.0****	**36**	**9.0***	**20**	**5.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.2**	**0**	**0.0**
250 µg/mL	A	100	7.7	8	8.0	6	6.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	7.6	6	6.0	5	5.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**7.7**	**14**	**7.0**	**11**	**5.5**	**6**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Positive control CPP																			
0.5 µg/mL	A	50	11.4	8	16.0	8	16.0	3	6.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	11.1	14	28.0	14	28.0	9	18.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100**	**11.3**	**22**	**22.0****	**22**	**22.0****	**12**	**12.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Table 5.8.1-23: Chromosome aberration test with Reg.No. 298327 with metabolic activation (18 hours treatment, harvest after 18 hours) - Experiment 2

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	9.2	6	6.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	11.0	5	5.0	4	4.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**10.1**	**11**	**5.5**	**5**	**2.5**	**3**	**1.5**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Reg.No. 298327																			
100 µg/mL	A	100	5.4	12	12.0	12	12.0	6	6.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	7.5	8	8.0	6	6.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**6.5**	**20**	**10.0**	**18**	**9.0***	**7**	**3.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
150 µg/mL	A	100	5.6	13	13.0	5	5.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	6.7	7	7.0	4	4.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**6.2**	**20**	**10.0**	**9**	**4.5**	**6**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
200 µg/mL	A	100	8.5	8	8.0	5	5.0	3	3.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	B	100	11.7	10	10.0	8	8.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**10.1**	**18**	**9.0**	**13**	**6.5**	**7**	**3.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**
250 µg/mL	A	100	8.4	21	21.0	15	15.0	7	7.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	5.5	13	13.0	4	4.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**7.0**	**34**	**17.0****	**19**	**9.5***	**9**	**4.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Positive control EMS1																			
350.0 µg/mL	A	50	4.2	14	28.0	14	28.0	10	20.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	8.6	13	26.0	12	24.0	8	16.0	1	2.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100#**	**6.4**	**27**	**27.0****	**26**	**26.0****	**18**	**18.0****	**1**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Report:	CA 5.8.1/19 [redacted] 2013a Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2013/1026779
Guidelines:	OECD 474 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 298327 (metabolite of BAS 500 F, pyraclostrobin; batch: L84-174, purity: 99.6%) was tested for chromosomal damage (clastogenicity) and for the ability to induce spindle poison effects (aneugenic activity) in NMRI mice using the micronucleus test method. For this purpose, the test substance was administered once orally to groups of 5 male mice at dose levels of 500, 1000 and 2000 mg/kg body weight. The vehicle served as negative and cyclophosphamide and vincristine sulfate as positive controls. The animals were sacrificed 24 or 48 (additional high dose and vehicle group) hours after the administration and the bone marrow of the two femora was prepared and investigated for micronuclei.

The oral administration of Reg.No. 298327 did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing either small or large micronuclei. The rate of micronuclei was mostly close to the concurrent negative control and was within the range of the historical control data. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur. Weak signs of systemic toxicity were observed in all dose groups consisting of piloerection and hunched posture.

Both of the positive control chemicals, i.e. cyclophosphamide for clastogenic effects and vincristine for induction of spindle poison effects, led to the expected increase in the rate of polychromatic erythrocytes containing small (cyclophosphamide) or small and large (vincristine sulphate) micronuclei, thus demonstrating the sensitivity of the test system.

In conclusion, under the the experimental conditions of this study, the test substance Reg. No. 298327 (Metabolite of BAS 500 F, pyraclostrobin) does not induce cytogenetic damage in bone marrow cells of NMRI mice in vivo.

(BASF DocID 2013/1026779)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

	Reg.No. 298327 (Metabolite of pyraclostrobin)
Description:	Solid, yellow
Lot/Batch #:	L84-174
Purity:	99.6% (tolerance ± 1.0%)
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Apr 2020 as indicated by the sponsor. Homogeneity of the test substance was guaranteed on account of the high purity and was ensured by mixing before test substance preparation.
Vehicle used:	DMSO and subsequent emulsion in corn oil

2. Control Materials:

Negative:	No negative control was employed in this study.
Vehicle control:	DMSO and subsequent emulsion in corn oil
Positive control:	Cyclophosphamide (CCP) 20 mg/kg for the determination of clastogenic effects Vincristine sulphate (VCR) 0.15 mg/kg for the determination aneugenic effects

3. Test animals:

Species:	Albino mice
Strain:	Crl:NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	5 - 8 weeks
Mean body weight at dosing:	28.8 g
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	Not indicated in the report
Micronucleus assay:	5 males/dose
Acclimation period:	At least 5 days
Diet:	Standardized pelleted feed (Maus/Ratte Haltung "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland)
Water:	Drinking water in bottles, ad libitum
Housing:	During the study the mice were housed individually in Makrolon cages, type MII

4. Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	frequency not indicated (fully air-conditioned rooms)
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	2000 mg/kg
Micronucleus assay:	500, 1000 and 2000 mg/kg The test substance was administered once by oral gavage using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 30-Jul-2012 to 10-Jan-2013

2. Preliminary range finding test:

Male and female NMRI mice were treated once by oral gavage with a test substance dose of 2000 mg/kg bw.

3. Micronucleus test:

Treatment and sampling:	Groups of 5 male mice were treated once with either vehicle or 500, 1000 or 2000 mg test substance/kg bw by oral gavage. Additional test groups treated with the vehicle control and the high dose were used for the second sampling period. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substances CCP and VCR were administered once by oral gavage (CCP) or i.p. injection (VCR). The animals were observed for evident clinical signs of toxicity throughout the study.

Twenty-four hours after the administration the mice were sacrificed and the two femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 300xg for 5 minutes. The supernatant was discharged and the pellet resuspended in about 50 µl fresh FCS. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.

Slide preparation:

One drop of the suspension was applied on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May Grünwald solution, rinsed, and finally stained with Giemsa solution (7.5%). After rinsing and clarifying in xylene, the preparations were mounted. The slides were coded prior to microscopic evaluation.

Slide evaluation:

In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored.

The increase in the number of micronuclei in polychrometic erythrocytes of treated animals as compared to the vehicle control group provides an index of a chromosome-breaking (clastogenic) effect or of a spindle activity (aneugenic) of the substance tested.

In addition, the number of small micronuclei ($d < D/4$) and of large micronuclei ($d \geq D/4$) (d = diameter of micronucleus, D = cell diameter) was determined: The size of micronuclei may indicate the possible mode of action of the test substance, i.e. a clastogenic or a spindle poison effect.

The ratio of polychromatic to normochromatic erythrocytes was calculated. An alteration of this ratio indicates a toxic effect on erythropoieses and thus, that the test substance actually reached the target organ.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test (modified rank test according to Wilcoxon). Here, the relative frequencies of cells with micronuclei of each animal were used.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and significant increase in the number of micronucleated polychromatic erythrocytes was observed.
- The proportion of cells containing micronuclei exceeded both the values of the concurrent vehicle control range and the vehicle historical control range.

A test substance is generally considered negative in this test system if:

- There was no significant increase in the number of micronucleated polychromatic erythrocytes at any dose above concurrent control frequencies.
- The frequencies of cells containing micronuclei were within the historical control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle was verified analytically. The homogeneity of the test substance in the vehicle was guaranteed by constant stirring during the removal and administration of the test substance formulation and by analytical determination of 3 individual samples of each concentration. The mean test substance concentrations were determined as 53.8, 95.7 and 193.3 mg/mL at nominal concentrations of 50, 100 and 200 mg/mL, respectively. This corresponds to a recovery rate ranging between 96% to 108% and is thus, within the expected range (90-110%).

B. PRELIMINARY RANGE FINDING TEST

In the pretest for the determination of the acute oral toxicity in males and females, the animals were treated with 2000 mg/kg bw. All animals survived with weak signs of toxicity. The clinical signs observed were piloerection and hunched posture. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

Micronucleus frequencies of the animals treated with the indicated doses of Reg.No. 298327 were near to the concurrent vehicle control values and within the historical control range (see Table 5.8.1-24). A single statistically significant increase in the number of micronucleated polychromatic erythrocytes was obtained at 24-hour sacrifice interval at low dose group (500 mg/kg bw). This micronucleus rate (1.1‰) was within the laboratory's historical vehicle control data range (0.3 - 3.0‰ micronucleated PCEs). Therefore, this observation is considered as biologically irrelevant. Large micronuclei (d > D/4) were neither observed in the negative control group nor in the test substance groups.
The PCE/NCE ratio was not affected by treatment with the test substance and were in the range of the vehicle control values. Thus, there was no indication that erythropoiesis was inhibited. At 2000 mg/kg body weight at 48 hours preparation interval the number of mature normochromatic erythrocytes was slightly reduced compared to the respective vehicle control group as indication of bone marrow toxicity, which is interpreted as indication that the test-item reached its target organ. This interpretation is supported by the results of a toxicokinetics study with Reg.No. 298327 in mice [see below; DocID 2012/1278425].
The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE containing mainly small micronuclei (24.4**, total 26.3‰). The administration of the spindle poison vincristine resulted in an incidence of micronuclei in polychromatic erythrocytes of 35.0‰. This included 12.5‰ of PCE with large micronuclei. The positive controls thus demonstrated the sensitivity of the test system.
The administration of the test substance was well tolerated by all animals with weak clinical signs of toxicity observed in all dose groups consisting of piloerection and hunched posture. No signs of systemic toxicity were observed in any of the animals treated with the positive control substances or the vehicle.

Table 5.8.1-24: Micronucleus test in mice administered Reg.No. 298327 by oral gavage

Treatment	PCE				NCE		PCE/NCE ratio
	scored	With MN			Number	With MN	
		total [‰]	small [‰]	large [‰]		[‰]	
DMSO emulsified in corn oil corn oil	10000	0.3	0.3	0.0	4743	0.8	2.1
Reg.No. 298327							
500 mg/kg	10000	1.1**	1.1**	0.0	4597	0.4	2.2
1000 mg/kg	10000	1.1	1.1	0.0	4873	0.4	2.1
2000 mg/kg	10000	0.4	0.4	0.0	4702	0.4	2.1
Positive controls							
Cyclophosphamide	10000	**26.3****	**24.4****	1.9	3158	1.9	3.2
Vincristine	10000	**35.0****	**22.5****	**12.5****	5877	0.3	1.7
48 h preparation interval							
DMSO emulsified in corn oil corn oil	10000	1.3	1.3	0.0	4639	1.5	2.1
Reg.No. 298327							
2000 mg/kg	10000	2.2	2.2	0.0	2923	0.7	3.4

** $p \leq 0.01$ (Wilcoxon Test; one sided)
PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes; MN: micronuclei

III. CONCLUSION

Based on the result of this study 500M04 (Reg.No. 298327, metabolite of pyraclostrobin) does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study, i.e. is devoid of clastogenic or aneugenic activity in vivo. A kinetic study demonstrated that the Reg.No. 298327 reached the target organ (bone marrow). Thus, the clastogenicity observed in vitro is not relevant under in vivo conditions.

Studies on Absorption, Distribution, Metabolism and Excretion of 500M04 (Reg.No. 298327, BF 500-5, 'Pyrazolon') in Mammals

Report:	CA 5.8.1/20 [redacted], 2013a 14C-Reg.No. 298327 (metabolite of BAS 500 F, Pyraclostrobin) - Study on the kinetics in mice 2012/1278425
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142, OECD 417, EPA 870.7485, JMAFF Guidelines on the Compiling of Test Results on Toxicity - Tests on In Vivo Fate in Animals (2001)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The distribution of Reg.No. 298327 (Metabolite of pyraclostrobin) in mouse blood cells, plasma and bone marrow after a single oral administration was determined using a test substance formulation containing radiolabelled ^{14}C-Reg.No. 298327 (Metabolite of pyraclostrobin). For this purpose, 5 male mice were treated once orally with a nominal dose of 1000 mg/kg bw. The target quantity of radioactivity was about 10 MBq per animal. After 5 hours the animals were sacrificed and samples of the indicated tissues were analysed for test substance content.

The analysis of the test substance formulation showed that the animals 1 to 4 received an actual dose of 986.2, 1035.1 and 1047.6 and 1082.7 mg/kg bw, respectively. Since in a first experiment the dosing failed for one animal due to technical problems, this animal was excluded from analysis. The mean radioactive dose of the 4 remaining animals was 9.25 MBq/animal. The mean total radioactive residues (TRR) in the bone marrow (25.30 µg Eq/g) after 5 hours were 0.001% of the applied dose, 0.008% in the plasma (29.83 µg Eq/g) and 0.003% in the blood cells (7.62 µg Eq/g).

Therefore, it is concluded that Reg. No. 298327 is systemically bioavailable and its presence in the bone marrow and blood after an oral administration is confirmed.

BASF DocID 2012/1278425

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

	a) ^{14}C-Reg.No. 298327 (Metabolite of BAS 500F)
	b) Reg.No. 298327 (Metabolite of BAS 500F)
Description:	a) Not indicated
	b) Solid, yellow
Lot/Batch #:	a) 724-2102
	b) L84-174
Purity:	a) radiochemical purity: >98%; specific activity: 8.99 MBq/mg
	b) 99.6% (tolerance +/- 1.0%)
Stability of test compound:	a) The stability of ^{14}C-Reg.No. 298327 was demonstrated analytically.
	b) The stability of Reg.No. 298327 was guaranteed by the sponsor (expiry date: 01-Apr-2020).

2. Vehicle: 0.5% CMC in drinking water

3. Test animals:

Species:	Albino mice
Strain:	Crl:NMRI
Number:	5
Sex:	Male
Age:	Not indicated
Weight at dosing:	about 30 - 40 g
Source:	Charles River Laboratories Germany GmbH
Acclimation period:	at least 8 days
Diet:	Kliba lab diet (mouse / rat “GLP”), pelleted (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water:	drinking water, ad libitum
Housing:	During acclimatization and prior to the experiment animals were housed individually in Macrolon cages type M III. During the experiments animals were housed individually in steel wire mesh cages.

4. Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	15 / hour
Lighting:	12 hours light / 12 hours darkness

5. Dose selection

A nominal dose of 1000 mg/kg bw was selected for this study based on repeated dose studies with Reg.No. 298327.

6. Preparation of the dosing solutions

In order to administer a target quantity of 10 MBq per animal, a stock solution of ^{14}C-Reg.No. 298327 was prepared in acetone. Reg.No. 298327 was added to this solution with an end nominal concentration of 100 mg/mL. The solvent was then evaporated and the mixture formulated in 0.5% CMC in drinking water.

7. Analyses

The stability, homogeneity and correctness of the concentration of the test substance preparation as well as the purity of ^{14}C-Reg.No. 298327 were determined analytically under the conditions described below:

HPLC (HP1100) analysis

Column:	Ascentis Express C18, 150 x 4.6 mm, 2.7 µm
Eluent:	A) acetonitrile / formic acid (1000 mL+1 mL) B) deionized water / formic acid (1000 mL+1 mL), %B: 60%, isocratic
Flow:	1.0 mL/min
Detection:	UV-Extinction at 274 nm HPLC Radioactivity Monitor LB 509 (Cell: 150)

B. TEST PERFORMANCE.

1. Dates of experimental work: 07-Aug-2012 to 03-Apr-2013

2. Study design:

Overall, 5 males were weighed and treated orally with a single nominal dose of 1000 mg/kg bw of the test substance preparation. The application volume was 10 mL/kg. After 5 hours the animals were sacrificed and samples of blood cells, plasma and the bone marrow were weighed and analyzed for total radioactive residues using a liquid scintillation counter. Since in a first experiment the dosing failed for one animal due to technical problems, the amount of test substance preparation for analysis after administration was insufficient. Therefore, only two animals were investigated (animals 1 and 3) and reanalysis of the test substance preparation after administration was not available. In a second experiment, two additional animals were dosed (animals 2 and 4) and the homogeneity, the correctness of the concentration and of the radiochemical purity was demonstrated at least before and after test substance administration. However, based on the current data, radioactivity was clearly detectable in blood, plasma and bone marrow of orally dosed animals.

3. Calculations:

Depending on the preparation of the samples the appropriate formulas were chosen.

Key of abbreviations		Dimension
DPM	= disintegrations per minute (abs. or per sample)	[DPM]
LSC	= weight of sample, prepared for liquid scintillation counting (LSC)	[g]
SOL	= weight of soluene	[g]
TRR	= total radioactive residues	[µg/g]
FRE	= weight of freeze drying sample	[g]
SAM	= weight of organs/Tissue	[g]
AQU	= weight of double distilled water	[g]
ACT	= specific activity of test article	[DPM/µg]
EQUTIS	= equivalents of test article per tissue weight	[µg/g]
D_{rad}	= dose of radioactivity administered	[DPM]

Formula I

$$\%\ of\ D_{rad} = \frac{\sum_{n=1}^{n} DPM_n / LSC_n}{n} \times \frac{SOL}{FRE} \times (SAM + AQU) \times \frac{100}{D_{rad}}$$

Formula II

$$EQUTIS = \frac{\sum_{n=1}^{n} DPM_n / LSC_n}{n} \times \frac{SAM + AQU}{SAM \times ACT}$$

Formula III

$$DPM = \left(\left(TRR\left[\frac{\mu g}{g}\right] \right) * \left(Spec.activity\left[\frac{MBq}{g}\right] * 60 \right) * Poolsampleweight[g] \right)$$

Formula IV

$$\%Dose = \left(DPM\left(Radioact.dose\left[\frac{MBq}{animal}\right] * 60000000 \right) \right) * 100\%$$

II. RESULTS AND DISCUSSION

STABILITY, HOMOGENEITY AND CONTENT OF THE TEST SUBSTANCE IN THE APPLICATION MEDIUM

The stability, homogeneity of ^{14}C- Reg.No. 298327 in the vehicle was confirmed by analysis. The achieved doses of the test substance in the individual mice were 986.2, 1035.1, 1047.6, 1082.7 and 1037.9 mg/kg bw for mice 1 to 4, respectively.

BIOAVAILABILITY DATA

The data in Table 5.8.1-25 demonstrate that after 5 h various amounts of residual radioactivity were present in blood, plasma and bone marrow.

Table 5.8.1-25: Radioactive residues in various tissues of male mice after a single treatment with ^{14}C-Reg.No. 298327

Animal No.		1	2	3	4	MEAN	SD
Animal weight [g]		37.7	34.7	32.9	32.3	34.4	2.41
Spec. activity [MBq/g]		44.11	75.62	44.11	75.62	59.87	18.19
Dose admin. [mg/kg bw]		986.2	035.1	047.6	082.7	037.9	39.90
Radioact. dose [MBq/animal]		9.06	9.90	8.41	9.63	9.25	0.66
Plasma	[µg Eq/g]	7.56	38.25	35.23	38.28	29.83	4.92
	[(%*)]	(0.002%)	0.010%)	0.010%)	0.011%)	0.008%)	0.004%)
Blood cells	[µg Eq/g]	1.68	6.83	5.54	6.45	7.62	6.47
	[(%*)]	(0.001%)	0.006%)	0.003%)	0.003%)	0.003%)	0.002%)
Bone marrow	[µg Eq/g]	4.20	36.83	20.60	39.58	25.30	6.37
	[(%*)]	(0.000%)	0.001%)	0.001%)	0.001%)	0.001%)	0.000%)

*: percent of the total administered dose

III. CONCLUSION

Analysis of the systemic availability of 500M04 (Reg.No. 298327) using a radiolabelled mixture showed that 5 h after a single oral administration the test substance could be detected in all examined tissues (blood cells, plasma and bone marrow). Thus, 500M04 is considered to be bioavailable to these tissues after oral application, i.e. the target tissue investigated in the in vivo micronucleus test was exposed to Reg.No. 298327.

Overall toxicological evaluation of 500M04 (Reg.No. 298327, BF 500-5, 'Pyrazolon')

500M04 was of low acute oral toxicity, was not-irritating to the skin and the eye and was not a skin sensitizer.

In a 90-day rat study 500M04 induced a slight regenerative normochromic-macrocytic anemia in males accompanied by an adaptive increase of extramedullary hematopoiesis in the spleen. Furthermore, high dose animals displayed increased drinking water consumption, which resulted in an increased discharge of less concentrated urine. In mid and high dose males a strong increase of unknown crystals was noted in the urine sediment. These changes were seen in a probable relation to the histopathological lesions observed in the kidneys at this dose level. Thus the NOAEL was 103 mg/kg bw/d (target dose level of 100 mg/kg bw/d) in male and 316 mg/kg bw/d (target dose level of 300 mg/kg bw/d) in female Wistar rats, respectively.

The QSAR evaluations of 500M04 were of low reliability and by weight of evidence there was no conclusive alert for genotoxicity.

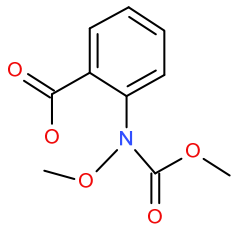
By weight of evidence 500M04 is not genotoxic based on the in vitro and in vivo studies conducted. A mouse kinetic study clearly confirmed that 500M04 is systemically bioavailable and thus present in bone marrow and blood after oral administration.

Compared to the active ingredient pyraclostrobin 500M04 is less toxic. Based on the available toxicological data it is considered to be **not toxicologically relevant** and an **ADI of 0.52 mg/kg bw/day** was established taking into account the NOAEL of 103 of the 90-day rat study and an assessment factor of 200 in order to adopt from a sub-chronic to a live-long exposure period.

The predicted chronic exposure for 500M04 (and its derivatives) does not exceed 0.1% of the proposed ADI of 0.52 mg/kg bw/day.

Group 3: Anthranilic acid derivatives

500M24 (other denominator: Reg.No. 5916421)



500M24 (Reg.No. 5916421) is a metabolite found in the rat as well as in the wheat metabolism studies.

A) QSAR Predicitions on 500M24 (Reg.No. 5916421)

OECD Toolbox (Version: 3.2) [see DocID 2014/1172955]
The OECD Toolbox revealed no DNA alerts for Ames, MN and CA or for DNA or protein binding.

OASIS TIMES (MIX V.2.27.13 TB; Mutagenicity S-9 activated v08.08) [see molecule 7 of report DocID 2014/1172952 and 2014/1172953]

The Ames mutagenicity predicition for 500M24 (Reg.No. 5916421) and its 8 in-silico generated metabolites was negative (out of domain). This holds true for the prediction of in vitro CA mutagenicity.

VEGA: Mutagenicity model (CAESAR, version 2.1.9) [see molecule 7 of report DocID 2014/1172954; for both VEGA models]

The prediction for 500M24 (Reg.No. 5916421) is mutagen (out of model applicability domain). The prediction is based on 6 molecules with the prediction mutagen, which was experimentally confirmed for 4 of them. The similarity indices ranged from 0.657 to 0.739 and thus not very good.

Discussion: The prediction is in contrast to the 500M24 (Reg.No. 5916421) experimental result and thus rejected.

VEGA: Mutagenicity model (SarPy; version 1.0.4-BETA)

500M24 (Reg.No. 5916421) is out of model applicability domain. The prediction is 'mutagen' with the structural alert SA 98. As discussed above, this alert was also obtained for pyraclostrobin and a number of pyraclostrobin metabolites, which like 500M24 (Reg.No. 5916421) were actually tested negative. The predicition is therefore rejected.

DEREK (Version 2.0.0.201011301322; Knowledge base version: 11_20_05_2010)

The prediction for genotoxicity and neurotoxicity is negative.

Genotoxicity studies on 500M24 (Reg.No. 5916421)

Report:	CA 5.8.1/21 Woitkowiak C., 2014a Reg.No. 5916421 (Metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium/Escherichia coli, reverse mutation test 2013/1323364
Guidelines:	OECD 471 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium strains TA98, TA100, TA 1535 and TA 1537 and E. coli strain WP2 uvrA were exposed to Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin); batch: L82-125, purity: 98.8%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent experiments. Vehicle and positive controls were included in each experiment.

In both assays, the plate incorporation assay and the preincubation assay, the test substance was tested in concentrations of 33, 100, 333, 1000, 2500 and 5000 µg/plate. No biologically relevant increase in the number of revertant colonies was noted in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.

A bacteriotoxic effect was occasionally observed in the standard plate test depending on the strain and test conditions from about 1000 µg/plate onwards. No precipitation of the test substance was found up to the highest tested concentration.

The test substance Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin) is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2013/1323364)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

test substance Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin)

Description: Solid, brownish
Lot/Batch #: L82-125
Purity: 98.8% (tolerance +/- 1.0%)
Stability of test compound: Stable - expiry date 01-Sep-2015. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance solutions. The stability of the test substance in the vehicle DMSO was verified analytically.
Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).
Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration: 100 µL/plate
Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60 µg/plate

3. Activation:

S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received on three consecutive days intraperitoneal injections of 80 mg phenobarbital and 80 mg β-naphthoflavone orally per kg body weight. 24 hours after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9 mix, was kept on ice until used.

The S9 mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

To demonstrate the efficacy of the S9 mix in this assay, the S9 batch was further characterized with benoz(a)pyrene in the strains TA 100 and TA 98 as required in OECD 471.

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
Salmonella typhimurium:
The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay: In the first experiment triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay: In the second experiment the test article / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains.

B. TEST PERFORMANCE:

1. Dates of experimental work: 27-Aug-2013 to 06-Sep-2013

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Merckoplate® plates (minimal glucose agar plates). In the experiments with E. coli the warm soft agar contains 0.5 mM tryptophan instead of histidine + biotin.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37°C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects, the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37°C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions. The stability of the test substance in the vehicle DMSO was verified analytically (BASF study 01Y0173/13Y006).

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A weak bacteriotoxic effect as indicated by a slight decrease in the number of his^+ or trp^+ revertants or slight reduction in the titer was occasionally observed in the standard plate test conditions at concentrations ≥1000 µg/plate and in all strains with and without metabolic activation in the preincubation at ≥ 2500 g/plate.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiment with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested [see Table 5.8.1-26]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system. Precipitation was not observed up to the maximum concentration.

Table 5.8.1-26: Bacterial gene mutation assay with Reg.No. 5916421 - Mean number of revertants

Experiment 1/2[#]: Plate incorporation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	26	19	54	52	13	11	13	7	81	81
Reg.No. 5916421										
33 µg/plate	26	21	52	55	13	10	12	8	94	76
100 µg/plate	26	21	50	51	14	13	13	8	86	83
333 µg/plate	27	17	47	49	12	8	12	8	80	74
1000 µg/plate	26	20	41	53	11	7	11	8	87	85
2500 µg/plate	25	19	36	51	11	7	11	6	64	70
5000 µg/plate	20	11	29	28	10	7	7	5	39	43
Pos. control[§]	1318	611	1555	1385	412	1337	266	680	726	996
Experiment 3: Preincubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	17	18	43	40	11	11	6	7	40	70
Reg.No. 5916421										
33 µg/plate	17	17	37	39	10	11	6	6	38	68
100 µg/plate	17	14	39	37	10	10	8	6	38	67
333 µg/plate	17	17	41	37	12	11	7	8	41	70
1000 µg/plate	17	15	44	40	10	12	9	7	39	73
2500 µg/plate	8[B]	7[B]	14[B]	11[B]	10[B]	5[B]	2[B]	2[B]	26[B]	21[B]
5000 µg/plate	0[B]	0[B]	0[B]	0[B]	4[B]	0[B]	1[B]	0[B]	21[B]	13[B]
Pos. control[§]	1387	844	1259	770	440	1457	254	777	465	945

[§] Compound and concentrations see Material and Methods (I.A.2.) above
[B] reduced background growth
[#] Due to a contamination of TA98 plates in the plate incorporation assay, the repetition of the TA98 experiment was performed as second experiment. Accordingly, the preincubation assay was the 3rd experiment

III. CONCLUSION

According to the results of the present study, the test substance 500M24 (Reg.No. 5916421, metabolite of pyraclostrobin) is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen.

Report:	CA 5.8.1/22 Schulz M., Landsiedel R., 2014f Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells (TK+/- locus assay, microwell version) 2013/1298449
Guidelines:	OECD 476 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 5916421 (Metabolite of 500 F; pyraclostrobin; batch: L82-125, purity: 98.8%) was tested in vitro for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the $TK^{+/-}$ locus. Two independent experiments were conducted in the presence and absence of metabolic activation with concentrations from 143.8 to 2300 µg/mL. The treatment intervals were 4 or 24 hours. Methyl methanesulfonate (MMS) and cyclophosphamide (CPP) served as positive controls in the experiments without and with metabolic activation, respectively.

Reg.No. 5916421 did not cause any biologically relevant increase in the mutant frequencies with or without a metabolizing system in two independently performed experiments. The positive control substances induced a marked increase in mutant frequency thus demonstrating the sensitivity of the test system.
Based on the results of the study it is concluded that under the conditions of the test Reg.No. 5916421(Metabolite of BAS 500 F; pyraclostrobin) does not induce forward mutations in mammalian cells in vitro.

(BASF DocID 2013/1298449)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Reg.No. 5916421 (Metabolite of 500 F; pyraclostrobin)
Description:	Solid, brownish
Lot/Batch #:	L82-125
Purity:	98.8% (tolerance ± 1.0%)
Stability of test compound:	Stable - Expiry date: 01-Sep-2015. The homogeneity of the test substance was guaranteed on account of the high purity and ensured by mixing prior to preparation of test substance solutions. The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	The vehicle controls with and without S9 mix contained the vehicle selected for the test substance at the same volume and concentration as used in the test cultures.
Positive control -S9:	Methyl methanesulfonate (MMS) 15 µg/mL (4-hour exposure period) and 5 µg/mL (24-hour exposure period)
Positive control +S9:	Cyclophosphamide (CPP) 2.5 µg/mL

3. Activation:

S9 was produced from the livers of at least 5 induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organism:

Mouse lymphoma L5178Y $TK^{+/-}$ cell line:
This is a cell line with a high proliferation rate (doubling time of about 9 - 10 hours), a high plating efficiency and a stable karyotype with a near diploid number of 40 ± 1 chromosome. Each batch used for mutagenicity testing was checked for mycoplasma contamination.

5. Culture media:

Culture medium:

RPMI 1640 medium including stable glutamine supplemented with:
1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL) and 1% (v/v) sodium pyruvate (10 mM) = RPMI-0
For treatment medium (with S9 mix):
RPMI-0 supplemented with 5% (v/v) fetal calf serum (FCS) = RPMI-5

	For treatment medium (without S9 mix) and subculturing cells: RPMI-0 is supplemented with 10% (v/v) FCS = RPMI-10 For cloning efficiency and selection medium: RPMI-0 supplemented with 20% (v/v) FCS = RPMI-20
Pretreatment medium:	A ("THMG" medium): RPMI-10 supplemented with thymidine (3.0 µg/mL), hypoxanthine (5.0 µg/mL), methotrexate (0.1 µg/mL) and glycine (7.5 µg/mL) B ("THG" medium): RPMI-10 supplemented with thymidine (3.0 µg/mL), hypoxanthine (5.0 µg/mL), and glycine (7.5 µg/mL)
Selection medium:	("TFT" medium): RPMI-20 supplemented with trifluorthymidine (TFT, 4.0 µg/mL)

6. Locus examined: thymidine kinase (TK)

7. Test concentrations:

a) Preliminary toxicity assay: Nine concentrations ranging from 9.0 to 2300 µg/mL
b) Mutation assay:

1st experiment:	287.5, 575, 1150, and 2300 µg/mL with and without metabolic activation (4-hour exposure period)
2nd experiment:	143.8, 287.5, 575, 1150 and 2300 µg/mL without (24-hour exposure period) and 431.3, 862.5, 1725 and 2300 µg/mL with metabolic activation (4-hour exposure period)

B. TEST PERFORMANCE:

1. Dates of experimental work: 24-Jun-2013 to 16-Jan-2014

2. Preliminary

cytotoxicity assay: In the pretest for toxicity based on the purity and the molecular weight of the test substance 2300 µg/mL (approx. 10 mM) Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin) was used as top concentration both with and without S9 mix at 4 hour exposure time and without S9 mix at 24 hour exposure time.

The pretest was performed following the method described for the main experiment. The relative suspension growth (RSG) was determined as toxicity indicator for dose selection and various parameters were checked for all or at least for some selected doses. Additionally, pH and solubility were determined.

3. Mutation Assay:

Pretreatment of Cells: During the week prior to treatment, spontaneous TK deficient mutants ($TK^{-/-}$) were eliminated from the stock cultures by incubating 3 x 10^5 cells per 75 cm² flask in a total volume of 30 mL for 1 day in "THMG" medium (pretreatment medium A), and for the following 3 days in "THG" medium (pretreatment medium B).

Cell treatment: For each test group, about $1.5x10^7$ cells per flask were seeded into 75 cm^2 flasks containing RPMI-10 for exposures without S9 mix and RPMI-5 medium for exposures with S9 mix and incubated for the respective exposure period (1st experiment: 4-hour exposure with and without S9 mix and 2nd experiment 24 hours without S9 mix and 4 hours with S9 mix) with 5% CO_2 at 37°C and ≥90% humidity for cell attachment. 2 cultures were treated in parallel for each test group.

Expression: At the end of the exposure period, the cells were transferred in tubes, centrifuged for 5 minutes at and were resuspended in RPMI-5 medium. The washing of the cells was repeated at least once. Then the cells were centrifuged and were resuspended in RPMI-10 medium. From each culture a sample of treated cells ($2x10^5$ cells/mL or $6x10^6$ cells/flask) were pipetted in 75 cm² flasks and were incubated for a 2-day expression period. To maintain exponential growth during this phase, each culture was counted daily and the cell numbers were adjusted at each day to $2x10^5$ cells/mL in 30 mL RPMI-10 medium.

Selection: For the mutant selection, the cells were resuspended in selection medium ("TFT" medium) and two 96-well plates per cultures were seeded with 2000 cells/well. After incubation of at least 9 days, both the number of negative wells and the number of wells containing small or large colonies were scored for calculation of the mutant frequency (MF).

Size distribution of the colonies:	The colonies are classified into large colonies (indication of gene mutation) and small colonies (indication of chromosome breakage). Small colonies are defined as less than 1/4 of the diameter of the well. Size is the key factor and morphology (the optical density of the small colonies is considerably higher) should be secondary.
Determination of Cytotoxicity:	Cloning efficiency 1 (survival): The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. At the end of the exposure period, the cells were centrifuged and 400 cells from each culture were resuspended in 50 mL RPMI-20 medium (8 cells/mL). Per culture 200 µL were dispensed in each well of two 96-well plates (1.6 cells/well). After incubation for 9 - 11 days the plates were scored for empty wells.
Cloning efficiency 2 (viability):	The viability (cloning efficiency 2; CE_2) was determined after the expression period. 2 days after end of exposure, the cells were centrifuged and 400 cells from each culture were resuspended in 50 mL RPMI-20 medium (8 cells/mL). Per culture 200 µL were dispensed in each well of two 96-well plates (1.6 cells/well). After incubation for at least 9 days the plates were scored for empty wells. Suspension growth (RSG) and relative total growth (RTG): For calculation of the suspension growth (SG) and the relative total growth (RTG) the cell counts determined within the expression period at 2nd and 3rd passage after exposure in the case of 4-hour exposure and 1st, 2nd and 3rd passage after exposure in the case of 24-hour exposure were used. This measure includes the relative growth in suspension during the expression period (RSG) and the relative cloning efficiency (RCE_2; viability) at the time the mutants are selected. For taking into account any loss of cells during the 4-hour treatment period a relative growth during treatment factor (RGDT, %) was calculated by comparing the growth of each treated culture relative control.

Calculations:

Mutant frequency:
Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{-\ln(\text{total number of empty well s} \div \text{total number of seeded wells (96)})}{\text{number of seeded cells per well (2000)}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_2} \times 100$$

Cloning efficiency (CE, %) absolute:

$$CE_x = \frac{-\ln(\text{total number of empty well s} \div \text{total number of seeded wells (96)})}{\text{number of seeded cells per well (1.6)}} \times 100$$

Cloning efficiency relative, in comparison to control:

$$RCE_x = \frac{CE_x \text{ of the test group}}{CE_x \text{ of the negative / vehicle control}} \times 100$$

Relative total growth:

Relative growth during treatment (RGDT, %)

$$\text{RGDT} = \frac{\text{Cell count after 4 h of the test group}}{\text{Cell count after 4 h of the negative/v ehicle control}} \times 100$$

Total suspension growth (SG) after 4 hour-exposure:

$$SG = \frac{\text{Cell count after 24 h}}{2 \text{ x } 10^5 \text{ cells per mL}^{1,2}} \times \frac{\text{Cell count after 48 h}}{2 \text{ x } 10^5 \text{ cells per mL}^2} \times \frac{\text{RGDT}}{100}$$

Total suspension growth (SG) after 24 hour-exposure:

$$SG = \frac{\text{Cell count after 24 h}}{2 \text{ x } 10^5 \text{ cells per mL}} \times \frac{\text{Cell count after 48 h}}{2 \text{ x } 10^5 \text{ cells per mL}^2} \times \frac{\text{Cell count after 72 h}}{2 \text{ x } 10^5 \text{ cells per mL}^2}$$

$$\text{RSG} = \frac{\text{SG of the test group}}{\text{SG of the negative/vehicle control}} \times 100$$

$$RTG = \frac{RSG \times RCE_2}{100}$$

[1] Cell number seeded following 4-hour treatment
[2] If cell number was lower than 2 x 10^5 cells per mL all remaining cells were seeded

4. Statistics:

An appropriate statistical trend test was performed to assess a dose-related increase of mutant frequencies. The number of mutant colonies obtained for the test substance treated groups was compared with that of the respective vehicle control groups. A trend is judged as statistically significant whenever the one-sided p-value (probability value) is below 0.05 and the slope was greater than 0. Both, biological and statistical significance will be considered together. However, both, biological and statistical significance has been considered together.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- The mutation frequency exceeds a threshold of 126 mutant colonies per 10^6 cells (GEF: Global Evaluation Factor) above the concurrent negative/vehicle control value.

and

- Evidence of reproducibility of any increase in mutant frequencies, means the mutagenic response occurs at least in both parallel cultures of one experiment.

and

- A statistically significant dose-related increase in mutant frequencies using an appropriate statistical trend test.

A test substance is generally considered negative in this test system if:

- The mutation frequency is below a threshold of 126 mutant colonies per 10^6 cells (GEF) above the concurrent negative/vehicle control value.

or

- No evidence of reproducibility of an increase in mutant frequencies is obtained.

or

- No statistically significant dose-related increase in mutant frequencies using an appropriate statistical trend test is observed.

However, in the evaluation of the test results the historical variability of the mutation rates in negative and vehicle controls and the mutation rates of all negative and vehicle controls of this study were taken into consideration. Results of test groups have been rejected if the relative total growth (RTG) and/or the cloning efficiency 1 (CE_1) were less than 10% of the respective vehicle controls. Whenever a test substance is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose-related shift in the ratio of small versus large colonies clastogenic effects are indicated.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically (see BASF study code 01Y0173/13Y006).

B. PRELIMINARY CYTOTOXICITY ASSAY

After 4 hours treatment in the absence and presence of S9 mix no excessive cytotoxicity indicated by reduced relative suspension growth of about or below 20% was observed. After 24 hours treatment in the absence of S9 mix reduced relative suspension growth of 23.9% was observed at 2300 µg/mL.

The pH of the test substance preparation was adjusted by adding small amounts of NaOH. Osmolarity and pH values were not influenced by test substance treatment. In this study no precipitation of Reg.No. 5916421 in the vehicle DMSO or the culture medium was observed.
Based on these data the highest concentration tested in the mutagenicity experiments was 2300 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

No relevant increase in the number of mutant colonies was observed with and without metabolic activation after 4 or 24 hours of treatment [see Table 5.8.1-27]. The corrected mutant frequencies obtained for both experiments were always below the calculated threshold taking into consideration the Global Evaluation Factor (GEF). There was no statistically significant dose-related trend in both experiments with and without S9 mix. The mutant frequencies obtained were within the range of the historical negative control data.

In the 24h exposure experiment without metabolic activation the corrected mutant frequency at 2300 µg/mL exceeded the threshold of 164 colonies per 10^6 cells. However, as excessive cytotoxicity was observed at this concentration (relative total growth of 6.8%) this concentration was excluded from evaluation.

Table 5.8.1-27: Reg.No. 5916421 - Gene mutation in mammalian cells

Exp.	Exposure period [h]	Test groups [µg/mL]	S9 mix	Prec.*	Cytotoxicity**		Genotoxicity***	
					Relative cloning efficiency 1 RCE_1(%)	Relative total growth RTG (%)	Corrected mutant frequency (MF_{corr})	Mutant frequency threshold***
							(colonies per 10^6 cells)	
1	4	Vehicle control[1]	-	n.d.	100.0	100.0	40.3	166
		287.5	-	-	93.4	96.7	30.6	166
		575.0	-	-	91.4	102.3	25.8	166
		1150.0	-	-	82.7	108.5	37.9	166
		2300.0	-	-	85.1	73.9	48.6	166
		Positive control[2]	-	n.d.	64.5	37.3	264.8	166
2	24	Vehicle control[1]	-	n.d.	100.0	100.0	37.9	164
		143.8	-	-	89.7	79.4	45.3	164
		287.5	-	-	92.7	84.7	52.4	164
		575.0	-	-	96.6	83.8	31.7	164
		1150.0	-	-	76.3	59.8	37.2	164
		2300.0	-	-	19.8	6.8	308.6	164
		Positive control[3]	-	n.d.	70.9	38.9	537.9	164
1	4	Vehicle control[1]	+	n.d.	100.0	100.0	59.6	186
		287.5	+	-	86.5	114.0	45.1	186
		575.0	+	-	112.1	119.0	38.2	186
		1150.0	+	-	100.0	132.4	58.1	186
		2300.0	+	-	99.1	103.0	69.7	186
		Positive control[4]	+	n.d.	74.6	42.8	597.4	186
2	4	Vehicle control[1]	+	n.d.	100.0	100.0	41.6	168
		431.3	+	-	109.3	113.2	46.9	168
		862.5	+	-	109.3	127.6	40.3	168
		1725.0	+	-	92.3	102.6	33.2	168
		2300.0	+	-	115.3	132.6	36.8	168
		Positive control[4]	+	n.d.	76.2	64.8	411.8	168

* Precipitation in culture medium at the end of exposure period
** For calculation see above in this report
*** Mutant frequency threshold: number of mutant colonies per 10^6 cells of current vehicle control plus 126
n.d. Not determined
[1] DMSO 1% (v/v) [2] MMS 15.0 µg/mL
[3] MMS 5.0 µg/mL [4] CPP 2.5 µg/mL

Treatment with the positive controls MMS and CCP resulted in clearly increased mutant frequencies as expected. The values of the corrected mutant frequencies clearly exceeded the respective calculated thresholds for a mutagenic effect based on the GEF (126 plus the mutant frequency of the respective negative control).

The pH value of the test substance preparation was adjusted by adding small amounts of NaOH. Osmolarity and pH values were not influenced by test substance treatment. No precipitation of Reg.No. 5916421 in the vehicle DMSO or the culture medium was observed.

III. CONCLUSION

Based on the results of the study it is concluded that 500M24 (Reg.No. 5916421, metabolite of pyraclostrobin) does not induce forward mutations in vitro in the mouse lymphoma assay with L5178Y TK$^{+/-}$ cells in the absence and the presence of metabolic activation.

Report:	CA 5.8.1/23 Schulz M., Landsiedel R., 2014e Reg.No. 5916421 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells (cytokinesis block method) 2013/1363549
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin) (batch L82-125; purity 98.8%) was tested in vitro for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in three independent experiments with concentrations of 287 to 2300 µg/mL. The exposure periods were either 4 hours or 24 hours (without S9 only) and the preparation intevalls were 24 or 44 hours (with S9 only) after start of treatment. In order to confirm the results of the 2nd experiment in the absence of S9 mix a third experiment was performed.

The test substance induced a statistically significant and biologically relevant increase in the number of micronucleated cells after 24 hours continuous treatment without S9 mix. This observation was confirmed in a second experiment. No increased number of micronucleated cells was observed after the 4 hour treatment with and without metabolic activation.

In all three experiments with and without metabolic activation no cytotoxicity was observed up to the highest applied test substance concentration. Likewise, no test substance precipitation in culture medium was observed.

Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Based on the results of this study, Reg.No. 5916421 is considered to have a chromosome-damaging (clastogenic) effect or to induce numerical chromosomal aberrations (aneugenic activity) under in vitro conditions in V79 cells in the absence of metabolic activation.

(BASF DocID 2013/1363549)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin)

Description:	Solid, brownish
Lot/Batch #:	L82-125
Purity:	98.8% (tolerance ± 1.0%)
Stability of test compound:	Stable - Epxiry date 01-Sep-2015. The homogeneity of the test substance was guaranteed on account of the high purity and ensured by mixing prior to preparation of test substance solutions. The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO
Positive control, -S9:	Ethylmethanesulfonate (EMS, 300, 400 and 500 µg/mL, dissolved in MEM without FCS)
Positive control, +S9:	Cyclophosphamide (CPA, 1.0 and 2.5 µg/mL, dissolved in MEM without FCS)

3. Activation: S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction was thawed at room temperature and 1 volume of S9-fraction was mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9 mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms:

Chinese hamster V79 cells
This is a continuous cell line with a high proliferation doubling time of 12-14 hours, a high plating efficiency (≥90%) and a stable karyotype (modal number of 22 chromosomes).

5. Culture medium/conditions:

Culture media:

Minimal essential medium with Earle's salts (MEM) containing a L-glutamine source supplemented with 10% (v/v) fetal calf serum (FCS), 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL) and 1% (v/v) amphotericine B (250 µg/mL). During exposure to the test substance in the presence of S9 mix MEM medium was used without FCS supplementation.

Cell culture:

Deep-frozen cell stocks were thawed at 37°C in a water bath, and volumes of 0.5 mL were transferred into 25 cm^2 plastic flasks containing about 5 mL MEM supplemented with 10% (v/v) fetal calf serum (FCS). Cells were grown with 5% (v/v) CO2 at 37°C and ≥ 90% humidity and subcultured twice weekly. Cell monolayers were suspended in culture medium after detachment with 0.25% (w/v) trypsin solution.

Cell cycle and harvest time: The cell cycle of the untreated V79 cells lasts for about 12 to 14 hours under the selected culture conditions. Thus, a harvest time of 24 hours is about 2 times the normal cell cycle length. V79 cells are an asynchronous cell population, i.e. at the time of test substance treatment there are different cell stages. Since the effect on these cell stages may vary for different test substances, more than one harvest time after treatment may be appropriate. Furthermore, substance-induced mitotic delay may considerably delay the first post-treatment mitosis. Therefore, delayed harvest times (e.g. 44 hours) and prolonged exposure periods (e.g. 24 hours treatment) were considered.

6. Test concentrations:

a) Preliminary toxicity assay: 17.97 to 2300 µg/mL with and without metabolic activation
Exposure period: 4 hours with and without metabolic activation; 24 hours without metabolic activation
Harvest time: 24 hours

b) Cytogenicity assay:

1st experiment: 287, 575, 1150 and 2300 µg/mL with and without S9 mix (4 hours exposure, 24 hour preparation interval)

2nd experiment: 287, 575, 1150 and 2300 µg/mL with and without metabolic activation (24 and 4 hour exposure, 24- and 44-hour preparation interval)

3rd experiment: 575, 1150, 1725 and 2300 µg/mL without S9 mix (24 hour exposure, 24 hours preparation interval)

B. TEST PERFORMANCE:

1. Dates of experimental work: 24-Jun-2013 to 17-Jan-2014

2. Preliminary cytotoxicity assay:

In the pretest for toxicity based on the purity and the molecular weight of the test substance 2300 µg/mL (approx. 10 mM) Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin) was used as top concentration. The pretest was performed following the method described for the main experiment. As indication of test substance toxicity relative increase in cell count (RICC) and cell attachment (morphology) were determined for dose selection. pH and solubility were additionally determined.

3. Cytogenicity Assay:

In the beginning two independent experiments were performed. In experiment I, the exposure period was 4 hours with and without S9 mix. In experiment II, the exposure periods were 4 hours with S9 mix and 24 hours without S9 mix. The cells were prepared 24 hours after start of treatment with the test item, except for the 4 hour treatment interval in the presence of metabolic activation (experiment II). In this experimental part cells were harvested after 44 hours to consider possibly occurring mitotic delay. Since statistically significant and biologically relevant increase in the number of micronucleated cells was observed after 24 hours continuous treatment in the absence of a metabolizing system a third corroborate experiment was performed.

Cell seeding / treatment:	Routinely grown cells that did not exceed a max. of 15 passages and reached a confluency of at least 50%, were detached by trypsination and used to prepare a single cell suspension with the required cell count (3-5x10^5 cells per culture, depending on the schedule) in MEM incl. 10% (v/v) FCS. 5 mL cell suspension were transferred into 25 cm² cell culture flasks using a dispenser. Subsequently, the test cultures were incubated. After an attachment period of about 20 - 24 hours, the medium was removed from the flasks and the treatment medium was added. In case of experiments without metabolic activation the treatment medium consisted of 4 mL MEM medium with FCS plus 1 mL positive control or test substance preparation/ vehicle, respectively. In case of metabolic activation, the treatment medium consisted of 3 mL MEM medium without FCS, 1 mL positive control or test substance preparation/ vehicle and 1 mL S9-mix, respectively. The cultures were incubated for the respective exposure period at 37°C, 5% (v/v) CO2 and ≥ 90% humidity. At the end of the exposure period, the medium was removed and the cultures were rinsed twice with 5 mL HBSS (Hanks Balanced Salt Solution). Subsequently, 5 mL MEM incl. 10% FCS supplemented with Cytochalasin B (CytB, final concentration: 3 µg/mL; stock: 0.6 mg/mL in DMSO) was added and incubated for the respective recovery time. In the case of 24-hour continuous exposure, CytB was added to the treatment medium at start of treatment, and cell preparation was started directly at the end of exposure. At 44 hours preparation interval in the presence of S9 mix the supplementation of CytB was 24 hours before preparation of the cultures.

Cell harvest, preparation of slides and staining:	Just before preparation the culture medium was completely removed. Single cell suspensions were prepared from each test group by trypsination. Then, the cell numbers per flask of each single cell suspension were determined using a cell counter. Subsequently, $5x10^4$ cells per slide were centrifuged at 1400 rpm for 7 minutes onto labelled slides using a Cytospin centrifuge. After drying, the slides were fixed in 90% (v/v) methanol for 10 minutes. Before scoring, the slides were stained with a mixture of 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) and propidium iodide (PI) in Fluoroshield™ at a concentration of 0.25 µg/mL each. By the use of the combination of both fluorescence dyes it can be differentiated between DNA (DAPI; excitation: 350 nm, emission: 460 nm) and cytoplasm (PI; excitation: 488 nm, emission: 590 nm).
Analysis of micronuclei and cytotoxicity:	Cytospin slides were scored by fluorescence microscopy. At least 1000 cells per culture, means at least 2000 cells per test group, were evaluated and the number of micronuclei-containing binucleated cells was recorded. Analysis of micronuclei was carried out following the criteria: The diameter of the micronucleus is less than 1/3 of the main nucleus; the micronucleus and main nucleus retain the same color; the micronucleus is not linked to the main nucleus and is located within the cytoplasm of the cell; only cells clearly surrounded by a nuclear membrane were scored. Cultures with few isolated cells were not analyzed for micronuclei.

Relative increase in cell count (RICC):

$$RICC = \frac{(\text{Increase in number of cells in treated cultures (final} - \text{starting}))}{(\text{Increase in number of cells in control cultures (final} - \text{starting}))} \text{ x } 100$$

Thus, a RICC of 53% indicates 47% cytotoxicity/cytostasis.

Proliferation Index (CBPI):

$$CBPI = \frac{\big((\text{No. mononucleated cells}) + (2 \text{ x No. binucleated cells}\big) + (3 \text{ x No. mulitnucleated cells}))}{(\text{Total number of cells})}$$

The CBPI was used to calculate the % cytostasis (relative inhibition of cell growth compared to the respective vehicle control group) - a CBPI of 1 (all cells are mononucleate) is equivalent to 100% cytostasis.

% Cytostasis = $100 - 100 \{(CBPI_T - 1) / (CBPI_C - 1)\}$

(T- test substance treated culture; C = vehicle control)

Replicative Index (RI):

$$RI = \frac{(((\text{No. binucleate cells}) + (2\text{xmultinucleate cells}) \div (\text{Total number of cells}))_T}{(((\text{No. binucleate cells}) + (2\text{xmultinucleate cells}) \div (\text{Total number of cells}))_C} x100$$

(T = test substance treated culture; C = vehicle control culture)

Thus, an RI of 53% means that in comparison to the respective control cultures only 53% of the cells divided in the treated test group (= 47% cytotoxicity/cytostasis).

4. Statistics:

The statistical evaluation of the data was carried out using the MUVIKE program system (BASF SE). The proportion of cells containing micronuclei was calculated for each group. A comparison of each dose group with the concurrent vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test is Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided. If the results of this test were statistically significant compared with the respective vehicle control, labels (* $p \leq 0.05$, ** $p \leq 0.01$) have been be printed in the tables.

5. Evaluation criteria:

A test item is considered "positive" in this assay if the following criteria are met:

- A significant, dose-related and reproducible increase in the number of cells containing micronuclei was observed.
- The number of micronucleated cells exceeded both the value of the concurrent vehicle control and the range of our laboratory's recent negative control data.

A test substance is generally considered "negative" in this test system if:

- The number of micronucleated cells in the test groups is not distinctly increased above the concurrent vehicle control and is within our laboratory's recent negative control data range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically (see BASF study code 01Y0173/13Y006).

B. PRELIMINARY CYTOTOXICITY ASSAY:

A preliminary cytotoxicity assay was performed to determine appropriate test substance concentrations for the main test. After 4 hours treatment in the absence and presence of S9 mix no cytotoxicity as indicated by reduced RICC of ≤ 40 - 50% was observed.

C. CYTOGENICITY ASSAYS:

In all experimental parts after 4 hours test substance treatment in the absence and presence of 9 mix the values were close to the concurrent vehicle control values and within historical negative control data range (0.1 - 1.8% micronucleated cells) [see Table 5.8.1-28 and Table 5.8.1-29]. In contrast, a dose dependent and statistically and biologically significant incease in the number of micronucleated cells was observed after 24 hours continuous treatment in the absence of a metabolizing system in two independently performed experiments [see Table 5.8.1-28].

The positive control substances EMS (without S9 mix; 300 and 400 µg/mL) and CPP (with S9 mix; 1.0 µg/mL) induced statistically significant increased micronucleus frequencies in three independently performed experiments.

In all three experiments no growth inhibition was observed up to the highest applied test substance concentrations tested. To the contrary, in both experiments after 24 hours exposure in the absence of S9 mix enhanced cell growth was observed at concentrations ≥ 1150 µg/mL. Cell morphology/ attachment was not adversely influenced at any concentration tested for the occurrence of micronuclei. Osmolarity and pH values were not influenced by test substance treatment. To ensure a constant pH, the pH of the stock solutions was adjusted to a physiological value using small amounts of NaOH as the test-item is acidic. No precipitation of the test substance in culture medium was observed.

Table 5.8.1-28: Summary of results of micronucleus test with Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin) - without metabolic activation

1					Genotoxicity	Cytotoxicity		
Exp.	Exposure/ Preparation interval	Test groups	S9 mix	Prec.*	Micronucleated cells** [%]	Proliferation index cytostasis (CBPI) [%]	Replicative index (RI) [%]	Relative increase in cell count (RICC) [%]
1	4/24 hrs	Vehicle control[1]	-	n.d.	0.5	0.0	100.0	100.0
		287.50 µg/mL	-	-	n.d.	n.d.	n.d.	110.2
		575.00 µg/mL	-	-	0.9	1.21	98.8	115.3
		1150.00 µg/mL	-	-	0.6	-0.4	100.4	112.1
		2300.00 µg/mL	-	-	1.0	2.1	97.9	109.3
		Positive control[2]	-	n.d.	2.3[S]	5.7	94.3	123.4
2	24/24 hrs	Vehicle vcntrol[1]	-	n.d.	0.4	0.0	100.0	100.0
		287.50 µg/mL	-	-	n.d.	n.d.	n.d.	80.8
		575.00 µg/mL	-	-	0.4	4.0	96.0	74.6
		1150.00 µg/mL	-	-	1.5[S]	4.6	95.4	164.7
		2300.00 µg/mL	-	-	3.3[S]	21.6	78.4	187.1
		Positive control[2]	-	n.d.	2.5[S]	2.8	97.2	181.8
3	24/24 hrs	Vehicle control[1]	-	n.d.	0.5	0.0	100.0	100.0
		575.00 µg/mL	-	-	n.d.	n.d.	n.d.	110.5
		1150.00 µg/mL	-	-	1.5[S]	20.9	78.2	310.1
		1725.00 µg/mL	-	-	3.3[S]	15.5	83.7	153.9
		2300.00 µg/mL	-	-	4.0[S]	31.0	67.8	231.6
		Positive control[2]	-	n.d.	1.4[S]	-6.2	107.2	239.9
		Positive control[3]	-	n.d.	2.8[S]	2.7	98.2	219.3

* Precipitation in culture medium at the end of exposure period (macroscopic)
** Relative number of binucleated cells with micronuclei per 2000 cells scored per test group
S Frequency statistically significant higher than corresponding control values
n.d. Not determined; [1] DMSO 1% (v/v) [2] EMS 300 µg/mL; [3] EMS 400 µg/mL

Table 5.8.1-29: Summary of results of micronucleus test with Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin) - with metabolic activation

					Genotoxicity	Cytotoxicity		
Exp.	Exposure/ Preparation interval	Test groups	S9 mix	Prec.*	Micronucleated cells** [%]	Proliferation index cytostasis (CBPI) [%]	Replicative index (RI) [%]	Relative increase in cell count (RICC) [%]
1	4/24 hrs	vehicle control[1]	+	n.d.	0.8	0.0	100.0	100.0
		287.50 µg/mL	+	-	n.d.	n.d.	n.d.	86.8
		575.00 µg/mL	+	-	0.6	0.1	99.9	87.0
		1150.00 µg/mL	+	-	0.6	2.1	97.9	81.5
		2300.00 µg/mL	+	-	0.5	10.2	89.8	76.3
		positive control[2]	+	n.d.	6.4[S]	43.4	56.6	62.2
2	4/44 hrs	vehicle vcntrol[1]	+	n.d.	0.9	0.0	100.0	100.0
		287.50 µg/mL	+	-	n.d.	n.d.	n.d.	75.7
		575.00 µg/mL	+	-	1.1	-6.8	106.8	78.6
		1150.00 µg/mL	+	-	0.8	-5.6	105.6	68.2
		2300.00 µg/mL	+	-	0.4	-4.7	104.7	65.5
		positive control[2]	+	n.d.	9.9[S]	-2.5	102.5	53.9

* Precipitation in culture medium at the end of exposure period (macroscopic)
** Relative number of binucleated cells with micronuclei per 2000 cells scored per test group
S Frequency statistically significant higher than corresponding control values
n.d Not determined; [1] DMSO 1 % (v/v); [2] CPP 1.0 µg/mL

III. CONCLUSION

Based on the results of the study it is concluded that 500M24 (Reg.No. 5916421, metabolite of pyraclostrobin) has the potential to induce micronuclei (clastogenic and/or aneugenic activity) under the in vitro conditions in V79 cells in the absence of metabolic activation after 24 hours continuous treatment. The relevance of this in vitro result was evaluated in a higher tier in vivo study (see below) according to Commission Regulation (EU) No 283/2013, Section 5.4.2.

Report:	CA 5.8.1/24 [redacted] 2014a Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin): Micronucleus assay in bone marrow cells of the mouse 2013/1389659
Guidelines:	OECD 474 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Report:	CA 5.8.1/25 Grauert E., Kamp H., 2014a Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin) - Concentration control analyses in 30% Dimethylsulfoxide / 70% Polyethyleneglycol 400 (v/v) 2014/1105774
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	CA 5.8.1/26 Becker M., Kamp H., 2014a Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin) - Plasma analysis for external studies 2014/1145915
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 5916421 (metabolite of BAS 500 F, pyraclostrobin; batch: L82-125, purity: 98.8%) was tested for the potential to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of NMRI mice. For this purpose, the test substance was administered once orally to groups of 7 male mice at dose levels of 500, 1000 and 2000 mg/kg body weight. The vehicle served as negative and cyclophosphamide as positive controls. The animals were sacrificed 24 or 48 (additional high dose and vehicle group) hours after the administration and the bone marrow of the two femora was prepared and investigated for micronuclei.

There was no biologically relevant or statistically significant enhancement in the frequency of micronuclei at any preparation interval after administration of the test item at any dose level. Cyclophosphamide administered orally was used as positive control which showed a substantial increase of induced micronucleus frequency. After treatment with the test item the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that Reg.No. 5916421 did not exert any cytotoxic effects in the bone marrow.

No clinical signs were observed in the main experiment in all dose groups. However, analysis of plasma samples taken 24 hours after administration confirmed the presence of Reg.No. 5916421 in blood and thus confirmed that the test-item reaches its target-organ, i.e. the highly blood perfused bone marrow.

In conclusion, it can be stated that under the experimental conditions reported, the test item Reg.No. 5916421 did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse. Therefore, Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin) is considered to be non-mutagenic in this micronucleus assay.

(BASF DocID 2013/1389659)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 5916421 (Metabolite of pyraclostrobin)

Description:	Solid, brownish
Lot/Batch #:	L82-125
Purity:	98.8% (tolerance ± 1.0%)
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Sep 2015 as indicated by the sponsor.
Vehicle used:	DMSO / PEG 400 (3/7)

2. Control Materials:

Negative:	No negative control was employed in this study.
Vehicle control:	30% Dimethyl suphoxide, 70% Polyethylen glycol 400 (DMSO / PEG 400 (3/7))
Positive control:	Cyclophosphamide (CCP) 40 mg/kg bw

3. Test animals:

Species:	Albino mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 12 weeks
Mean body weight at dosing:	35.7 ± 1.8 g
Source:	Charles River Laboratories, Research Models and Services Germany GmbH
Number of animals per dose:	
Range finding study:	4 males and 4 females (2/sex for each pre-test)
Micronucleus assay:	7 males/dose
Acclimation period:	At least 5 days
Diet:	pelleted standard diet (certified), ad libitum
Water:	tap water, ad libitum
Housing:	During the study the mice were housed individually in Makrolon cages, type MIII

4. Environmental conditions:

Temperature:	20 - 24°C
Humidity:	45 - 65%
Air changes:	frequency not indicated (fully air-conditioned rooms)
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	2000 mg/kg
Micronucleus assay:	500, 1000 and 2000 mg/kg The test substance was administered once by oral gavage using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 05-Nov-2013 to 03-Dec-2013

2. Preliminary range finding test:

Male and female NMRI mice were treated once by oral gavage with a test substance dose of 2000 mg/kg bw.

3. Micronucleus test:

Treatment and sampling:	Groups of 5 or 7 male mice were treated once with either vehicle or 500, 1000 or 2000 mg test substance/kg bw by oral gavage. Additional test groups treated with the vehicle control and the high dose were used for the second sampling period. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substance CCP was administered once by oral gavage (CCP). The animals were observed for evident clinical signs of toxicity throughout the study. Twenty-four after the administration the mice were sacrificed and the two femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 390xg for 10 minutes. The supernatant was discarded and the pellet resuspended in about 50 µl fresh FCS. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide preparation:	One drop of the suspension was applied on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May Grünwald solution, rinsed, and finally stained with Giemsa solution (7.5%). Cover slips were mounted with EUKITT. The slides were coded prior to microscopic evaluation.
Slide evaluation:	In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). To describe a cytotoxic effect, the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes.

4. Statistics:

Statistical methods (nonparametric Mann-Whitney test) were used as an aid in evaluating the results, if necessary. However, the primary point of consideration is the biological relevance of the results.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and significant increase in the number of micronucleated polychromatic erythrocytes was observed.
- The proportion of cells containing micronuclei exceeded both the values of the concurrent vehicle control range and the vehicle historical control range.

A test substance is generally considered negative in this test system if:

- There was no significant increase in the number of micronucleated polychromatic erythrocytes at any dose above concurrent control frequencies.
- The frequencies of cells containing micronuclei were within the historical control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance is confirmed indirectly by dose formulation analytics (see report # 04Y0173/13Y053). The actual test-item concentrations were 99.1, 99.5 and 99.7% of the nominal concentrations of 50, 100 and 200 mg/mL.

B. PRELIMINARY RANGE FINDING TEST

In the pretest for the determination of the acute oral toxicity in males and females, the animals were treated with 1000 or 2000 mg/kg bw. All animals survived with weak signs of toxicity (ruffled fur). There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

In comparison to the corresponding vehicle controls there was no statistically significant or biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after treatment with Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin) were below or very close to the value of the vehicle control group [see Table 5.8.1-30]. Moreover, micronucleus values obtained in all dose groups were very well within the historical negative control range (0.112±0.088% micronucleated cells, range: 0-9).

Cyclophosphamide administered orally was used as positive control which showed a statistically significant increase of induced micronucleus frequency.

Table 5.8.1-30: Micronucleus test in mice administered Reg.No. 5916421 by oral gavage

Test group	Dose [mg/kg bw]	Sampling time [h]	PCEs with micronuclei [%]	Range [n]	PCE per 2000 erythrocytes
Vehicle control	0	24	0.120	1-6	1184
Test item	500	24	0.036	0-1	1260
	1000	24	0.043	0-2	1164
	2000	24	0.079	0-4	1197
Positive control	40	24	**1.960****	21-59	1168
Vehicle	0	48	0.040	0-2	1286
Test item	2000	48	0.064	0-3	1282

** $p \leq 0.01$ (nonparametric Mann-Whitney test)
PCE polychromatic erythrocytes

The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control indicating that Reg.No. 5916421 (Metabolite of BAS 500 F, pyraclostrobin) did not have any cytotoxic properties in the bone marrow. No clinical signs were observed in the main experiment in all dose groups. The analysis of plasma samples taken 24 hours after administration of 2000 mg/kg bw confirmed the presence of the test item in blood in 4 out of 7 high dose mice. Thus, there is proof that Reg.No. 5916421 reached the target organ since the bone marrow is highly blood perfused. The fact that no test-item was detected in the 3 remaining animals does not affect the principal conclusion as the blood samples were only taken at a single sampling time 24 hour after administration.
In a separate study (see below) the plasma levels of Reg.No. 5916421 were determined.

III. CONCLUSION

In conclusion, it can be stated that during the study described and under the experimental conditions reported, the test item 500M24 (Reg.No. 5916421, metabolite of pyraclostrobin) did not induce micronuclei as determined by the micronucleus test in the bone marrow cells of the mouse. The analysis of Reg.No. 5916421 in rat plasma demonstrated that the test item reached the target organ (bone marrow). Thus, the clastogenicity observed in vitro is not relevant under in vivo conditions.

Report:	CA 5.8.1/27 [redacted] et al., 2016 a Reg.No. 5916421 (metabolite of BAS 500 F, Pyraclostrobin) - Investigation of the bioavailability in blood of the mouse 2016/1236540
Guidelines:	OECD 474
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 5916421 (metabolite of BAS 500 F, pyraclostrobin; batch: L82-125, purity: 98.8%) was administered to 2 male and 2 female mice by oral gavage at a dose of 2000 mg/kg bw, using the same vehicle as in the in vivo mouse micronucleus assay. Blood was sampled 24 hours after administration and analyzed for test item plasma concentrations by a validated LC-MS method.
Reg.No. 5916421 (500M24) concentrations of 44.5 to 257.3 ng/mL were determined in plasma. This demonstrates that the highly blood perfused bone marrow as the target organ in the in vivo mouse micronucleus assay was exposed to Reg.No. 5916421.

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	Reg.No. 5916421 (metabolite of pyraclostrobin)
	Description:	Solid, brownish
	Lot/Batch #:	L82-125
	Purity:	98.8% (tolerance ± 1.0%)
	Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Nov 2017 as indicated by the sponsor (based on a reanalysis in October 2015).
	Vehicle used:	DMSO / PEG 400 (3/7)
2.	**Control Materials:**	
		Due to the special purpose of this study, i.e. the determination of test item plasma levels 24 hours after administration, no control materials were used in this study.

3. Test animals:

Species:	Albino mice
Strain:	NMRI
Sex:	Male and female
Age:	5 - 8 weeks
Mean body weight at dosing:	26.0 ± 2.45 g
Source:	Charles River Laboratories Germany GmbH
Number of animals:	2 male and 2 female mice
Acclimation period:	At least 5 days
Diet:	pelleted standard diet (Maus/Ratte Haltung "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water:	tap water, ad libitum
Housing:	During the study the mice were housed individually in Makrolon cages, type MII

4. Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	frequency not indicated (fully air-conditioned rooms)
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses: 2000 mg/kg; The test substance was administered once by oral gavage using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 20-Jun-2016 to 07-Sep-2016

2. Treatment of animals:

For the purpose of this study, i.e. to determine the plasma levels of Reg.No. 5916421 24 hours after oral gavage the use of a small number of animals and the highest dose used in the previous mouse micronucleus test (see BASF DocID 2014/1105774) was considered appropriate.

Male and female NMRI mice were treated once by oral gavage with a test substance dose of 2000 mg/kg bw based on actual body weights.

3. Observation of animals:

The animals were checked frequently for signs of systemic toxicity.

4. Sampling of blood:

24 hours administration of the test item the animals were sacrificed by decapitation under isofluorane anaesthesia. Per animal about 500 µL blood were sampled with EDTA as anticoagulant in ready-to-use tubes (Microvette® tubes, Sarstedt, Germany). After centrifugation of 2 minutes at 20000 g at 4°C the plasma was stored in separate tubes at -80°C until analysis.

In order to have plasma control samples of untreated animals (blank samples), about 100 µl blood per animal was taken under isoflurane anesthesia from the vena facialis the day before test substance administration.

5. Sample workup and analysis:

The analysis of the plasma samples for Reg.No. 5916421 was performed by using a validated analytical HPLC-MS method (see M-CA 4, BASF DocID 2016/1327657) with a Limit of Quantification of 50ng/mL plasma.
The samples were extracted by adding 270 µL acetonitrile to 30 µL of plasma (1:10 dilution). After thorough mixing in a 1.5 mL plastic tube and protein precipitation the samples were centrifuged at 13000 rpm for 5 minutes. The clear supernatant was directly used for LC-MS measurements.
The sample extracts were measured in a defined sequence with samples of the extraction solution (acetonitrile), blank matrix solution (extracted control plasma), system suitability samples (independently prepared calibration sample (100 ng/mL)), quality control samples (extracts of spiked control plasma samples containing nominally 14.58, 154.8 and 874.8 ng/mL) and calibration samples (5, 10, 25, 50, 75, 100, 250, 500 and 1000 ng/mL). It is important to note that the concentrations of the system suitability, quality and calibration solutions refer to the concentration in the sample for measurement. The LOQ mentioned above refers to the concentration of Reg.No. 5916421 in plasma, which is 1:10 diluted during extraction.

II. RESULTS AND DISCUSSION

A. Clinical signs of toxicity

No signs of systemic toxicity were noted after a single administration of 2000 mg/kg bw by gavage.

B. Analytical determinations

No analytical determination of the test item concentration in the dosing solution was performed. In the original micronucleus assay (see BASF DocID 2013/1389659 above) the concentrations of the dosing solutions were determined (BASF DocID 2014/1105774). The actual test-item concentrations were 99.1, 99.5 and 99.7% of the nominal concentrations of 50, 100 and 200 mg/mL, demonstrating that the laboratory was able to correctly prepare the dosing solutions. As this study was performed in the same laboratory, the lack of a dosing solution concentration control analysis is not considered to affect the validity of the plasma concentration analysis.

No test item was detected in the (blank) plasma samples retrieved 1 day before administration. The concentrations of Reg.No. 5916421 24 hours after administration of a dose of 2000 mg/kg bw were in the range of 44.5 to 257.3 ng/mL plasma [see Table 5.8.1-31]. The concentration in the plasma of female animal #4 was slightly below the wLOQ.

Table 5.8.1-31: Plasma concentrations of Reg.No. 5916421 twenty-four hours after administration of a dose of 2000 mg/kg bw by gavage

Animal No	Sample Name	Concentration [ng/ml]	Sample Name	Concentration [ng/ml]
1 (♂)	Sample 1L	n.d.	Sample 1	257.3
2 (♂)	Sample 2L	n.d.	Sample 2	156.2
3 (♀)	Sample 3L	n.d.	Sample 3	137.6
4 (♀)	Sample 4L	n.d.	Sample 4	44.5*

n.d. = not detected
* < wLOQ (working limit of quantification)
Samples 1L, 2L, 3L and 4L: Untreated control samples
Samples 1, 2, 3 and 4: 24 hours after 2000 mg/kg bw dosage

The quality control samples (analysed three times during the measurement of the sample, i.e. at the beginning, mid and end of the sequence) revealed Reg.No. 5916421 concentrations between 94.2 and 106.6% of the nominal values [see Table 5.8.1-32].

Table 5.8.1-32: Quality control samples: Recovery of Reg.No. 5916421 from plasma samples spiked at 3 concentrations

Sample identifier	Area [Au]	Determined concentration [ng/mL]	Nominal concentration [ng/mL]	Nominal concentration [%]
QC 1 1	81910	14.12	14.58	96.8
QC 1 2	85658	14.76	14.58	101.2
QC 1 3	83049	14.31	14.58	98.1
QC 2 1	877531	154.4	145.8	105.9
QC 2 2	878475	154.5	145.8	106.0
QC 2 3	847990	149.2	145.8	102.3
QC 3 1	5329929	932.6	874.8	106.6
QC 3 2	4709741	824.2	874.8	94.2
QC 3 3	5105592	893.4	874.8	102.1

III. CONCLUSION

Reg.No. 5916421 (500M24; metabolite of pyraclostrobin) concentrations of 44.5 to 257.3 ng/mL were determined in plasma of mice 24 hours after administration of a dose of 2000 mg/kg bw. This demonstrates that the highly blood perfused bone marrow as the target organ in the in vivo mouse micronucleus assay was exposed to Reg.No. 5916421.

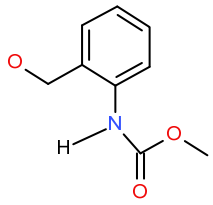
Overall toxicological evaluation of 500M24 (Reg.No. 5916421)

The QSAR evaluation of 500M24 is of low reliability and by weight of evidence there was no conclusive alert on genotoxicity.

500M24 is considered to be not genotoxic based on the in vitro and in vivo studies conducted. Therefore, 500M24 is considered to be **not toxicologically relevant**.

With regard to consumer exposure the TTC concept for a non-genotoxic compound was applied. Thus an ADI of 0.0015 mg/kg bw/day as well as an ARfD of 0.005 mg/kg bw/day was established. The estimated exposure to 500M24 is low (9% of TTC of 0.0015 mg/kg bw/day).

500M49 (other denominator: Reg.No. 5916420)



500M49 (Reg.No. 5916420) is a metabolite found in the hen metabolism study and in a hydrolysis study at exaggerated temperatures with olive oil (simulating oil deodorization).

A) QSAR Predications for 500M049 (Reg.No. 5916420)

OECD Toolbox (Version: 3.2) [see DocID 2014/1172955]

The OECD Toolbox revealed the alerts '*Radical|Radical >> Radical mechanism via ROS formation (indirect)|Radical >> Radical mechanism via ROS formation (indirect) >> Single-Ring Substituted Primary Aromatic Amines|SN1|SN1 >> Nucleophilic attack after metabolic nitrenium ion formation|SN1 >> Nucleophilic attack after metabolic nitrenium ion formation >> Single-Ring Substituted Primary Aromatic Amines*' and '*AN2|AN2 >> Carbamoylation after isocyanate formation|AN2 >> Carbamoylation after isocyanate formation >> N-Hydroxylamines|Radical |Radical >> Radical mechanism via ROS formation (indirect)|Radical >> Radical mechanism via ROS formation (indirect) >> N-Hydroxylamines|SN1|SN1 >> Nucleophilic attack after metabolic nitrenium ion formation|SN1 >> Nucleophilic attack after metabolic nitrenium ion formation >> N-Hydroxylamines*' as DNA Alert for Ames, MN and CA and DNA binding. For protein binding 3 alerts were provided: '*Acylation|Acylation >> Direct acylation involving a leaving group|Acylation >> Direct acylation involving a leaving group >> Carbamates*' and '*Schiff base formation|Schiff base formation >> Schiff base formation with carbonyl compounds|Schiff base formation >> Schiff base formation with carbonyl compounds >> Aldehydes*'. The third alert was a combination of the two above.

Discussion: At least the second identified alert for DNA binding and DNA Alert for Ames, MN and CA is present in a number of metabolites which - like 500M49 (Reg.No. 5916420) - were tested negative in the Ames test (500M02 (Reg No 3693159) and 500M62 (Reg No 412785)). Additionally, in view of the negative in mammalian gene mutation test with 500M49 (Reg.No. 5916420), the prediction does not adequately reflect the experimental database and therefore the prediction is rejected.

The situation for protein binding, which is also considered to be indicative for in vitro chromosome aberration, is similar. At least the first alert was also found for the metabolite 500M51 (Reg.No. 78810), which like 500M49 (Reg.No. 5916420) was negative in the in vivo micronucleus assay. Therefore, the prediction does not adequately reflect the experimental database and the prediction is rejected.

OASIS TIMES (MIX V.2.27.13 TB; Mutagenicity S-9 activated v08.08) [see molecule 5 of report DocID 2014/1172952 and 2014/1172953]

The Ames mutagenicity alert for 500M49 (Reg.No. 5916420) was negative (out of domain), however 3/13 in-silico generated metabolites had a positive alert (N-Hydroxylamines or Single-ring Substituted PrimaryAromatic Amines; in domoain).

The prediction of in vitro CA for 500M49 (Reg.No. 5916420) and 5/13 in-silico generated metabolites were positive. The identified alerts were carbamates, N-hydroxalamines and single-ring substituted primary aromatic anilines). Except for 500M49 (Reg.No. 5916420) all positive alerts were in domain.

Discussion: The alerts identified for the Ames test are also present in 500M62 (Reg.No. 412785) and/or 500M02 (Reg.No. 369315), which like 500M49 (Reg.No. 5916420) tested negative in the Ames test.

Single of the alerts identified for the in vitro chromosome aberration were also present in metabolites 500M51 (Reg.No. 78810) and 500M24 (Reg.No. 5916421). The in vitro CA for 500M51 (Reg.No. 78810) was - like that for 500M49 (Reg.No. 5916420) - negative. In the cases 500M24 (Reg.No.5916421) the positive in vitro CA was not confirmed in the negative higher tier in vivo mouse micronucleus test. Therefore, the prediction does not adequately reflect the experimental database and the prediction is rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.9) [see molecule 5 of report BASF DocID 2014/1172954; for both VEGA models]

The prediction for 500M49 (Reg.No. 5916420) is non-mutagen (out of model applicability domain). This is in agreement with the experimental data (see below).

VEGA: Mutagenicity model (SarPy; version 1.0.4-BETA)

500M49 (Reg.No. 5916420) is out of model applicability domain. The prediction is ‘mutagen’ with the structural alert SA 98. As discussed above, this alert was also obtained for pyraclostrobin and a number of pyraclostrobin metabolites, which like 500M49 (Reg.No. 5916420) were actually tested negative. The predicition is therefore rejected.

DEREK (Version 2.0.0.201011301322; Knowledge base version: 11_20_05_2010)

The prediction for genotoxicity and neurotoxicity is negative.

Genotoxicity studies on 500M49 (Reg.No. 5916420)

Report:	CA 5.8.1/28 Woitkowiak C., 2013b Reg.No. 5916420 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella Typhimurium / Escherichia Coli reverse mutation assay 2013/1281928
Guidelines:	OECD 471, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium strains TA98, TA100, TA 1535 and TA 1537 and E. coli strain WP2 uvrA were exposed to Reg.No. 5916420 (Metabolite of BAS 500 F, pyraclostrobin); batch: L82-115, purity: 100.0%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.

In both assays, the plate incorporation assay and the preincubation assay, the test substance was tested in concentrations of 33, 100, 333, 1000, 2500 and 5000 µg/plate.

No biologically relevant increase in the number of revertant colonies was noted in any of the strains tested in presence or absence of metabolic activation in both experiments. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.

Except for a slight decrease in the number of his^+ revertants in the plate incorporation assay with TA 1535 in presence of S9 mix and in the preincubation assay with TA 98 without S9 mix, both at 5000 µg/plate, no indication of bacteriotoxicity was observed. Precipitation of the test substance did not occur up to the highest concentration tested.

The test substance Reg.No. 5916420 (Metabolite of BAS 500 F, pyraclostrobin) is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2013/1281928)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg.No. 5916420 (metabolite of BAS 500 F, pyraclostrobin)

Description: Solid, white
Lot/Batch #: L82-115
Purity: 100.0% (tolerance +/- 1.0%)
Stability of test compound: The test substance was stable over the study period under the storage conditions and guaranteed until 01 Apr 2015. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance solutions and on account of the high purity. The stability of the test substance in the vehicle DMSO over a period of 4 h was verified analytically.
Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).
Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration: 100 µL/plate
Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60 µg/plate

3. Activation:

S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received on three consecutive days intraperitoneal injections of 80 mg phenobarbital and 80 mg β-naphthoflavone orally per kg body weight. 24 hours after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9 mix, was kept on ice until used.

The S9 mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

To demonstrate the efficacy of the S9 mix in this assay, the S9 batch was further characterized with benoz(a)pyrene in the strains TA 100 and TA 98 as required in OECD 471.

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay: In the first experiment triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay: In the second experiment the test article / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains.

B. TEST PERFORMANCE:

1. Dates of experimental work: 25-Jun-2013 to 04-Jul-2013

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Merckoplate® plates (minimal glucose agar plates). In the experiments with E. coli the warm soft agar contains 0.5 mM tryptophan instead of histidine + biotin.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37°C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects, the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37°C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A weak bacteriotoxic effect (slight decrease in the number of his^+ revertants) was observed in the standard plate test in tester strain TA1535 in the presence of S9 mix at 5000 µg/plate only. In the preincubation assay slight bacteriotoxicity (slight decrease in the number of his^+ revertants) was observed only in tester strain TA98 without S9 mix at 5000 µg/plate.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiment with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested [see Table 5.8.1-33]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Precipitation was not observed up to the maximum concentration.

Table 5.8.1-33: Bacterial gene mutation assay with Reg.No. 5916420 - Mean number of revertants

Experiment 1: Plate incorporation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	39	23	52	32	13	11	12	6	69	64
Reg.No. 5916420										
33 µg/plate	38	22	51	33	12	11	10	7	73	68
100 µg/plate	36	22	50	36	11	13	10	5	69	64
333 µg/plate	43	21	51	35	14	11	10	6	69	67
1000 µg/plate	35	20	51	35	12	11	9	5	71	68
2500 µg/plate	34	21	54	46	14	11	8	6	69	75
5000 µg/plate	34	19	49	45	10	11	9	5	75	67
Pos. control[§]	1282	1088	1448	1204	411	1025	257	396	218	861
Experiment 2: Preincubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	27	16	50	37	12	11	9	6	40	35
Reg.No. 5916420										
33 µg/plate	27	16	49	41	12	11	9	6	39	36
100 µg/plate	27	15	47	39	12	10	10	7	36	33
333 µg/plate	29	15	46	36	13	10	10	6	37	38
1000 µg/plate	28	14	52	38	12	9	8	6	40	32
2500 µg/plate	27	16	48	41	13	12	10	6	41	35
5000 µg/plate	22	11	47	44	11	11	8	5	39	34
Pos. control[§]	897	588	1275	1125	414	1012	231	319	230	528

[§] = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSION

According to the results of the present study, the test substance 500M49 (Reg.No. 5916420, metabolite of pyraclostrobin) is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/29 Schulz M., Landsiedel R., 2014d Reg.No. 5916420 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells (TK+/- locus assay, microwell version) 2013/1298447
Guidelines:	OECD 476, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 5916420 (metabolite of 500 F; pyraclostrobin; batch: L82-115, purity: 100.0%) was tested in vitro for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the $TK^{+/-}$ locus. Two independent experiments were conducted in the presence and absence of metabolic activation with treatment intervals of 4 or 24 hours and concentrations from 62.5 to 2000 µg/mL. Methyl methanesulfonate (MMS) and cyclophosphamide (CPP) served as positive controls in the experiments without and with metabolic activation, respectively.

The test substance did not cause any biologically relevant increase in the mutant frequencies neither without S9 mix nor after adding a metabolizing system in two experiments performed independently of each other. The positive control substances, however, induced a marked increase in mutant frequency and the negative controls gave mutant frequencies within the range expected for the L5178Y TK+/- mouse lymphoma cell line.

Cytotoxicity indicated by clearly reduced relative total growth (RTG) of below 20% of control was observed after 24 hours exposure in the 2nd experiment in the absence of metabolic activation only.

Based on the results of the study it is concluded that under the conditions of the test Reg.No. 5916420 (Metabolite of 500 F; pyraclostrobin) does not induce forward mutations in mammalian cells in vitro.

(BASF DocID 2013/1298447)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

	Reg.No. 5916420 (metabolite of 500 F; pyraclostrobin)
Description:	Solid, white
Lot/Batch #:	L82-115
Purity:	100.0% (tolerance ± 1.0%)
Stability of test compound:	Stable - expiry date: 01-Apr-2015. The homogeneity of the test substance was guaranteed on account of the high purity and ensured by mixing prior to preparation of test substance solutions. The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	The vehicle controls with and without S9 mix contained the vehicle selected for the test substance at the same volume and concentration as used in the test cultures.
Positive control -S9:	Methyl methanesulfonate (MMS) 15 µg/mL (4-hour exposure period) and 5 µg/mL (24-hour exposure period)
Positive control +S9:	Cyclophosphamide (CPP) 2.5 µg/mL

3. Activation:

S9 was produced from the livers of at least 5 induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organism: Mouse lymphoma L5178Y $TK^{+/-}$ cell line. This is a cell line with a high proliferation rate (doubling time of about 9 - 10 hours), a high plating efficiency (about 90%) and a stable karyotype with a near diploid number of 40 ± 1 chromosomes. Each batch used for mutagenicity testing was checked for mycoplasma contamination.

5. Culture media:

Culture medium:	RPMI 1640 medium including stable glutamine supplemented with: 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL) and 1% (v/v) sodium pyruvate (10 mM) = RPMI-0 For treatment medium (with S9 mix): RPMI-0 supplemented with 5% (v/v) fetal calf serum (FCS) = RPMI-5 For treatment medium (without S9 mix) and subculturing cells: RPMI-0 is supplemented with 10% (v/v) FCS = RPMI-10 For cloning efficiency and selection medium: RPMI-0 supplemented with 20% (v/v) FCS = RPMI-20
Pretreatment medium:	A ("THMG" medium): RPMI-10 supplemented with thymidine (3.0 µg/mL), hypoxanthine (5.0 µg/mL), methotrexate (0.1 µg/mL) and glycine (7.5 µg/mL) B ("THG" medium): RPMI-10 supplemented with thymidine (3.0 µg/mL), hypoxanthine (5.0 µg/mL), and glycine (7.5 µg/mL)
Selection medium:	("TFT" medium): RPMI-20 supplemented with trifluorthymidine (TFT, 4.0 µg/mL)

6. Locus examined: thymidine kinase (TK)

7. Test concentrations:

a) Preliminary toxicity assay:	Nine concentrations ranging from 7.8 to 2000 µg/mL
b) Mutation assay:	
1st experiment:	125.0, 250.0, 500.0, 1000.0 and 2000 µg/mL with and without metabolic activation (4-hour exposure)
2nd experiment:	62.5, 125.0, 250.0, 500.0, 1000 and 2000 µg/mL without (24-hour exposure) and 375.0, 750.0, 1500 and 2000 µg/mL with metabolic activation (4-hour exposure)

B. TEST PERFORMANCE:

1. Dates of experimental work: 17-Jun-2013 to 14-Jan-2014

2. Preliminary cytotoxicity assay:

In the pretest for toxicity based on the molecular weight of the test substance 2000 µg/mL (approx. 10 mM) Reg.No. 5916420 was used as top concentration both with and without S9 mix at 4 hour exposure time and without S9 mix at 24 hour exposure time.

The pretest was performed at 9 concentrations spaced by a factor of two following the method described for the main experiment. The relative suspension growth (RSG) was determined as toxicity indicator for dose selection and various parameters were checked for all or at least for some selected doses. Additionally, pH and solubility were determined.

3. Mutation Assay:

Pretreatment of Cells: During the week prior to treatment, spontaneous TK deficient mutants ($TK^{-/-}$) were eliminated from the stock cultures by incubating 3 x 10^5 cells per 75 cm² flask in a total volume of 30 mL for 1 day in “THMG" medium (pretreatment medium A), and for the following 3 days in “THG" medium (pretreatment medium B).

Cell treatment: For each test group, about 1.5 x 10^7 cells per flask were seeded into 75 cm^2 flasks containing RPMI-10 for exposures without S9 mix and RPMI-5 medium for exposures with S9 mix and incubated for the respective exposure period (1st experiment: 4-hour exposure with and without S9 mix and 2nd experiment 24 hours without S9 mix and 4 hours with S9 mix) with 5% CO_2 at 37°C and ≥90% humidity for cell attachment. 2 cultures were treated in parallel for each test group.

Expression:	At the end of the exposure period, the cells were transferred in tubes, centrifuged for 5 minutes at and were resuspended in RPMI-5 medium. The washing of the cells was repeated at least once. Then the cells were centrifuged and were resuspended in RPMI-10 medium. From each culture a sample of treated cells (2 x 10^5 cells/mL or 6 x 10^6 cells/flask) were pipetted in 75 cm² flasks and were incubated for a 2-day expression period. To maintain exponential growth during this phase, each culture was counted daily and the cell numbers were adjusted at each day to 2 x 10^5 cells/mL in 30 mL RPMI-10 medium.
Selection:	For the mutant selection, the cells were resuspended in selection medium ("TFT" medium) and two 96-well plates per cultures were seeded with 2000 cells/well. After incubation of at least 9 days, both the number of negative wells and the number of wells containing small or large colonies were scored for calculation of the mutant frequency (MF).
Size distribution of the colonies:	The colonies are classified into large colonies (indication of gene mutation) and small colonies (indication of chromosome breakage). Small colonies are defined as less than 1/4 of the diameter of the well. Size is the key factor and morphology (the optical density of the small colonies is considerably higher) should be secondary.
Determination of Cytotoxicity:	Cloning efficiency 1 (survival): The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. At the end of the exposure period, the cells were centrifuged and 400 cells from each culture were resuspended in 50 mL RPMI-20 medium (8 cells/mL). Per culture 200 µL were dispensed in each well of two 96-well plates (1.6 cells/well). After incubation for 9 to 11 days the plates were scored for empty wells.
Cloning efficiency 2 (viability):	The viability (cloning efficiency 2; CE_2) was determined after the expression period. 2 days after end of exposure, the cells were centrifuged and 400 cells from each culture were resuspended in 50 mL RPMI-20 medium (8 cells/mL). Per culture 200 µL were dispensed in each well of two 96-well plates (1.6 cells/well). After incubation for at least 9 days the plates were scored for empty wells.

Suspension growth (RSG) and relative total growth (RTG):

For calculation of the suspension growth (SG) and the relative total growth (RTG) the cell counts determined within the expression period at 2nd and 3rd passage after exposure in the case of 4-hour exposure and 1st, 2nd and 3rd passage after exposure in the case of 24-hour exposure were used.

This measure includes the relative growth in suspension during the expression period (RSG) and the relative cloning efficiency (RCE_2; viability) at the time the mutants are selected.

For taking into account any loss of cells during the 4-hour treatment period a relative growth during treatment factor (RGDT, %) was calculated by comparing the growth of each treated culture relative control.

Calculations:

Mutant frequency:

Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{-\ln(\text{total number of empty well s} \div \text{total number of seeded wells (96)})}{\text{number of seeded cells per well (2000)}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_2} \times 100$$

Cloning efficiency (CE, %) absolute:

$$CE_x = \frac{-\ln(\text{total number of empty well s} \div \text{total number of seeded wells (96)})}{\text{number of seeded cells per well (1.6)}} \times 100$$

Cloning efficiency relative, in comparison to control:

$$RCE_x = \frac{CE_x \text{ of the test group}}{CE_x \text{ of the negative / vehicle control}} \times 100$$

Relative total growth:

Relative growth during treatment (RGDT, %)

$$\text{RGDT} = \frac{\text{Cell count after 4 h of the test group}}{\text{Cell count after 4 h of the negative/v ehicle control}} \times 100$$

Total suspension growth (SG) after 4 hour-exposure:

$$SG = \frac{\text{Cell count after 24 h}}{2 \text{ x } 10^5 \text{ cells per mL}^{1,2}} \times \frac{\text{Cell count after 48 h}}{2 \text{ x } 10^5 \text{ cells per mL}^2} \times \frac{\text{RGDT}}{100}$$

Total suspension growth (SG) after 24 hour-exposure:

$$SG = \frac{\text{Cell count after 24 h}}{2 \text{ x } 10^5 \text{ cells per mL}} \times \frac{\text{Cell count after 48 h}}{2 \text{ x } 10^5 \text{ cells per mL}^2} \times \frac{\text{Cell count after 72 h}}{2 \text{ x } 10^5 \text{ cells per mL}^2}$$

$$\text{RSG} = \frac{\text{SG of the test group}}{\text{SG of the negative/v ehicle control}} \times 100$$

$$RTG = \frac{RSG \times RCE_2}{100}$$

[1] Cell number seeded following 4-hour treatment
[2] If cell number was lower than 2 x 10^5 cells per mL all remaining cells were seeded

4. Statistics:

An appropriate statistical trend test (MS EXCEL function RGP; 9) was performed to assess a dose-related increase of mutant frequencies. The number of mutant colonies obtained for the test substance treated groups was compared with that of the respective vehicle control groups. A trend is judged as statistically significant whenever the one-sided p-value (probability value) is below 0.05 and the slope was greater than 0. Both, biological and statistical significance will be considered together. However, both, biological and statistical significance has been considered together.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- The mutation frequency exceeds a threshold of 126 mutant colonies per 10^6 cells (GEF: Global Evaluation Factor) above the concurrent negative/vehicle control value.

and

- Evidence of reproducibility of any increase in mutant frequencies, means the mutagenic response occurs at least in both parallel cultures of one experiment.

and

- A statistically significant dose-related increase in mutant frequencies using an appropriate statistical trend test.

A test substance is generally considered negative in this test system if:

- The mutation frequency is below a threshold of 126 mutant colonies per 10^6 cells (GEF) above the concurrent negative/vehicle control value.

or

- No evidence of reproducibility of an increase in mutant frequencies is obtained.

or

- No statistically significant dose-related increase in mutant frequencies using an appropriate statistical trend test is observed.

However, in the evaluation of the test results the historical variability of the mutation rates in negative and vehicle controls and the mutation rates of all negative and vehicle controls of this study were also taken into consideration. Results of test groups have been rejected if the relative total growth (RTG) and/or the cloning efficiency 1 (CE_1) were less than 10% of the respective vehicle controls. Whenever a test substance is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose-related shift in the ratio of small versus large colonies clastogenic effects are indicated.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically (see BASF study code 01Y0174/13Y004).

B. PRELIMINARY CYTOTOXICITY ASSAY

Cytotoxicity - as determined by reduced relative suspension growth of about or below 20% - was only observed without metabolic activation. This was observed at 2000 µg/mL in the experiment with 4 treatment and at ≥ 1000 µg/ml after 24 hour treatment.

The pH value was not influenced by the addition of the test substance preparation to the culture medium at all concentrations. In addition, no test substance precipitation was observed in the vehicle DMSO or in culture medium up to the highest required test substance concentration.

Based on these data the highest concentration tested in the mutagenicity experiments was 2000 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

No relevant increase in the number of mutant colonies was observed without or with metabolic activation. The corrected mutant frequencies obtained for both experiments were always below the calculated threshold taking into consideration the Global Evaluation Factor (GEF) [see Table 5.8.1-36].

The statistical analyses by testing for linear trend led to a negative finding for the 1st experiment in the absence of S9 mix. In contrast, in the 2nd experiment in the absence of S9 mix and both experiments in the presence of S9 mix the mutant frequencies were statistically significant dose-related increased. However, all values were below the respective mutant frequency threshold and, therefore, the statistical significance was considered as biologically irrelevant.

Treatment with the positive controls MMS and CCP resulted in increased mutant frequencies as expected.

In both experiments after 4 hours exposure, no cytotoxic effects were observed neither in the presence nor in the absence of S9 mix. However, in the 2nd experiment after 24 hours exposure in the absence of metabolic activation the relative total growth (RTG) was reduced at 2000 µg/mL [see Table 5.8.1-36].

Osmolarity and pH values were not influenced by test substance treatment. Likewise, no precipitation of the test-item was observed in the vehicle DMSO or in culture medium at any concentration.

III. CONCLUSION

Based on the results of the study it is concluded that 500M49 (Reg.No. 5916420, metabolite of pyraclostrobin) does not induce forward mutations in vitro in the mouse lymphoma assay with L5178Y $TK^{+/-}$ cells in the absence and the presence of metabolic activation.

Report:	CA 5.8.1/30 Schulz M., Landsiedel R., 2014c Reg.No. 5916420 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells (cytokinesis block method) 2013/1361921
Guidelines:	OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 5916420 (Metabolite of BAS 500 F, pyraclostrobin) (batch L82-115; purity 100.0%) was tested in vitro for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation in two independent experiments. In the 1st experiment (62.5-2000 µg/mL), the exposure period was 4 hours with and without S9 mix. In 2nd experiment, the exposure periods were 4 hours with S9 mix (62.5-2000 µg/mL) and 24 hours without S9 mix (62.5-2000 µg/mL). The cells were prepared 24 hours after start of treatment with the test item, except for the 4 hour treatment interval in the presence of metabolic activation in the 2nd experiment. In this experimental part cells were harvested after 44 hours to consider possibly occurring mitotic delay.

No statistically significant or biologically relevant increase in the number of micronucleated cells was observed either without S9 mix or after the addition of a metabolizing system.

Cytotoxicity was only observed in the 2nd experiment after 24 hours continuous treatment in the absence of S9 mix. In both experiments no test substance precipitation in culture medium was noted.

Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Based on the results of this study, Reg.No. 5916420 is not considered to have neither chromosome-damaging (clastogenic) nor aneugenic activity under in vitro conditions in V79 cells in the absence and the presence of metabolic activation.

(BASF DocID 2013/1361921)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 5916420 (metabolite of pyraclostrobin)

Description:	Solid, white
Lot/Batch #:	L82-115
Purity:	100.0% (tolerance ± 1.0%)
Stability of test compound:	Stable - expiry date: 01 Apr 2015. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance preparations. The stability of the test substance dissolved in the vehicle DMSO over a period of 4 hours was verified analytically.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO
Positive control, -S9:	Ethylmethanesulfonate (EMS, 300, 400 and 500 µg/mL, dissolved in MEM without FCS)
Positive control, +S9:	Cyclophosphamide (CPA, 1.0 and 2.5 µg/mL, dissolved in MEM without FCS)

3. Activation: S9 was prepared from the liver of male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction was thawed at room temperature and 1 volume of S9-fraction was mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9 mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms:

Chinese hamster V79 cells
This is a continuous cell line with a high proliferation doubling time of 12-14 hours, a high plating efficiency (≥ 90%) and a stable karyotype (modal number of 22 chromosomes).

5. Culture medium/conditions:

Culture media:

Minimal essential medium with Earle's salts (MEM) containing a L-glutamine source supplemented with 10% (v/v) fetal calf serum (FCS), 1% (v/v) penicillin/streptomycin (10 000 IU / 10000 µg/mL) and 1% (v/v) amphotericine B (250 µg/mL). During exposure to the test substance in the presence of S9 mix MEM medium was used without FCS supplementation.

Cell culture:

Deep-frozen cell stocks were thawed at 37°C in a water bath, and volumes of 0.5 mL were transferred into 25 cm^2 plastic flasks containing about 5 mL MEM supplemented with 10% (v/v) fetal calf serum (FCS). Cells were grown with 5% (v/v) CO2 at 37°C and ≥ 90% humidity and subcultured twice weekly. Cell monolayers were suspended in culture medium after detachment with 0.25% (w/v) trypsin solution.

Cell cycle and harvest time:	The cell cycle of the untreated V79 cells lasts for about 12 to 14 hours under the selected culture conditions. Thus, a harvest time of 24 hours is about 2 times the normal cell cycle length. V79 cells are an asynchronous cell population, i.e. at the time of test substance treatment there are different cell stages. Since the effect on these cell stages may vary for different test substances, more than one harvest time after treatment may be appropriate. Furthermore, substance-induced mitotic delay may considerably delay the first post-treatment mitosis. Therefore, delayed harvest times (e.g. 44 hours) and prolonged exposure periods (e.g. 24 hours treatment) were considered.

6. Test concentrations:

a) Preliminary toxicity assay:	15.6 to 2000.0 µg/mL with and without metabolic activation Exposure period: 4 hours with and without metabolic activation; 24 hours without metabolic activation Harvest time: 24 hours
b) Cytogenicity assay:	
1st experiment:	62.5, 125, 250, 500, 1000 and 2000 µg/mL with and without S9 mix (4 hours exposure, 24 hour preparation interval)
2nd experiment:	125, 250, 500, 1000 and 2000 µg/mL with and without metabolic activation (24 hour exposure, 24- and 44-hour preparation interval)

B. TEST PERFORMANCE:

1. Dates of experimental work: 10-Jun-2013 to 20-Nov-2013

2. Preliminary cytotoxicity assay:

In the pretest for toxicity based on the purity and the molecular weight of the test substance 2000 µg/mL (approx. 10 mM) Reg.No. 5916420 (Metabolite of BAS 500 F, pyraclostrobin) was used as top concentration. The pretest was performed following the method described for the main experiment. As indication of test substance toxicity relative increase in cell count (RICC) and cell attachment (morphology) were determined for dose selection. pH and solubility were additionally determined.

3. Cytogenicity Assay:

Two independent experiments were performed. In experiment I, the exposure period was 4 hours with and without S9 mix. In experiment II, the exposure periods were 4 hours with S9 mix and 24 hours without S9 mix. The cells were prepared 24 hours after start of treatment with the test item, except for the 4 hour treatment interval in the presence of metabolic activation (experiment II). In this experimental part cells were harvested after 44 hours to consider possibly occurring mitotic delay.

Cell seeding / treatment:	Routinely grown cells that did not exceed a max. of 15 passages and reached a confluency of at least 50%, were detached by trypsination and used to prepare a single cell suspension with the required cell count (3-5x10^5 cells per culture, depending on the schedule) in MEM incl. 10% (v/v) FCS. 5 mL cell suspension was transferred into 25 cm² cell culture flasks using a dispenser. Subsequently, the test cultures were incubated. After an attachment period of about 20 - 24 hours, the medium was removed from the flasks and the treatment medium was added. In case of experiments without metabolic activation the treatment medium consisted of 5 or 4 mL MEM medium with FCS plus 1 mL positive control or 0.05 mL test substance preparation/ vehicle, respectively. In case of metabolic activation, the treatment medium consisted of 4 or 3 mL MEM medium without FCS, 1 mL positive control or 0.05 mL test substance preparation/ vehicle and 1 mL S9-mix, respectively. The cultures were incubated for the respective exposure period at 37°C, 5% (v/v) CO_2 and ≥ 90% humidity. At the end of the exposure period, the medium was removed and the cultures were rinsed twice with 5 mL HBSS (Hanks Balanced Salt Solution). Subsequently, 5 mL MEM incl. 10% FCS supplemented with Cytochalasin B (CytB, final concentration: 3 µg/mL; stock: 0.6 mg/mL in DMSO) was added and incubated for the respective recovery time. In the case of 24-hour continuous exposure, CytB was added to the treatment medium at start of treatment, and cell preparation was started directly at the end of exposure. At 44 hours preparation interval in the presence of S9 mix the supplementation of CytB was 24 hours before preparation of the cultures.

Cell harvest, preparation of slides and staining:	Just before preparation the culture medium was completely removed. Single cell suspensions were prepared from each test group by trypsination. Then, the cell numbers per flask of each single cell suspension were determined using a cell counter. Subsequently, $5x10^4$ cells per slide were centrifuged at 1400 rpm for 7 minutes onto labelled slides using a Cytospin centrifuge. After drying, the slides were fixed in 90% (v/v) methanol for 10 minutes. Before scoring, the slides were stained with a mixture of 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) and propidium iodide (PI) in Fluoroshield™ at a concentration of 0.25 µg/mL each. By the use of the combination of both fluorescence dyes it can be differentiated between DNA (DAPI; excitation: 350 nm, emission: 460 nm) and cytoplasm (PI; excitation: 488 nm, emission: 590 nm).
Analysis of micronuclei and cytotoxicity:	Cytospin slides were scored by fluorescence microscopy. At least 1000 cells per culture, means at least 2000 cells per test group, were evaluated and the number of micronuclei-containing binucleated cells was recorded. Analysis of micronuclei was carried out following the criteria: The diameter of the micronucleus is less than 1/3 of the main nucleus; the micronucleus and main nucleus retain the same color; the micronucleus is not linked to the main nucleus and is located within the cytoplasm of the cell; only cells clearly surrounded by a nuclear membrane were scored. Cultures with few isolated cells were not analyzed for micronuclei.

Relative increase in cell count (RICC):

$$RICC = \frac{\text{(Increase in number of cells in treated cultures (final} - \text{starting))}}{\text{(Increase in number of cells in control cultures (final} - \text{starting))}} \text{x } 100$$

Thus, a RICC of 53% indicates 47% cytotoxicity/cytostasis.

Proliferation Index (CBPI):

$$CBPI = \frac{\big(\text{(No. mononucleated cells)} + \text{(2 x No. binucleated cells}\big) + \text{(3 x No. mulitnucleated cells))}}{\text{(Total number of cells)}}$$

The CBPI was used to calculate the % cytostasis (relative inhibition of cell growth compared to the respective vehicle control group) - a CBPI of 1 (all cells are mononucleate) is equivalent to 100% cytostasis.

% Cytostasis = 100 - 100 $\{(CBPI_T - 1) / (CBPI_C - 1)\}$

(T- test substance treated culture; C = vehicle control)

Replicative Index (RI):

$$RI = \frac{\text{(((No. binucleate cells)} + \text{(2xmultinucleate cells)} \div \text{(Total number of cells))}_T}{\text{(((No. binucleate cells)} + \text{(2xmultinucleate cells)} \div \text{(Total number of cells))}_C} x100$$

(T = test substance treated culture; C = vehicle control culture)

Thus, an RI of 53% means that in comparison to the respective control cultures only 53% of the cells divided in the treated test group (= 47% cytotoxicity/cytostasis).

4. Statistics:

The statistical evaluation of the data was carried out using the MUVIKE program system (BASF SE). The proportion of cells containing micronuclei was calculated for each group. A comparison of each dose group with the concurrent vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test is Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided. If the results of this test were statistically significant compared with the respective vehicle control, labels (* $p \leq 0.05$, ** $p \leq 0.01$) have been be printed in the tables.

5. Evaluation criteria:

A test item is considered "positive" in this assay if the following criteria are met:

- A significant, dose-related and reproducible increase in the number of cells containing micronuclei was observed.
- The number of micronucleated cells exceeded both the value of the concurrent vehicle control and the range of our laboratory's recent negative control data.

A test substance is generally considered "negative" in this test system if:

- The number of micronucleated cells in the test groups is not distinctly inceased above the concurrent vehicle control and is within our laboratory's recent negative control data range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically (see BASF study code 01Y0174/13Y004).

B. PRELIMINARY CYTOTOXICITY ASSAY:

A preliminary cytotoxicity assay was performed to determine appropriate test substance concentrations for the main test. After 4 hours treatment in the absence and presence of S9 mix no cytotoxicity was observed. In the pretest with 24 hours continuous treatment in the absence of S9 mix, the relative increase in cell count was clearly reduced at 2 000 µg/mL.

C. CYTOGENICITY ASSAYS:

In this study, no statistically significant or biologically relevant increase in the number of micronucleated cells was observed either without S9 mix or after the addition of a metabolizing system [see Table 5.8.1-34 and Table 5.8.1-35]. In both experiments the incidences of micronucleated cells (0.2 - 1.3%) were close to the concurrent vehicle control values (0.4 - 1.2%) and clearly within historical negative control data range (0.1 - 1.8%).

The positive control substances EMS (without S9 mix; 300 µg/mL) and CPP (with S9 mix; 1.0 µg/mL) induced statistically significant increased micronucleus frequencies in both independently performed experiments.

Growth inhibition as indicated by reduced cell numbers or RICC was observed in the 1st experiment in the absence of S9 mix at ≥ 1000 µg/mL and likewise in the 2nd experiment in the absence of S9 mix after 24 hours continuous treatment at ≥ 1000 µg/mL. In the latter case a reduced proliferative activity (CBPI) and reduced replicative index (RI) was also observed. Likewise, cell morphology/attachment was adversely influenced (grade > 2).

In both experiments with and without metabolic activation neither test substance precipitation nor changes of osmolarity and pH values were observed.

Table 5.8.1-34: Summary of results of micronucleus test with Reg.No. 5916420 (Metabolite of BAS 500 F, pyraclostrobin) - without metabolic activation

					Genotoxicity	Cytotoxicity		
Exp.	Exposure/ Preparation interval	Test groups	S9 mix	Prec.*	Micronucleated cells** [%]	Proliferation index cytostasis (CBPI) [%]	Replicative index (RI) [%]	Relative increase in cell count (RICC) [%]
1	4/24 hrs	vehicle control[1]	-	n.d.	0.4	0.0	100.0	100.0
		62.5 µg/mL	-	-	n.d.	n.d.	n.d.	106.1
		125.0 µg/mL	-	-	n.d.	n.d.	n.d.	116.9
		250.0 µg/mL	-	-	n.d.	n.d.	n.d.	85.9
		500.0 µg/mL	-	-	0.9	4.8	95.2	87.7
		1000.0 µg/mL	-	-	1.0	2.8	97.2	49.3
		2000.0 µg/mL	-	-	0.7	5.6	94.4	55.4
		positive control[2]	-	n.d.	3.3[S]	5.0	95.0	104.0
2	24/24 hrs	vehicle vcntrol[1]	-	n.d.	0.4	0.0	100.0	100.0
		125.0 µg/mL	-	-	n.d.	n.d.	n.d.	80.9
		250.0 µg/mL	-	-	0.5	10.5	89.5	83.0
		500.0 µg/mL	-	-	0.4	36.4	63.6	73.3
		1000.0 µg/mL	-	-	0.2	66.2	33.8	32.8
		2000.0 µg/mL	-	-	n.e.	n.e.	n.e.	-36.2
		positive control[2]	-	n.d.	4.3[S]	14.3	85.7	98.9

Precipitation in culture medium at the end of exposure period (macroscopic)
** Relative number of binucleated cells with micronuclei per 2000 cells scored per test group
S Frequency statistically significant higher than corresponding control values
n.d. Not determined
n.e. Not evaluated due to strong toxicity
1 DMSO 1% (v/v)
2 EMS 300 µg/mL

Table 5.8.1-35: Summary of results of micronucleus test with Reg.No. 5916420 (Metabolite of BAS 500 F, pyraclostrobin) - with metabolic activation

					Genotoxicity	Cytotoxicity		
Exp.	Exposure/ Preparation interval	Test groups	S9 mix	Prec.*	Micronucleated cells** [%]	Proliferation index cytostasis (CBPI) [%]	Replicative index (RI) [%]	Relative increase in cell count (RICC) [%]
1	4/24 hrs	vehicle control[1]	+	n.d.	1.2	0.0	100.0	100.0
		62.5 µg/mL	+	-	n.d.	n.d.	n.d.	110.3
		125.0 µg/mL	+	-	n.d.	n.d.	n.d.	96.0
		250.0 µg/mL	+	-	n.d.	n.d.	n.d.	91.3
		500.0 µg/mL	+	-	0.6	5.2	94.8	108.0
		1000.0 µg/mL	+	-	0.8	8.6	91.4	97.0
		2000.0 µg/mL	+	-	0.4	14.8	85.2	65.2
		positive control[2]	+	n.d.	3.9[S]	48.6	51.4	102.2
2	4/44 hrs	vehicle vcntrol[1]	+	n.d.	1.1	0.0	100.0	100.0
		125.0 µg/mL	+	-	n.d.	n.d.	n.d.	89.0
		250.0 µg/mL	+	-	1.0	-1.5	101.5	78.4
		500.0 µg/mL	+	-	1.3	6.6	93.4	84.1
		1000.0 µg/mL	+	-	1.1	-1.8	101.8	79.0
		2000.0 µg/mL	+	-	0.6	2.6	97.4	76.1
		positive control[2]	+	n.d.	11.3[S]	8.2	91.8	65.7

* Precipitation in culture medium at the end of exposure period (macroscopic)
** Relative number of binucleated cells with micronuclei per 2000 cells scored per test group
[S] Frequency statistically significant higher than corresponding control values
n.d. Not determined
n.e. Not evaluated due to strong toxicity
[1] DMSO 1% (v/v)
[2] CPP 1.0 µg/mL

Table 5.8.1-36: Reg.No. 5916420 - Gene mutation in mammalian cells

Exp.	Exposure period [h]	Test groups [µg/mL]	S9 mix	Prec.*	Cytotoxicity**		Genotoxicity***	
					Relative cloning efficiency 1 RCE_1(%)	Relative total growth RTG (%)	Corrected mutant frequency (MF_{corr})	Mutant frequency threshold***
							(colonies per 10^6 cells)	
1	4	Vehicle control[1]	-	n.d.	100.0	100.0	59.1	185
		125.0	-	-	87.4	111.0	42.9	185
		250.0	-	-	69.9	103.8	57.6	185
		500.0	-	-	81.5	111.1	47.3	185
		1000.0	-	-	66.0	108.6	40.2	185
		2000.0	-	-	70.5	87.5	51.5	185
		Positive control[2]	-	n.d.	63.9	40.0	772.2	185
2	24	Vehicle control[1]	-	n.d.	100.0	100.0	39.3	165
		62.5	-	-	102.7	94.2	39.4	165
		125.0	-	-	99.1	83.9	39.2	165
		250.0	-	-	88.2	78.2	48.6	165
		500.0	-	-	88.2	64.7	48.2	165
		1000.0	-	-	82.1	48.9	43.8	165
		2000.0	-	-	59.1	13.8	84.7	165
		Positive control[3]	-	n.d.	71.2	38.8	442.7	165
1	4	Vehicle control[1]	+	n.d.	100.0	100.0	35.1	161
		125.0	+	-	72.2	91.6	53.5	161
		250.0	+	-	92.6	88.4	38.3	161
		500.0	+	-	124.0	85.1	68.2	161
		1000.0	+	-	147.7	95.3	45.9	161
		2000.0	+	-	91.0	77.1	67.2	161
		Positive control[4]	+	n.d.	80.2	78.3	226.0	161
2	4	Vehicle control[1]	+	n.d.	100.0	100.0	51.2	177
		375.0	+	-	111.9	103.8	42.4	177
		750.0	+	-	90.3	106.9	41.9	177
		1500.0	+	-	106.1	82.8	63.1	177
		2000.0	+	-	92.3	63.2	69.2	177
		Positive control[4]	+	n.d.	75.6	41.7	421.5	177

* Precipitation in culture medium at the end of exposure period
** For calculation see above in this report
*** Mutant frequency threshold: number of mutant colonies per 10^6 cells of current vehicle control plus 126
n.d. Not determined
[1] DMSO 1% (v/v) [2] MMS 15.0 µg/mL
[3] MMS 5.0 µg/mL [4] CPP 2.5 µg/mL

III. CONCLUSION

Based on the results of the study it is concluded that 500M49 (Reg.No. 5916420, metabolite of pyraclostrobin) has not the potential to induce micronuclei (clastogenic and/or aneugenic activity) under the in vitro conditions of this study.

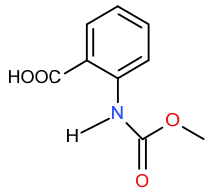
Overall toxicological evaluation of 500M49 (Reg.No. 5916420)

The QSAR evaluation of 500M49 is of low reliability and by weight of evidence there was no conclusive alert for genotoxicity.

500M49 is considered to be not genotoxic based on the in vitro studies conducted. Therefore, 500M49 is considered to be **not toxicologically relevant**.

With regard to consumer exposure the TTC concept for a non-genotoxic compound was applied. Thus an ADI of 0.0015 mg/kg bw/day as well as an ARfD of 0.005 mg/kg bw/day was established. The estimated exposure (0.2% of TTC of 0.005 mg/kg bw/day for acute and 0.1% of TTC 0.0015 mg/kg bw/day for chronic exposure) is low.

500M51 (other denominator: Reg.No. 78810)



500M51 (Reg.No. 78810) is a metabolite of pyraclostrobin found in rat and goat metabolism studies.

A) QSAR Predicitions for 500M51 (Reg.No. 78810)

OECD Toolbox (Version: 3.2) [see DocID 2014/1172955]

In the OECD toolbox no DNA alerts for genotoxicity (Ames, MN, CA) or alerts for DNA binding were observed. For protein binding, which is also taken as supportive evidence for a positive in vitro CA, the alert was '*Acylation|Acylation >> Direct acylation involving a leaving group|Acylation >> Direct acylation involving a leaving group >> Carbamates*'. It is unclear how to interpret the positive protein binding alert in absence of a DNA alert for Ames, MN and CA.

Discussion: The same alert was also found for 500M49 (Reg.No. 5916420), which like 500M51 (Reg.No. 78810) was tested negative for in vitro chromosome aberration. The positive prediction is there rejected.

OASIS TIMES (MIX V.2.27.13 TB; Mutagenicity S-9 activated v08.08) [see molecule 6 of report DocID 2014/1172952 and 2014/1172953]

There were no Ames mutagenicity alerts for 500M51 (Reg.No. 78810) or in-silico generated metabolites. In all cases the structures were out of domain. For in vitro chromosome aberration the prediction for 500M51 (Reg.No. 78810) was positive with the alert info 'carbamates' (out of domain). In contrast all 8 in-silico metabolites were predicted negative (out of domain).

Discussion: The result of the in vitro micronucleus assay (see CA 5.8.1/2 below) revealed no chromosome damaging potential of 500M51 (Reg.No. 78810). Therefore, the positive prediction for CA is not reflecting the toxicological database and is therefore rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.9) [see molecule 6 of report DocID 2014/1172954 for both VEGA models]

500M51 (Reg.No. 78810) is out of model applicability domain. The prediction is 'non mutagen' with no specific structural alerts.

VEGA: Mutagenicity model (SarPy; version 1.0.4-BETA)

500M51 (Reg.No. 78810) is out of model applicability domain. The prediction is 'mutagen' with the structural alert SA 98. As discussed above, this alert was also obtained for pyraclostrobin and a number of pyraclostrobin metabolites, which like 500M51 (Reg.No. 78810) were actually tested negative. The predicition is therefore rejected.

DEREK (Version 2.0.0.201011301322; Knowledge base version: 11_20_05_2010)

The prediction for genotoxicity and neurotoxicity is negative.

Genotoxicity studies on 500M51 (Reg.No. 78810)

Report:	CA 5.8.1/31 Woitkowiak C., 2013a Reg.No. 78810 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium / Escherichia coli reverse mutation assay 2013/1255749
Guidelines:	OECD 471, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium (strains TA98, TA 100, TA 1535 and TA 1537) and E. coli strain WP2 uvrA were exposed to Reg.No. 78810 (Metabolite of BAS 500 F, pyraclostrobin); batch: L82-117, purity: 97.9%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. In the plate incorporation assay Reg.No. 78810 was tested in concentrations of 33, 100, 333, 1000, 2600 and 5200 µg/plate. In the preincubation test Reg.No. 78810 were tested in concentrations of 10, 33, 100, 333, 1000 and 2600 µg/plate. In both, the plate incorporation assay and the preincubation assay occasionally a weak bacteriotoxic effect was observed depending on the strain and test conditions at concentrations from about 1000 µg/plate onwards. Precipitation of the test substance did not occur up to the highest tested concentration.

A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.

According to the results of the study the test substance Reg.No. 78810 was not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2013/1255749)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

	Reg.No. 78810 (Metabolite of BAS 500 F, pyraclostrobin)
Description:	Solid, white
Lot/Batch #:	L82-117
Purity:	97.9% (tolerance +/- 1.0%)
Stability of test compound:	The test substance was stable over the study period under the storage conditions and guaranteed until 01 May 2015. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance solutions. The stability of the test substance in the vehicle DMSO over a period of 4 h was verified in the analytical study project 01Y0175/13Y005.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).
Vehicle control:	The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

To demonstrate the efficacy of the S9 mix in this assay, the S9 batch was further characterized with benoz(a)pyrene in the strains TA 100 and TA 98 as required in OECD 471.

3. Activation:

S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received on three consecutive days intraperitoneal injections of 80 mg phenobarbital and 80 mg β-naphthoflavone orally per kg body weight. 24 hours after the last administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium: The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay: In the first experiment triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2600 and 5200 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay: In the second experiment the test article / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 10, 33, 100, 333, 1000 and 2600 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains.

B. TEST PERFORMANCE:

1. Dates of experimental work: 25-Jun-2013 to 04-Jul-2013

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Merckoplate® plates (minimal glucose agar plates). In the experiments with E. coli the warm soft agar contained 0.5 mM tryptophan instead of histidine + biotin.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37°C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects, the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37°C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically (see above).

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

A bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed in the standard plate test and in the preincubation test depending on the strain and test conditions from about 1 000 µg/plate onwards.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiment with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested [see Table 5.8.1-37]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Precipitation was not observed up to the maximum concentration.

Table 5.8.1-37: Bacterial gene mutation assay with Reg.No. 78810 - Mean number of revertants

Experiment 1: Plate incorporation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	39	23	52	32	13	11	12	6	69	64
Reg.No. 078810										
33 µg/plate	33	24	47	33	14	12	11	7	68	65
100 µg/plate	40	22	47	32	13	10	12	6	72	58
333 µg/plate	35	23	47	34	11	10	12	7	61	70
1000 µg/plate	21	25	45	33	10	10	10	5	32	43
2600 µg/plate	9	12	25	23	9	10	5	4	5	15
5200 µg/plate	0	0	0	0	0	0	0	0	0	4
Pos. control[§]	1282	1088	1797	1204	411	1025	257	396	218	861
Experiment 2: Preincubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	27	16	50	37	12	11	9	6	40	35
Reg.No. 078810										
10 µg/plate	28	16	47	37	13	11	9	7	41	38
33 µg/plate	29	16	49	39	12	11	11	6	40	36
100 µg/plate	28	18	53	37	11	12	9	7	38	36
333 µg/plate	28	16	49	38	13	12	10	7	40	35
1000 µg/plate	18	12	43	29	12	7	7	7	17	18
2600 µg/plate	0	0	0	0	0	4	0	0	0	0
Pos. control[§]	897	588	1275	1125	414	1014	231	319	230	528

[§] = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSION

According to the results of the present study, the test substance Reg.No. 78810 is not mutagenic in the Ames standard plate test and in the preincubation test under the experimental conditions chosen here.

Report:	CA 5.8.1/32 Schulz M., Landsiedel R., 2014b Reg.No. 78810 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells (TK+ locus assay, microwell version) 2013/1298448
Guidelines:	OECD 476, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 78810 (Metabolite of 500 F; pyraclostrobin; batch: L82-117, purity: 97.9%) was tested in vitro for its ability to induce forward mutations in L5178Y cells by assessing the mutation of the $TK^{+/-}$ locus. Two independent experiments were conducted in the presence and absence of metabolic activation with concentrations of 7.8 to 2000 µg/mL. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. The treatment interval for cultures treated with the test substance in the presence of metabolic activation was 4 h for both experiments. Methyl methanesulfonate (MMS) and cyclophosphamide (CPP) served as positive controls in the experiments without and with metabolic activation, respectively.

Cytotoxicity indicated by clearly reduced relative total growth (RTG) of below 20% of control was observed after 24 hours exposure in the 2nd experiment in the absence of metabolic activation only.

The test substance did not cause any biologically relevant increase in the mutant frequencies neither without S9 mix nor after adding a metabolizing system in two experiments performed independently of each other. The positive control substances, however, induced a marked increase in mutant frequency and the negative controls gave mutant frequencies within the range expected for the L5178Y $TK^{+/-}$ mouse lymphoma cell line.

Based on the results of the study it is concluded that under the conditions of the test Reg.No. 78810 (metabolite of 500 F; pyraclostrobin) does not induce forward mutations in mammalian cells in vitro.

(BASF DocID 2013/1298448)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Description:	Reg.No. 78810 (metabolite of pyraclostrobin) Solid, white
Lot/Batch #:	L82-117
Purity:	97.9% (tolerance ± 1.0%)
Stability of test compound:	The test substance was stable over the study period under the storage conditions and guaranteed until 01 May 2015. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance solutions. The stability of the test substance in the vehicle DMSO over a period of 4 h was verified analytically.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	The vehicle controls with and without S9 mix contained the vehicle selected for the test substance at the same volume and concentration as used in the test cultures.
Positive control -S9:	Methyl methanesulfonate (MMS) 15 µg/mL (4-hour exposure period) and 5 µg/mL (24-hour exposure period)
Positive control +S9:	Cyclophosphamide (CPP) 2.5 µg/mL

3. Activation:

S9 was produced from the livers of at least 5 induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4.	**Test organism:**	Mouse lymphoma L5178Y $TK^{+/-}$ cell line: This is a cell line with a high proliferation rate (doubling time of about 9 - 10 hours), a high plating efficiency (about 90%) and a stable karyotype with a near diploid number of 40 ± 1 chromosome. Each batch used for mutagenicity testing was checked for mycoplasma contamination.
5.	**Culture media:**	
	Culture medium:	RPMI 1640 medium including stable glutamine supplemented with: 1% (v/v) penicillin/streptomycin (10 000 IU / 10 000 µg/mL) and 1% (v/v) sodium pyruvate (10 mM) = RPMI-0 For treatment medium (with S9 mix): RPMI-0 supplemented with 5% (v/v) fetal calf serum (FCS) = RPMI-5 For treatment medium (without S9 mix) and subculturing cells: RPMI-0 is supplemented with 10% (v/v) FCS = RPMI-10 For cloning efficiency and selection medium: RPMI-0 supplemented with 20% (v/v) FCS = RPMI-20
	Pretreatment medium:	A ("THMG" medium): RPMI-10 supplemented with thymidine (3.0 µg/mL), hypoxanthine (5.0 µg/mL), methotrexate (0.1 µg/mL) and glycine (7.5 µg/mL) B ("THG" medium): RPMI-10 supplemented with thymidine (3.0 µg/mL), hypoxanthine (5.0 µg/mL), and glycine (7.5 µg/mL)
	Selection medium:	("TFT" medium): RPMI-20 supplemented with trifluorthymidine (TFT, 4.0 µg/mL)
6.	**Locus examined:**	thymidine kinase (TK)

7. Test concentrations:

a) Preliminary toxicity assay: Nine concentrations ranging from 7.8 to 2000 µg/mL

b) Mutation assay:
1st experiment: 250.0, 500.0, 1000.0 and 2000 µg/mL with and without metabolic activation

2nd experiment: 7.8, 15.6, 31.3, 62.5, 125.0, 250.0, 500.0, 1000 and 2000 µg/mL without metabolic activation and 375.0, 750.0, 1500 and 2000 µg/mL with metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 10-Jun-2013 to 14-Jan-2014

2. Preliminary cytotoxicity assay:

In the pretest for toxicity based on the molecular weight of the test substance 2 000 µg/mL (approx. 10 mM) Reg.No. 78810 (Metabolite of BAS 500 F, pyraclostrobin) was used as top concentration both with and without S9 mix at 4 hour exposure time and without S9 mix at 24 hour exposure time.
The pretest was performed following the method described for the main experiment. The relative suspension growth (RSG) was determined as toxicity indicator for dose selection and various parameters were checked for all or at least for some selected doses. Additionally, pH and solubility were determined.

3. Mutation Assay:

Pretreatment of Cells: During the week prior to treatment, spontaneous TK deficient mutants ($TK^{-/-}$) were eliminated from the stock cultures by incubating 3 x 10^5 cells per 75 cm² flask in a total volume of 30 mL for 1 day in “THMG" medium (pretreatment medium A), and for the following 3 days in “THG" medium (pretreatment medium B).

Cell treatment:	For each test group, about 1.5 x 10^7 cells per flask were seeded into 75 cm^2 flasks containing RPMI-10 for exposures without S9 mix and RPMI-5 medium for exposures with S9 mix and incubated for the respective exposure period (1st experiment: 4-hour exposure with and without S9 mix and 2nd experiment 24 hours without S9 mix and 4 hours with S9 mix) with 5% CO_2 at 37°C and ≥90% humidity for cell attachment. 2 cultures were treated in parallel for each test group.
Expression:	At the end of the exposure period, the cells were transferred in tubes, centrifuged for 5 minutes at 1000 rpm and were resuspended in RPMI-5 medium. The washing of the cells was repeated at least once. Then the cells were centrifuged and were resuspended in RPMI-10 medium. From each culture a sample of treated cells (2 x 10^5 cells/mL or 6 x 10^6 cells/flask) were pipetted in 75 m^2 flasks and were incubated for a 2-day expression period. To maintain exponential growth during this phase, each culture was counted daily and the cell numbers were adjusted at each day to 2 x 10^5 cells/mL in 30 mL RPMI-10 medium.
Selection:	For the mutant selection, the cells were resuspended in selection medium ("TFT" medium) and two 96-well plates per cultures were seeded with 2000 cells/well. After incubation of at least 9 days, both the number of negative wells and the number of wells containing small or large colonies were scored for calculation of the mutant frequency (MF).
Size distribution of the colonies:	The colonies are classified into large colonies (indication of gene mutation) and small colonies (indication of chromosome breakage). Small colonies are defined as less than 1/4 of the diameter of the well. Size is the key factor and morphology (the optical density of the small colonies is considerably higher) should be secondary.
Determination of Cytotoxicity:	Cloning efficiency 1 (survival): The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. At the end of the exposure period, the cells were centrifuged and 400 cells from each culture were resuspended in 50 mL RPMI-20 medium (8 cells/mL). Per culture 200 µL were dispensed in each well of two 96-well plates (1.6 cells/well). After incubation for 9 - 11 days the plates were scored for empty wells.

Cloning efficiency 2 (viability):

The viability (cloning efficiency 2; CE_2) was determined after the expression period. 2 days after end of exposure, the cells were centrifuged and 400 cells from each culture were resuspended in 50 mL RPMI-20 medium (8 cells/mL). Per culture 200 µL were dispensed in each well of two 96-well plates (1.6 cells/well). After incubation for at least 9 days the plates were scored for empty wells.

Suspension growth (RSG) and relative total growth (RTG):
For calculation of the suspension growth (SG) and the relative total growth (RTG) the cell counts determined within the expression period at 2nd and 3rd passage after exposure in the case of 4-hour exposure and 1st, 2nd and 3rd passage after exposure in the case of 24-hour exposure were used.

This measure includes the relative growth in suspension during the expression period (RSG) and the relative cloning efficiency (RCE_2; viability) at the time the mutants are selected.

For taking into account any loss of cells during the 4-hour treatment period a relative growth during treatment factor (RGDT, %) was calculated by comparing the growth of each treated culture relative control.

Calculations:

Mutant frequency:
Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{-\ln(\text{total number of empty well s} \div \text{total number of seeded wells (96)})}{\text{number of seeded cells per well (2000)}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_2} \times 100$$

Cloning efficiency (CE, %) absolute:

$$CE_x = \frac{-\ln(\text{total number of empty wells} \div \text{total number of seeded wells (96)})}{\text{number of seeded cells per well (1.6)}} \times 100$$

Cloning efficiency relative, in comparison to control:

$$RCE_x = \frac{CE_x \ of \ the \ test \ group}{CE_x \ of \ the \ negative \ / \ vehicle \ control} \times 100$$

Relative total growth:

Relative growth during treatment (RGDT, %)

$$\text{RGDT} = \frac{\text{Cell count after 4 h of the test group}}{\text{Cell count after 4 h of the negative/v ehicle control}} \times 100$$

Total suspension growth (SG) after 4 hour-exposure:

$$SG = \frac{\text{Cell count after 24 h}}{2 \text{ x } 10^5 \text{ cells per mL}^{1,2}} \times \frac{\text{Cell count after 48 h}}{2 \text{ x } 10^5 \text{ cells per mL}^2} \times \frac{\text{RGDT}}{100}$$

Total suspension growth (SG) after 24 hour-exposure:

$$SG = \frac{\text{Cell count after 24 h}}{2 \text{ x } 10^5 \text{ cells per mL}} \times \frac{\text{Cell count after 48 h}}{2 \text{ x } 10^5 \text{ cells per mL}^2} \times \frac{\text{Cell count after 72 h}}{2 \text{ x } 10^5 \text{ cells per mL}^2}$$

$$\text{RSG} = \frac{\text{SG of the test group}}{\text{SG of the negative/v ehicle control}} \times 100$$

$$RTG = \frac{RSG \times RCE_2}{100}$$

[1] Cell number seeded following 4-hour treatment
[2] If cell number was lower than 2 x 10^5 cells per mL all remaining cells were seeded

4. Statistics:

An appropriate statistical trend test (MS EXCEL function RGP; 9) was performed to assess a dose-related increase of mutant frequencies. The number of mutant colonies obtained for the test substance treated groups was compared with that of the respective vehicle control groups. A trend is judged as statistically significant whenever the one-sided p-value (probability value) is below 0.05 and the slope was greater than 0. Both, biological and statistical significance will be considered together. However, both, biological and statistical significance has been considered together.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- The mutation frequency exceeds a threshold of 126 mutant colonies per 10^6 cells (GEF: Global Evaluation Factor) above the concurrent negative/vehicle control value.

and

- Evidence of reproducibility of any increase in mutant frequencies, means the mutagenic response occurs at least in both parallel cultures of one experiment.

and

- A statistically significant dose-related increase in mutant frequencies using an appropriate statistical trend test.

A test substance is generally considered negative in this test system if:

- The mutation frequency is below a threshold of 126 mutant colonies per 10^6 cells (GEF) above the concurrent negative/vehicle control value.

or

- No evidence of reproducibility of an increase in mutant frequencies is obtained.

or

- No statistically significant dose-related increase in mutant frequencies using an appropriate statistical trend test is observed.

However, in the evaluation of the test results the historical variability of the mutation rates in negative and vehicle controls and the mutation rates of all negative and vehicle controls of this study were taken into consideration. Results of test groups have been rejected if the relative total growth (RTG) and/or the cloning efficiency 1 (CE_1) were less than 10% of the respective vehicle controls. Whenever a test substance is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose-related shift in the ratio of small versus large colonies clastogenic effects are indicated.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically (see BASF study code 01Y0175/13Y005).

B. PRELIMINARY CYTOTOXICITY ASSAY

After 4 hours treatment in the absence and presence of S9 mix no cytotoxicity indicated by reduced relative suspension growth of about or below 20% was observed. After 24 hours treatment in the absence of S9 mix reduced relative suspension growth of about or below 20% was observed after treatment with 125 µg/mL and above.

With exception of the highest concentration (2000 µg/ml) the pH value was not influenced by the addition of the test substance preparation to the culture medium at all concentrations. At the highest concentrationthe pH was adjusted to a physiological value by addition of small amounts of 2N NaOH. In this study, in the absence and the presence of S9 mix neither in the vehicle DMSO nor in culture medium test substance precipitation was observed up to the highest required test substance concentration.

Based on these data the highest concentration tested in the mutagenicity experiments was 2000 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

In this study, no relevant increase in the number of mutant colonies was observed either without S9 mix or after the addition of a metabolizing system. The corrected mutant frequencies obtained for both experiments were always below the calculated threshold taking in consideration the Global Evaluation Factor (GEF).

In both experiments after 4 hours treatment in the absence and presence of metabolic activation the values for the corrected mutation frequencies (MFcorr.: 32.6 - 62.0 per 10^6 cells) were close to the respective vehicle control values (MFcorr.: 49.8 - 58.1 per 10^6 cells, see Table 5.8.1-38) and clearly within the range of historical negative control data (without S9 mix: MFcorr.: 23.7 - 92.4 per 10^6 cells; with S9 mix: MFcorr.: 21.4 - 103.0 per 10^6 cells). In addition, in the 2nd experiment after 24 hours treatment in the absence of metabolic activation the values for the corrected mutation frequencies (MFcorr.: 55.8 - 109.8 per 10^6 cells) were close to the respective vehicle control value (MFcorr.: 52.6 per 10^6 cells, see Table 5.8.1-38) and nearby the range of historical negative control data range (without S9 mix: MFcorr.: 12.2 - 80.6 per 10^6 cells). There was no statistically significant dose-related increase in both experiments with and without S9 mix.

Treatment with the positive controls MMS and CCP resulted in clearly increased mutant frequencies as expected. The values of the corrected mutant frequencies (MFcorr.: 465.0 - 674.5 per 10^6 cells) clearly exceeded the respective calculated thresholds for a mutagenic effect based on the GEF (126 plus the mutant frequency of the respective negative control).

In both experiments after 4 hours exposure, no cytotoxic effects indicated by reduced cloning efficiencies or reduced relative total growth of below 20% of control were observed neither in the presence nor in the absence of S9 mix. In contrast, in the 2nd experiment after 24 hours exposure in the absence of metabolic activation the RTG was clearly reduced from 250 µg/mL (RTG: 20.8%) onwards.

The pH value of the test substance preparation was adjusted by adding small amounts of NaOH. In this study, in the absence and the presence of S9 mix neither in the vehicle DMSO nor in culture medium test substance precipitation was observed up to the highest required test substance concentration.

Table 5.8.1-38: Gene mutation in mammalian cells

Exp.	Exposure period [h]	Test groups [µg/mL]	S9 mix	Prec.*	Cytotoxicity**		Genotoxicity***	
					Relative cloning efficiency 1 RCE_1(%)	Relative total growth RTG (%)	Corrected mutant frequency (MF_{corr})	Mutant frequency threshold***
							(colonies per 10^6 cells)	
1	4	Vehicle control[1]	-	n.d.	100.0	100.0	49.9	176
		250.0	-	-	100.3	113.3	44.5	176
		500.0	-	-	111.3	101.4	62.0	176
		1000.0	-	-	118.3	106.7	49.9	176
		2000.0	-	-	104.1	91.2	32.6	176
		Positive control[2]	-	n.d.	98.4	39.3	674.5	176
2	24	Vehicle control[1]	-	n.d.	100.0	100.0	52.6	179
		7.8	-	-	113.1	86.1	55.8	179
		15.6	-	-	112.1	93.2	58.3	179
		31.3	-	-	96.7	87.0	63.0	179
		62.5	-	-	91.4	61.1	57.6	179
		125.0	-	-	86.6	29.6	85.3	179
		250.0	-	-	73.4	20.8	109.8	179
		500.0	-	-	76.2	20.3	90.8	179
		1000.0	-	-	73.4	22.1	76.9	179
		2000.0	-	-	42.6	7.3	86.9	179
		Positive control[3]	-	n.d.	76.2	79.3	467.6	179

Table 5.8.1-38: Gene mutation in mammalian cells

Exp.	Exposure period [h]	Test groups [µg/mL]	S9 mix	Prec.*	Cytotoxicity**		Genotoxicity***	
					Relative cloning efficiency 1 RCE_1(%)	Relative total growth RTG (%)	Corrected mutant frequency (MF_{corr})	Mutant frequency threshold***
							(colonies per 10^6 cells)	
1	4	Vehicle control[1]	+	n.d.	100.0	100.0	49.8	176
		250.0	+	-	107.2	103.2	48.2	176
		500.0	+	-	102.3	93.3	49.1	176
		1000.0	+	-	110.7	101.5	40.9	176
		2000.0	+	-	106.4	100.5	46.9	176
		Positive control[4]	+	n.d.	80.2	57.9	465.0	176
2	4	Vehicle control[1]	+	n.d.	100.0	100.0	58.1	184
		375.0	+	-	114.9	132.9	39.4	184
		750.0	+	-	103.8	102.7	55.0	184
		1500.0	+	-	105.4	116.6	51.7	184
		2000.0	+	-	95.6	86.8	56.8	184
		Positive control[4]	+	n.d.	70.8	52.1	555.0	184

* Precipitation in culture medium at the end of exposure period
** For calculation see above in this report
*** Mutant frequency threshold: number of mutant colonies per 10^6 cells of current vehicle control plus 126
n.d. Not determined
[1] DMSO 1% (v/v) [2] MMS 15.0 µg/mL
[3] MMS 5.0 µg/mL [4] CPP 2.5µg/mL

III. CONCLUSION

Based on the results of the study it is concluded that 500M51 (Reg.No. 78810, metabolite of pyraclostrobin) does not induce forward mutations in vitro in the mouse lymphoma assay with L5178Y $TK^{+/-}$ cells in the absence and the presence of metabolic activation.

Report: CA 5.8.1/33
Schulz M., Landsiedel R., 2014a
Reg.No. 78810 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro micronucleus assay in V79 cells (cytokinesis block method)
2013/1361922

Guidelines: OECD 487 (2010), Commission Regulation EU No. 640/2012 of 06 July 2012 - B.49: In vitro Mammalian Cell Micronucleus Test

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 78810 (Metabolite of BAS 500 F, pyraclostrobin) (batch L82-117; purity 97.9%) was tested in vitro for its potential to induce micronuclei in V79 cells of the Chinese hamster in the absence and presence of metabolic activation by S9 mix in two independent experiments with different exposure periods (4 and 24 hours).

Cytotoxicity as indicated by clearly reduced relative increase in cell count (RICC), proliferation index (CBPI) or replicative index (RI) was only observed at 2000 µg/mL in the 2nd experiment after 24 hours continuous treatment in the absence of S9 mix. In both experiments with and without metabolic activation, no test substance precipitation in culture medium at the end of exposure period was macroscopically observed. No statistically significant or biologically relevant increase in the number of micronucleated cells was observed either without S9 mix or after the addition of a metabolizing system.

Vehicle and positive controls revealed the expected results of micronucleated cells thereby ensuring the validity of the test.

Based on the results of this study, Reg.No. 78810, metabolite of pyraclostrobin, is considered not to have a chromosome-damaging (clastogenic) effect nor to induce numerical chromosomal aberrations (aneugenic activity) under in vitro conditions in V79 cells in the absence and the presence of metabolic activation.

(BASF DocID 2013/1361922)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 78810 (Metabolite of BAS 500 F, pyraclostrobin)

Description:	Solid, white
Lot/Batch #:	L82-117
Purity:	97.9% (tolerance ± 1.0%)
Stability of test compound:	The test substance was stable over the study period under the storage conditions and guaranteed until 01 May 2015. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance solutions. The stability of the test substance in the vehicle DMSO over a period of 4 h was verified in the analytical study project 01Y0175/13Y005.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO
Positive control, -S9:	Ethylmethanesulfonate (EMS, 300, 400 and 500 µg/mL, dissolved in MEM without FCS)
Positive control, +S9:	Cyclophosphamide (CPA, 1.0 and 2.5 µg/mL, dissolved in MEM without FCS)

3. Activation: S9 was produced from male Wistar rats. The rats were induced by intraperitoneal applications of 80 mg/kg bw phenobarbital and by oral administrations of 80 mg/kg bw β-naphthoflavone each, on three consecutive days. The livers were prepared 24 hours after the last treatment. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction was thawed at room temperature and 1 volume of S9-fraction was mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9 mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms:

Chinese hamster V79 cells
This is a continuous cell line with a high proliferation doubling time of 12-14 hours, a high plating efficiency (≥ 90%) and a stable karyotype (modal number of 22 chromosomes).

5. Culture medium/conditions:

Culture media:

Minimal essential medium with Earle's salts (MEM) containing a L-glutamine source supplemented with 10% (v/v) fetal calf serum (FCS), 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL) and 1% (v/v) amphotericine B (250 µg/mL). During exposure to the test substance in the presence of S9 mix MEM medium was used without FCS supplementation.

Cell culture:

Deep-frozen cell stocks were thawed at 37°C in a water bath, and volumes of 0.5 mL were transferred into 25 cm^2 plastic flasks containing about 5 mL MEM supplemented with 10% (v/v) fetal calf serum (FCS). Cells were grown with 5% (v/v) CO2 at 37°C and ≥ 90% humidity and subcultured twice weekly. Cell monolayers were suspended in culture medium after detachment with 0.25% (w/v) trypsin solution.

Cell cycle and harvest time: The cell cycle of the untreated V79 cells lasts for about 12 - 14 hours under the selected culture conditions. Thus, a harvest time of 24 hours is about 2 times the normal cell cycle length. V79 cells are an asynchronous cell population, i.e. at the time of test substance treatment there are different cell stages. Since the effect on these cell stages may vary for different test substances, more than one harvest time after treatment may be appropriate. Furthermore, substance-induced mitotic delay may considerably delay the first post-treatment mitosis. Therefore, delayed harvest times (e.g. 44 hours) and prolonged exposure periods (e.g. 24 hours treatment) were considered.

6. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations spaced by a factor of 2 ranging von 15.6 to 2000 µg/ml were used with and without metabolic activation
Exposure period: 4 hours with and without metabolic activation; 24 hours without metabolic activation
Harvest time: 24 hours

b) Cytogenicity assay:
1st experiment: 62.5, 125, 250, 500, 1000 and 2000 µg/mL with and without S9 mix (4 hours exposure, 24 hour preparation interval)
2nd experiment: 62.5, 125, 250, 500, 1000 and 2000 µg/mL without metabolic activation (24 hour exposure, 24 hour preparation interval)
125, 250, 500, 1000 and 2000 µg/mL with metabolic activation (4 hour exposure, 44 hour preparation interval)

B. TEST PERFORMANCE:

1. Dates of experimental work: 17-Jun-2013 to 21-Nov-2013

2. Preliminary cytotoxicity assay:

In the pretest for toxicity based on the purity and the molecular weight of the test substance 2000 µg/mL (approx. 10 mM) Reg.No. 78110 (Metabolite of BAS 500 F, pyraclostrobin) was used as top concentration. The pretest was performed following the method described for the main experiment. As indication of test substance toxicity relative increase in cell count (RICC) and cell attachment (morphology) were determined for dose selection. pH and solubility were additionally determined.

3. Cytogenicity Assay:

Two independent experiments were performed. In experiment I, the exposure period was 4 hours with and without S9 mix. In experiment II, the exposure periods were 4 hours with S9 mix and 24 hours without S9 mix. The cells were prepared 24 hours after start of treatment with the test item, except for the 4 hour treatment interval in the presence of metabolic activation (experiment II). In this experimental part cells were harvested after 44 hours to consider possibly occurring mitotic delay.

Cell seeding / treatment:	Routinely grown cells that did not exceed a max. of 15 passages and reached a confluency of at least 50%, were detached by trypsination and used to prepare a single cell suspension with the required cell count (3-5x10^5 cells per culture, depending on the schedule) in MEM incl. 10% (v/v) FCS. 5 mL cell suspension were transferred into 25 cm² cell culture flasks using a dispenser. Subsequently, the test cultures were incubated. After an attachment period of about 20 - 24 hours, the medium was removed from the flasks and the treatment medium was added. In case of experiments without metabolic activation the treatment medium consisted of 5 or 4 mL MEM medium with FCS plus 1 mL positive control or 0.05 mL test substance preparation/vehicle, respectively. In case of metabolic activation, the treatment medium consisted of 4 or 3 mL MEM medium without FCS, 1 mL positive control or 0.05 mL test substance preparation/vehicle and 1 mL S9-mix, respectively. The cultures were incubated for the respective exposure period at 37°C, 5% (v/v) CO_2 and ≥ 90% humidity. At the end of the exposure period, the medium was removed and the cultures were rinsed twice with 5 mL HBSS (Hanks Balanced Salt Solution). Subsequently, 5 mL MEM incl. 10% FCS supplemented with Cytochalasin B (CytB, final concentration: 3 µg/mL; stock: 0.6 mg/mL in DMSO) was added and incubated for the respective recovery time. In the case of 24-hour continuous exposure, CytB was added to the treatment medium at start of treatment, and cell preparation was started directly at the end of exposure. At 44 hours preparation interval in the presence of S9 mix the supplementation of CytB was 24 hours before preparation of the cultures.

Cell harvest, preparation of slides and staining:	Just before preparation the culture medium was completely removed. Single cell suspensions were prepared from each test group by trypsination. Then, the cell numbers per flask of each single cell suspension were determined using a cell counter. Subsequently, $5x10^4$ cells per slide were centrifuged at 1400 rpm for 7 minutes onto labelled slides using a Cytospin centrifuge. After drying, the slides were fixed in 90% (v/v) methanol for 10 minutes. Before scoring, the slides were stained with a mixture of 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) and propidium iodide (PI) in Fluoroshield™ at a concentration of 0.25 µg/mL each. By the use of the combination of both fluorescence dyes it can be differentiated between DNA (DAPI; excitation: 350 nm, emission: 460 nm) and cytoplasm (PI; excitation: 488 nm, emission: 590 nm).
Analysis of micronuclei and cytotoxicity:	Cytospin slides were scored by fluorescence microscopy. At least 1000 cells per culture, means at least 2000 cells per test group, were evaluated and the number of micronuclei-containing binucleated cells was recorded. Analysis of micronuclei was carried out following the criteria: The diameter of the micronucleus is less than 1/3 of the main nucleus; the micronucleus and main nucleus retain the same color; the micronucleus is not linked to the main nucleus and is located within the cytoplasm of the cell; only cells clearly surrounded by a nuclear membrane were scored. Cultures with few isolated cells were not analyzed for micronuclei.

Relative increase in cell count (RICC):

$$RICC = \frac{(\text{Increase in number of cells in treated cultures (final} - \text{starting}))}{(\text{Increase in number of cells in control cultures (final} - \text{starting}))} \text{x } 100$$

Thus, a RICC of 53% indicates 47% cytotoxicity/cytostasis.

Proliferation Index (CBPI):

$$CBPI = \frac{\big((\text{No. mononucleated cells}) + (2 \text{ x No. binucleated cells}\big) + (3 \text{ x No. mulitnucleated cells}))}{(\text{Total number of cells})}$$

The CBPI was used to calculate the % cytostasis (relative inhibition of cell growth compared to the respective vehicle control group) - a CBPI of 1 (all cells are mononucleate) is equivalent to 100% cytostasis.

% Cytostasis = 100 - 100 $\{(CBPI_T - 1) / (CBPI_C - 1)\}$

(T- test substance treated culture; C = vehicle control)

Replicative Index (RI):

$$RI = \frac{(((\text{No. binucleate cells}) + (2\text{xmultinucleate cells}) \div (\text{Total number of cells}))_T}{(((\text{No. binucleate cells}) + (2\text{xmultinucleate cells}) \div (\text{Total number of cells}))_C} x100$$

(T = test substance treated culture; C = vehicle control culture)

Thus, an RI of 53% means that in comparison to the respective control cultures only 53% of the cells divided in the treated test group (= 47% cytotoxicity/cytostasis).

4. Statistics:

The statistical evaluation of the data was carried out using the MUVIKE program system (BASF SE). The proportion of cells containing micronuclei was calculated for each group. A comparison of each dose group with the concurrent vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test is Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided. If the results of this test were statistically significant compared with the respective vehicle control, labels (* $p \leq 0.05$, ** $p \leq 0.01$) have been be printed in the tables.

5. Evaluation criteria:

A test item is considered "positive" in this assay if the following criteria are met:

- A significant, dose-related and reproducible increase in the number of cells containing micronuclei was observed.
- The number of micronucleated cells exceeded both the value of the concurrent vehicle control and the range of our laboratory's recent negative control data.

A test substance is generally considered "negative" in this test system if:

- The number of micronucleated cells in the test groups is not distinctly increased above the concurrent vehicle control and is within our laboratory's recent negative control data range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically (see BASF study code 01Y0175/13Y005).

B. PRELIMINARY CYTOTOXICITY ASSAY:

A preliminary cytotoxicity assay was performed to determine appropriate test substance concentrations for the main test. After 4 hours treatment in the absence and presence of S9 mix no cytotoxicity was observed as indicated by a reduced RICC of about or below 40 - 50%. In contrast, in the pretest with 24 hours continuous treatment in the absence of S9 mix, the relative increase in cell count was clearly reduced after treatment with ≥ 1000 µg/mL (RICC 32.9 and 27.2% at 1000 and 2000 µg/mL).

C. CYTOGENICITY ASSAYS:

In this study, no statistically significant or biologically relevant increase in the number of micronucleated cells was observed either without S9 mix or after the addition of a metabolizing system. In both experiments in the absence and presence of metabolic activation after 4 and 24 hours treatment with the test substance the values (0.2 - 1.5% micronucleated cells) were close to the concurrent vehicle control values (0.5 - 1.5% micronucleated cells) and clearly within our historical negative control data range (0.1 - 1.8% micronucleated cells) [see Table 5.8.1-39 and Table 5.8.1-40].

In the 1st experiment with S9 mix a statistically significant percentage of micronucleated cells was obtained when compared to the respective vehicle control value at an intermediate concentration of 1000 µg/mL (1.5% micronucleated cells). This significant difference was due to the low rate of micronucleated cells in the concurrent vehicle control group (0.5%). The value at 1000 µg/ml was clearly within the historical negative control data range and the increase was not dose-dependent, therefore, this finding was regarded as biologically irrelevant.

The positive control substances EMS (without S9 mix; 300 and 400 µg/mL) and CPP (with S9 mix; 1.0 µg/mL) induced statistically significant increased micronucleus frequencies in both independently performed experiments.

Growth inhibition indicated by reduced cell counts (RICC: -30.7%) was only observed in the 2nd experiment in the absence of S9 mix after 24 hours continuous treatment at the highest applied test substance concentration [see Table 5.8.1-39].

After 4 hours treatment in absence or presence of S9 mix, no reduced proliferative activity was observed at the test groups scored for cytogenetic damage. Contrary, in the 2nd experiment after 24 hours treatment in the absence of metabolic activation the proliferation index (CBPI) was decreased at 250 µg/mL and above.

In addition, in the 2nd experiment in the absence of S9 mix growth inhibition indicated by strongly reduced replicative index (RI) was observed at 500 µg/mL (32.2%). Cell morphology /attachment was only adversely influenced (grade > 2) in the 2nd experiment in the absence of S9 mix after 24 hours continuous treatment at the highest applied concentration of 2000 µg/mL.

In both experiments with and without metabolic activation, test substance precipitation was observed neither in the vehicle DMSO nor in culture medium. Osmolarity and pH values were not influenced by test substance treatment. The pH of the stock solutions was adjusted to a physiological value using small amounts of NaOH.

Table 5.8.1-39: Summary of results of micronucleus test with Reg.No. 78810 (Metabolite of BAS 500 F, pyraclostrobin)

					Genotoxicity	Cytotoxicity		
Exp.	Exposure/ Preparation interval	Test groups	S9 mix	Prec.*	Micronucleated cells** [%]	Proliferation index cytostasis (CBPI) [%]	Replicative index (RI) [%]	Relative increase in cell count (RICC) [%]
1	4/24 hrs	vehicle control[1]	-	n.d.	0.9	0.0	100.0	100.0
		62.5 µg/mL	-	-	n.d.	n.d.	n.d.	120.4
		125.0 µg/mL	-	-	n.d.	n.d.	n.d.	137.8
		250.0 µg/mL	-	-	n.d.	n.d.	n.d.	135.6
		500.0 µg/mL	-	-	1.0	-0.1	100.1	125.5
		1000.0 µg/mL	-	-	0.8	3.9	96.1	112.1
		2000.0 µg/mL	-	-	0.4	7.2	92.8	120.8
		positive control[2]	-	n.d.	2.5[S]	3.7	96.3	120.0
2	24/24 hrs	vehicle control[1]	-	n.d.	0.5	0.0	100.0	100.0
		62.5 µg/mL	-	-	n.d.	n.d.	n.d.	96.5
		125.0 µg/mL	-		0.2	11.5	88.5	98.6
		250.0 µg/mL	-	-	0.9	42.4	57.6	113.7
		500.0 µg/mL	-	-	0.3	67.7	32.3	120.8
		1000.0 µg/mL	-	-	0.3	46.7	53.3	120.9
		2000.0 µg/mL	-	-	n.d.	n.d.	n.d.	-30.7
		positive control[2]	-	n.d.	1.7[S]	4.9	95.1	134.9
		positive control[3]	-	n.d.	2.9[S]	6.0	94.0	146.4

* Precipitation in culture medium at the end of exposure period (macroscopic)
** Relative number of binucleated cells with micronuclei per 2000 cells scored per test group
S Frequency statistically significant higher than corresponding control values
n.d. Not determined
1 DMSO 1% (v/v)
2 EMS 300 µg/mL
3 EMS 400 µg/mL

Table 5.8.1-40: Summary of results of micronucleus test with Reg.No. 78810 (Metabolite of BAS 500 F, pyraclostrobin)

					Genotoxicity	Cytotoxicity		
Exp.	Exposure/ Preparation interval	Test groups	S9 mix	Prec.*	Micronucleated cells** [%]	Proliferation index cytostasis (CBPI) [%]	Replicative index (RI) [%]	Relative increase in cell count (RICC) [%]
1	4/24 hrs	vehicle control[1]	+	n.d.	0.5	0.0	100.0	100.0
		62.5 µg/mL	+	-	n.d.	n.d.	n.d.	77.8
		125.0 µg/mL	+	-	n.d.	n.d.	n.d.	98.8
		250.0 µg/mL	+	-	n.d.	n.d.	n.d.	86.5
		500.0 µg/mL	+	-	0.6	8.8	91.2	95.9
		1000.0 µg/mL	+	-	1.5[S]	4.6	95.4	69.7
		2000.0 µg/mL	+	-	0.6	5.5	94.5	86.7
		positive control[2]	+	n.d.	6.4[S]	50.2	49.8	88.4
2	4/44 hrs	vehicle conntrol[1]	+	n.d.	1.5	0.0	100.0	100.0
		125.0 µg/mL	+	-	n.d.	n.d.	n.d.	87.5
		250.0 µg/mL	+	-	n.d.	n.d.	n.d.	98.2
		500.0 µg/mL	+	-	1.3	3.1	96.9	86.0
		1000.0 µg/mL	+	-	0.6	-0.5	100.5	79.6
		2000.0 µg/mL	+	-	1.0	3.9	96.1	85.8
		positive control[2]	+	n.d.	13.8[S]	4.0	96.0	60.9

* Precipitation in culture medium at the end of exposure period (macroscopic)
** Relative number of binucleated cells with micronuclei per 2000 cells scored per test group
[S] Frequency statistically significant higher than corresponding control values
n.d. Not determined
n.e. Not evaluated due to strong toxicity
[1] DMSO 1% (v/v)
[2] CPP 1.0 µg/mL

III. CONCLUSION

Based on the results of the study it is concluded that 500M51 (Reg.No. 78810, metabolite of pyraclostrobin) does not induce micronuclei (clastogenic and/or aneugenic activity) under the in vitro conditions of this study.

Toxicological evaluation of 500M51 (Reg.No. 78810)

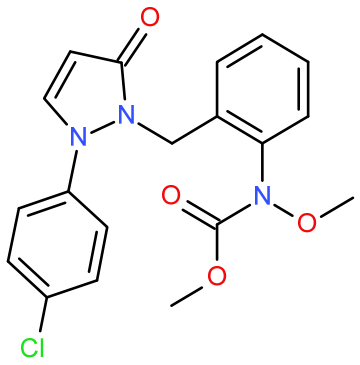
The QSAR evaluation of 500M51 is of low reliability and by weight of evidence there was no conclusive alert for genotoxity.

500M51 is considered to be not genotoxic based on the in vitro studies conducted. Therefore, 500M51 is considered to be **not toxicologically relevant**.

With regard to consumer exposure the TTC concept for a non-genotoxic compound was applied. Thus an ADI of 0.0015 mg/kg bw/day as well as an ARfD of 0.005 mg/kg bw/day was established. 500M51 which was identified in livestock metabolism only, accounts for 0.2% of the ARfD and has a negligible chronic exposure.

Group 6: Photometabolite 500M76

500M76 (other denominators: Reg.No. 413038, BF 500-14)



500M76 (Reg.No. 413038) is a photolysis metabolite found in surface water. The highest calculated concentration (PEC_{sw}) is 0.352 µg/L. In contrast to the other two surface water photolysis metabolites, 500M76 (Reg.No. 413038) is also found as a plant metabolite in wheat forage.

A) QSAR Predicitions for 500M76 (Reg.No. 413038)

OECD Toolbox (Version: 3.2) [see DocID 2014/1172955]
For 500M76 (Reg.No. 413038) the OECD toolbox does not indicate any alerts.

OASIS TIMES (MIX V.2.27.13 TB; Mutagenicity S-9 activated v08.08) [see molecule 4 of report DocID 2014/1172952 and 2014/1172953]

There were no Ames mutagenicity alerts for 500M76 (Reg.No. 413038) or in-silico generated metabolites. In all cases the structures were out of domain. The prediction in vitro chromosome aberration for 500M76 (Reg.No. 413038) was negative (out of domain). However, the CA prediction was positive for 1 out of 14 in-silico generated metabolites. The respective alert was ‘phenols’ (in domain).

Discussion: The alert ‘phenols’ identified for the in vitro chromosome aberration was also present for pyraclostrobin and metabolite 500M60 (Reg.No. 411847). Whereas the in vitro study with pyraclostrobin was negative, the in vitro study with 500M60 (Reg.No. 411847) - like that with 500M76 (Reg.No. 413038) - was positive. However, the in vitro results were not confirmed in the higher tier in vivo mouse micronucleus studies. Therefore, the weight of evidence indicates that 500M76 (Reg.No. 413038) is not genotoxic.

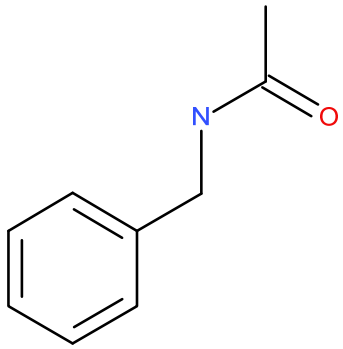
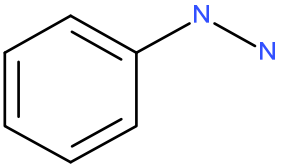
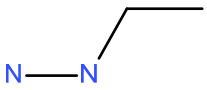
VEGA: Mutagenicity model (CAESAR, version 2.1.9) [see molecule 4 of report DocID 2014/1172954 for both VEGA models]

500M76 (Reg.No. 413038) is out of model applicability domain. The prediction is 'non mutagen'.

VEGA: Mutagenicity model (SarPy; version 1.0.4-BETA)

500M76 (Reg.No. 413038) is out of model applicability domain. The prediction is 'mutagen' with the structural alerts SA 47, SA 87, SA98 and SA 103. As discussed above, the alert SA 98 was also obtained for pyraclostrobin and a number of pyraclostrobin metabolites, which like 500M76 (Reg.No. 413038) were actually tested negative.

The substructures for the other alerts are depicted below. The predictions of the structural alerts SA 47, SA 87 and SA 103 are each based on 3 molecules of the training set which structurally were not very similar to 500M76 (Reg.No. 413038) as the similarity ranged from 0.515 to 0.637). For the alert SA 47 2 of the 3 predicted molecules were actually tested positive. This ratio was 1 of 3 for the alerts SA 87 and SA 103.

SA 47	SA 87	SA 103
		

Discussion: In view of the negative Ames test (see below) the prediction of gene mutation in bacteria is rejected.

DEREK (Version 2.0.0.201011301322; Knowledge base version: 11_20_05_2010)

The prediction for genotoxicity and neurotoxicity is negative.

Genotoxicity studies on 500M76 (Reg.No. 413038)

Study already submitted:

In the original submission an Ames test on Reg.No. 413038 was submitted and assessed (BASF DocID 2000/1000005). A standard plate test and a pre-incubation test with and without metabolic activation was performed at concentrations of 22 - 5500 µg/plate in S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 as well as in E. coli WP2 uvrA. A slight bacteriotoxicity was noted at 5500 µg/plate in the standard plate test and at concentrations ≥ 2750 µg/plate in the pre-incubation assay, while no precipitations were noted at any concentration. No increase of revertant colonies was observed in any of the tests performed.

New studies submitted in this dossier:

Report:	CA 5.8.1/34 Engelhardt G., Leibold E., 2003b In vitro gene mutation test with Reg.No. 413038 in CHO cells (HPRT locus assay) 2003/1004384
Guidelines:	OECD 476, EEC 2000/32 B.17
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

Reg. No. 413038 (Metabolite of BAS 500F; batch: 01586-236, purity: 94.5%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells. Two independent experiments were conducted in the presence and absence of metabolic activation at concentrations of up to 1000 µg/mL. The treatment interval in the absence and presence of metabolic activation was 4 h in both experiments. Ethylmethanesulfonate (EMS) and 3-methylcholanthrene (MCA) served as positive controls in the experiments without and with metabolic activation, respectively.

Cytotoxic effects indicated by reduced cloning efficiencies below 20% of the respective vehicle control were observed in the experiments without metabolic activation at concentrations ≥ 300 µg/mL and in the experiments with metabolic activation at 1000 µg/mL.

Neither in the original nor in the confirmatory experiments a relevant increase in the mutant frequency was observed. The positive control substances, however, induced a marked increase in mutant frequency.

Based on the results of the study, it is concluded that under the conditions of the test Reg. No. 413038 does not induce forward mutations in mammalian cells in vitro.

(BASF DocID 2003/1004384)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

	Reg. No. 413038 (Metabolite of BAS 500F)
Description:	Solid, lightbrown
Lot/Batch #:	01586-236
Purity:	94.5%
Stability of test compound:	Expiry date: November 2003. The homogeneity of the test substance was guaranteed by grinding before preparation of the test substance formulations. The stability of a comparable batch (01586-90) at room temperature in the vehicle DMSO over a period of 4 hours and in water over a period of 96 hours was verified analytically.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	1% (v/v) DMSO in culture medium
Positive control -S9:	Ethyl methanesulfonate (EMS) 300 µg/mL
Positive control +S9:	3-Methylcholantrene (MCA) 10 µg/mL

3. Activation:

S9 was produced from the livers of 5 induced male Wistar Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg Aroclor 1254 (as solution in corn oil with a concentration of 20 g/100 mL) per kg body weight. 5 days after administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction was thawed at room temperature and 1 volume of S9-fraction was mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	50 µM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	30 mM
$MgCl_2$	10 mM
$CaCl_2$	10 mM
S9	10%

4. Test organism:

Chinese hamster CHO cells (sub-strain K1). Stocks of the CHO cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).

5. Culture media:

Culture medium: Ham's F12 medium with L-glutamine and hypoxanthine supplemented with 10% (v/v) fetal calf serum (FCS).

Pretreatment medium: ("HAT" medium): FCS-supplemented Ham's F12 medium with L-glutamine containing per mL 13.6 µg hypoxanthine, 0.18 µg aminopterin and 3.88 µg thymidine.

Selection medium: ("TG" medium): L-Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL

All media were supplemented with
- 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL)
- 1% (v/v) amphotericine B (250 µg/mL)

During pulse exposure (4 hours) to the test substance, Ham‘s F12 medium was used without FCS supplementation. In the case of continuous treatment (24 hours) Ham‘s F12 medium with FCS supplementation was used.

6. Locus examined:

hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

7. Test concentrations:

a) Preliminary toxicity assay:	Nine concentrations ranging from 5 to 2000 µg/mL
b) Mutation assay:	
1st experiment:	12.5, 25.0, 50.0, 100.0, 200.0 and 400.0 µg/mL without and 62.5, 125.0, 250.0, 500.0, 750.0 and 1000.0 µg/mL with metabolic activation
2nd experiment	9.38, 18.75, 37.5, 75.0, 150.0 and 300.0 µg/mL without and 62.5, 125.0, 250.0, 500.0, 750.0 and 1000.0 µg/mL with metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 04-Nov-2002 to 20-Jan-2003

2. Preliminary cytotoxicity assay:

Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25-cm² flasks with 9 test substance concentrations (5 - 2000 µg/mL) in serum-free Ham's F12 medium for about 4 hours (with and without metabolic activation) after an attachment period of 24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS), covered with Ham's F12 and incubated for a further 5 - 7 days. After this incubation period, colonies were fixed, stained and counted. In addition to the cloning efficiency the following parameters were measured: pH, osmolality and the determination of precipitates (solubility).

3. Mutation Assay:

Pretreatment of Cells:	After thawing from the frozen stock, cells were seeded into flasks and incubated with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants.
Cell treatment:	For each test group, about $5x10^5$ cells per flask were seeded into 75 cm^2 flasks containing about 10 mL Ham's F12 medium supplemented with 10% FCS and incubated for about 24 hours with 5% CO_2 at 37°C and ≥ 90% humidity for cell attachment. 2 x 2 flasks were used for each test group.

After the cell attachment period, the medium was replaced by the fresh (treatment) medium. In case of experiments without metabolic activation the treatment medium consisted of 9.9 mL Ham's F12 medium without FCS plus 100 µL positive control or test substance. In case of metabolic activation the treatment medium consisted of 7.9 mL Ham's F12 medium without FCS, 100 µL positive control or test substance preparation and 2 mL S9-mix.

The vehicle controls with and without S-9 mix only contained the vehicle DMSO at the same concentration and volume used in the cultures treated with either test substance or positive control (1%).

Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix at 5% CO_2, 37°C and ≥ 90% humidity.

Expression:

After incubation for 4 hours, the treatment medium was replaced by 10 mL Ham's F12 medium with 10% FCS after having been rinsed twice with Hanks' balanced salt solution (HBSS). Subsequently, the flasks were incubated for another 17 - 24 hours; two flasks were pooled in each case and then subcultured (1^{st} passage). After two further passages (duration of the expression period is about 7-9 days), cells were transferred into selection medium (TG medium) at the 4^{th} passage.

Selection:

For the mutant selection, six 75-cm^2 flasks each were seeded with $3x10^5$ cells from each treatment group in selection medium (TG medium) and incubated for about 1 week. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.

Determination of Cytotoxicity:

Cloning efficiency 1 (survival):

The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25 cm^2 flasks using 5 mL Ham`s F12 medium with 10% FCS. After an approximately 24-hour attachment period, the cells were incubated with vehicle, test substance or the positive control for 4 hours. At the end of the treatment period the cells were washed twice with HBSS and the treatment medium was replaced by 5 mL Ham's F12 medium with 10% FCS. After a further incubation for 1 week the colonies were fixed, stained and counted.

Cloning efficiency 2 (viability):

The viability (cloning efficiency 2; CE_2) was determined in parallel to the selection of mutants. For each treatment group approx. 200 cells of each dose (cells pooled from 2 flasks) were taken in duplicate, seeded into Ham's F12 medium with 10% FCS (25-cm² flasks) and allowed to form colonies (incubator; 1 week). Afterwards, the colonies were fixed, stained and counted.

Calculations:

Mutant frequency:

Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{total\,number\,of\ mutant\ colonies}{number\,of\ seeded\,cells} x\,10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\,absolute}} x\,100$$

Cloning efficiency (CE, %)

absolute:

$$CE_{absolute} = \frac{total\,number\,of\,colonies\,in\,the\,test\,group}{total\,number\,of\,seeded\,cells\,in\,the\,test\,group} x100$$

relative, in comparison to control:

$$CE_{relative} = \frac{CE\,of\,the\,dose\,group}{CE\,of\,the\,vehicle\,control} x100$$

4. Statistics:

Due to the negative findings, a statistical evaluation was not carried out.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies ($MF_{corr.}$) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose-response relationship. Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose-response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

A test substance is generally considered negative in this test system if:

- The corrected mutation frequency ($MF_{corr.}$) in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance under storage conditions throughout the study period was guaranteed (Expiry date of the test item: November 2003). The stability of a comparable batch (01586-90) at room temperature in the vehicle DMSO over a period of 4 hours and in water over a period of 96 hours was verified analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY

In the preliminary experiment, a test substance induced cytotoxicity leading to a reduction of relative cloning efficiency below 20% was observed at ≥ 500 µg/mL in the absence of metabolic activation. With metabolic activation the relative cloning efficiency was markedly reduced at ≥ 1000 µg/ml.

Precipitation of the test substance was only observed in the experiment without metabolic activation at concentrations ≥ 1000 µg/mL. No marked effect on osmolality and pH were observed.
Based on these data the highest concentration tested in the mutagenicity experiments without and with metabolic activation was 400 and 1000 µg/mL, respectively.

C. MUTAGENICITY ASSAYS

No relevant increase in the number of mutant colonies was observed in the original and confirmatory experiments with and without metabolic activation [see Table 5.8.1-41 and Table 5.8.1-42]. The mutant frequencies obtained at any tested concentration with or without metabolic activation were within the range of the historical negative control data.

Cytotoxicity as determined by the relative cloning efficiency was decreased at concentrations > 200 µg/mL in the first and second experiment without metabolic activation. With metabolic activation cytotoxicity was observed at 1000 µg/mL in both experiments.

Treatment with the positive controls EMS and MCA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

The pH and osmolality of the tested concentrations were not altered at the concentrations tested. Test substance precipitation did not occur up the highest tested concentration of 1000 µg/mL.

Table 5.8.1-41: Reg.No. 413038 - Gene mutation in mammalian cells - 1st experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	5	1.39	1.62	93.7	100.0	86.9	100.0
Reg. No. 413038							
12.5 µg/mL	10	2.78	3.24	96.8	103.3	86.7	99.8
25.0 µg/mL	11	3.06	3.63	94.7	101.1	84.8	97.6
50.0 µg/mL	21	5.83	6.27	99.0	105.7	93.5	107.6
100.0 µg/mL	18	5.00	5.65	93.4	99.7	87.5	100.7
200.0 µg/mL	10	2.78	3.41	68.3	72.9	80.7	92.9
400.0 µg/mL	-	-	-	0.0	0.0	-	-
Positive control EMS							
300.0 µg/mL	812	225.56	253.11	76.7	81.9	88.2	101.5
With metabolic activation[1)]; 4-hour exposure period							
Vehicle (DMSO)	4	1.11	1.20	97.1	100.0	95.6	100.0
Reg. No. 413038							
62.5 µg/mL	2	0.56	0.54	93.8	96.6	96.8	101.3
125.0 µg/mL	22	6.11	7.12	98.9	101.9	87.4	91.4
250.0 µg/mL	4	1.11	1.28	98.9	101.9	83.2	87.0
500.0 µg/mL	5	1.39	1.55	90.9	93.6	93.4	97.7
750.0 µg/mL	8	2.22	2.36	59.0	60.8	92.8	97.1
1000.0 µg/mL	21	5.84	6.72	5.3	5.5	88.1	92.2
Positive control MCA							
10.0 µg/mL	619	171.95	223.63	85.9	88.5	77.9	81.5

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
- due to high toxicity the cultures were not continued
[1)] S-9 fraction : cofactors = 3 : 7

Table 5.8.1-42: Reg.No. 413038 - Gene mutation in mammalian cells - 2nd experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	Relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	11	3.06	3.38	98.4	100.0	90.6	100.0
Reg. No. 413038							
9.38 µg/mL	10	2.78	3.33	95.4	97.0	81.3	89.7
18.75 µg/mL	10	2.78	3.09	98.2	99.8	90.9	100.3
37.5 µg/mL	4	1.12	1.33	102.4	104.1	86.0	94.9
75.0 µg/mL	0	0.00	0.00	96.8	98.4	82.7	91.3
150.0 µg/mL	2	0.56	0.63	98.8	100.4	88.9	98.1
300.0 µg/mL	-	-	-	0.5	0.5	-	-
Positive control EMS							
300 µg/mL	762	211.67	286.23	98.1	99.7	73.9	81.6
With metabolic activation[1)]; 4-hour exposure period							
Vehicle (DMSO)	13	3.61	4.16	98.6	100.0	85.4	100.0
Reg. No. 413038							
62.5 µg/mL	2	0.56	0.62	102.3	103.8	87.9	102.9
125.0 µg/mL	0	0.00	0.00	99.5	100.9	77.5	90.7
250.0 µg/mL	3	0.84	1.06	97.7	99.1	77.8	91.1
500.0 µg/mL	1	0.28	0.37	87.4	88.6	79.2	92.7
750.0 µg/mL	12	3.34	4.23	80.7	81.8	80.9	94.7
1000.0 µg/mL	3	0.84	1.13	1.6	1.6	74.3	87.0
Positive control MCA							
10 µg/mL	259	71.95	94.92	96.8	98.2	75.8	88.8

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
- due to high toxicity the cultures were not continued
[1)] S-9 fraction : cofactors = 1 : 9

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test 500M76 (Reg. No. 413038, metabolite of pyraclostrobin) does not induce forward mutations in the HPRT locus in CHO cells in vitro.

Report:	CA 5.8.1/35 Schulz M., Landsiedel R., 2013b Reg.No. 413038 (Metabolite of BAS 500 F, Pyraclostrobin) - In vitro chromosome aberration assay in V79 cells 2012/1044766
Guidelines:	OECD 473, EEC 2000/32 B.10, EPA 870.5375, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.10
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 5.8.1/36 Schulz M., Landsiedel R., 2014g Amendment No. 1 - Reg.No. 413038 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro chromosome aberration assay in V79 cells 2014/1145892
Guidelines:	OECD 473, EEC 2000/32 B.10, EPA 870.5375, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.10
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

Reg.No. 413038 (metabolite of pyraclostrobin; batch: 01586-236, purity: 94.5%) was tested in vitro for the ability to induce chromosome and numerical aberrations in Chinese Hamster V79 cells in two independent experiments in the presence and absence of metabolic activation at concentrations of 62.5 µg/mL to 850 µg/mL with a pulse treatment of 4 hours. The cells were prepared 18 h post treatment-begin. Vehicle (DMSO) and positive controls (cyclophosphamide (CPP) and ethylmethanesulfonate (EMS) for the experiment with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system.

Strong cytotoxicity was found from concentrations of ≥ 600 µg/mL in experiments without metabolic activation and ≥ 800 µg/mL in the experiments with metabolic activation.

The test substance caused a statistically significant increase in the number of structurally aberrant metaphases incl. and excl. gaps after 4 hours treatment at 18-hour sampling time in the absence and presence of a metabolizing system. In the presence of metabolic activation, the increase was confirmed in an independently performed repeat experiment. No biologically relevant increase in the frequency of cells containing numerical chromosome aberrations was observed.
Based on the results of this study, Reg.No. 413038 is considered to have a clastogenic potential in vitro in Chinese hamster V79 cells in the presence or absence of metabolic activation.

(BASF DocID 2012/1044766)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 413038 (Metabolite of BAS 500 F, pyraclostrobin)

Description:	Solid, lightbrown
Lot/Batch #:	01586-236
Purity:	94.5%
Stability of test compound:	The test substance was stable over the study period under the storage conditions (Expiry date: 01-Nov-2003). The homogeneity of the test substance was guaranteed, by grinding before preparation of the test substance preparations. The stability of a comparable batch (01586-90) at room temperature in the vehicle DMSO over a period of 4 hours and in water over a period of 96 hours was verified analytically (BASF study code 40M0249/994128).
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	DMSO
Positive control, -S9:	Ethylmethanesulfonate 350 µg/mL
Positive control, +S9:	Cyclophosphamide 0.5 µg/mL

3. Activation: S9 was produced from the livers of 5 induced male Wistar Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg Aroclor 1254 (as solution in corn oil with a concentration of 20 g/100 mL) per kg body weight. 5 days after administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction was thawed at room temperature and 1 volume of S9-fraction was mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms: Chinese hamster V79 cells

5. Culture medium: MEM medium containing a L-glutamine source supplemented with
- 10% (v/v) fetal calf serum (FCS)
- 1% (v/v) penicillin/streptomycin (10000 IU/10000 µg/mL)
- 1% (v/v) amphotericine B (250 µg/mL)

During exposure to the test substance (only 4-hour treatment), MEM medium was used without FCS supplementation.

6. Test concentrations:

a) Preliminary toxicity assay: Eight to nine concentrations ranging from 5 to 4000 µg/mL were used in pretests for dose selection for the main experiments. V79 cells were prepared at a sampling time of 18 hours after 4 and 18 hours exposure time without metabolic activation and after 4 hours exposure time with metabolic activation.

b) Mutation assay:

1st experiment: 62.5, 125, 250, 500 and 750 µg/mL with and without metabolic activation (18 h preparation interval)

2nd experiment: 400, 450, 500, 550, 600 and 650 µg/mL without metabolic activation (18 h preparation interval)
600, 650, 700, 750, 800 and 850 µg/mL with metabolic activation (18 h preparation interval)

B. TEST PERFORMANCE:

1. Dates of experimental work: 25-Sep-2002 to 17-Jan-2003

2. Preliminary cytotoxicity assay:

A range-finding cytotoxicity test was conducted with V79 cultures exposed to test substance concentrations of 5 - 4000 µg/mL for 4 and 18 hours without metabolic activation and for 4 hours with metabolic activation, respectively. At the end of the exposure period, cell count, cell attachment, mitotic index and the quality of slides were determined in order to derive appropriate test substance concentrations for the main test.

3. Cytogenicity Assay:

Cell treatment:	Cells were exposed to the test substance, solvent or positive control in pulse treatment experiments for 4 hours with or without metabolic activation. The cells were incubated in Quadriperm® dishes at 37°C, 5% CO_2 and ≥ 90% humidity. Two chambers of a Quadriperm dish were used for each concentration (=duplicate cultures). The preparation interval was 18 h post treatment-begin. For determination of cytotoxicity, additional cell cultures (using 25 cm² plastic flasks) were treated in the same way as in the main experiment. Growth inhibition was estimated by comparing the cell number in the treated groups with the concurrent control.
Spindle inhibition:	100 µL colcemide (stock: 10 µg/mL phosphate buffered saline) was added to the cultures 2 - 3 hours prior to harvesting.
Cell harvest:	At the end of the incubation time the culture medium was completely removed. For hypotonic treatment, 5 mL of a 0.4% KCl solution (37°C) was added for about 20 minutes. The cells were fixed by addition of 5 mL methanol/glacial acetic acid (3:1 v/v). The fixative was changed twice.
Slide preparation:	The slides were removed from the Quadriperm chambers, briefly allowed to drip off and passed through a Bunsen burner flame. After drying, the cells were stained with Giemsa and Titrisol. After rinsing and clarifying in Xylene the cover slips were mounted in Corbit-Balsam.

Metaphase analysis:	Slides were coded prior to analysis. As a rule, the first 100 consecutive well spread metaphases of each culture were counted for all test groups, and if cells had 20 - 22 chromosomes, they were analyzed for structural chromosome aberrations. Numerical chromosome aberrations were also recorded. If there is a clear increase in chromosomally damaged cells, the number of metaphases to be analyzed can be reduced to at least 50 mitoses/test group. A mitotic index based on 1000 cells per culture was determined for all evaluated test groups in both experiments.

4. Statistics:

The proportion of metaphases with aberrations was calculated for each group.

A comparison of each dose group with the vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test was Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A statistically significant, dose-related, and reproducible increase in the number of structural chromosomal aberrations (excl. gaps).
- The proportion of aberrations (excl. gaps) exceeded both the concurrent negative control range and the historical negative control range.

A test substance is generally considered negative in this test system if:

- The number of cells with structural aberrations (excl. gaps) in the dose groups is not statistically significant increased above the concurrent vehicle control value and is within the historical negative control data range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance under storage conditions throughout the study period was proven. The stability of a comparable batch (01586-90) at room temperature in the vehicle DMSO over a period of 4 hours and in water over a period of 96 hours was verified analytically (BASF study code 40M0249/994128).

B. PRELIMINARY CYTOTOXICITY ASSAY:

A preliminary cytotoxicity assay was performed to determine appropriate test substance concentrations for the main test. A recduction of cell counts or mitotic indices below 50% of the control value were observed at concentrations ≥ 500 µg/mL in the absence and at condentrations ≥ 750 µg/mL in the presence of metabolic activation. Accordingly, 750 µg/mL with and without metabolic activation was selected as the top dose for the 1st experiment of the main test. 850 µg/mL with metabolic activation and 650 µg/mL without metabolic activation were selected as the top doses for the 2nd experiment of the main test.

C. CYTOGENICITY ASSAYS:

In this study, cells could be evaluated up to 600 µg/mL in the experiments without metabolic activation and up to 800 µg/mL in the experiments with metabolic activation. Higher concentrations were not scorable due to strong cytotoxicity and/or poor metaphase quality.

In the first experiment, a staticitcally significant increase in structural aberrant metaphase cells was observed after 4 hours of exposure in the absence of metabolic activation at a concentration of 500 µg/mL. This increase was not confirmed under the same conditions in the 2nd experiment. In the 1st experiment with metabolic activation a statistically significant increase of structural chromosome aberrations was noted at 750 µg/mL. In the 2nd experiment performed with metabolic activation, the aberration rates at 700 and 750 µg/mL were below the concurrent vehicle control, but at 800 µg/mL an increased aberration rate was observed, exceeding the vehicle control and the historical vehicle control data [see Table 5.8.1-43 to Table 5.8.1-46].

With regard to numerical chromosome abberations, an increase in endopolyploid cells of about 6.1% was observed in the 1st experiment after 4 hours of exposure without metabolic activation at 500 µg/mL and an increase of 2.4% at 750 µg/mL with metabolic activation. Whereas the former exceeded the negative historical control range the latter was well within the historical control. As these increases were not reproducible in the second experiment they were regarded as incidental and not biologically relevant.

The osmolarity and pH values were not relevantly influenced by the test substance treatment. Precipitation of the test substance did not occur up to the highest tested concentration. Vehicle and positive controls were all in a range to ensure the validity of the test.

III. CONCLUSION

500M76 (Reg.No. 413038, a metabolite of pyraclostrobin) is considered to have a clastogenic potential under in vitro conditions in V79 cells at least in the presence of metabolic activation. The situation without metabolic activation is unclear as the clastogenic activity observed after 4 hours could not be confirmed in the second experiment under the same conditions. In absence of an experiment with 18 hour exposure without metabolic activation a final assessment can not be conducted. Nonetheless, this does not affect the principal fact that Reg.No. 413038 has clastogenic activity in vitro.

The relevance of this in vitro result was evaluated in a higher tier in vivo study (see below) according to Commission Regulation (EU) No 283/2013, Section 5.4.2.

Table 5.8.1-43: Chromosome aberration test with Reg.No. 413038 without metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	11.2	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	B	100	10.8	2	2.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**11.0**	**3**	**1.5**	**3**	**1.5**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**
Reg.No. 413038																			
125 µg/mL	A	100	9.1	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	8.5	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**8.8**	**4**	**2.0**	**3**	**1.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
250 µg/mL	A	100	10.1	4	4.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0
	B	100	9.0	4	4.0	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	2.9
	A + B	**200**	**9.6**	**8**	**4.0**	**6**	**3.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**4**	**2.0**
500 µg/mL	A	100	9.5	6	6.0	5	5.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	6	5.7
	B	100	9.1	10	10.0	9	9.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	7	6.5
	A + B	**200**	**9.3**	**16**	**8.0****	**14**	**7.0***	**6**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**13**	**6.1**
Positive control EMS																			
350 µg/mL	A	50	11.5	7	14.0	7	14.0	3	6.0	1	2.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	11.0	8	16.0	8	16.0	4	8.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100**	**11.3**	**15**	**15.0****	**15**	**15.0****	**7**	**7.0****	**1**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)

Table 5.8.1-44: Chromosome aberration test with Reg.No. 413038 with metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	13.2	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	12.5	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**12.9**	**4**	**2.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Reg.No. 413038																			
250 µg/mL	A	100	12.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	2.0
	B	100	12.9	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**12.5**	**4**	**2.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**2**	**1.0**
500 µg/mL	A	100	8.7	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	11.7	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**10.2**	**5**	**2.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
750 µg/mL	A	100	7.0	8	8.0	7	7.0	3	3.0	0	0.0	0	0.0	1	1.0	1	1.0	2	1.9
	B	100	6.8	6	6.0	5	5.0	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	3	2.9
	A + B	**200**	**6.9**	**14**	**7.0***	**12**	**6.0****	**6**	**3.0***	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**1**	**0.5**	**5**	**2.4**
Positive control CPP																			
0.5 µg/mL	A	50	9.9	9	18.0	9	18.0	2	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	10.1	9	18.0	9	18.0	4	8.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100**	**10.0**	**18**	**18.0****	**18**	**18.0****	**6**	**6.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)

Table 5.8.1-45: Chromosome aberration test with Reg.No. 413038 without metabolic activation (18 hours treatment, harvest after 18 hours) - Experiment 2

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	10.7	2	2.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	B	100	13.1	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**11.9**	**3**	**1.5**	**3**	**1.5**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**
Reg.No. 413038																			
500 µg/mL	A	100	9.2	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	8.5	2	2.0	2	2.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**8.9**	**3**	**1.5**	**3**	**1.5**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
550 µg/mL	A	100	11.5	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	12.7	3	3.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**12.1**	**4**	**2.0**	**3**	**1.5**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
600 µg/mL	A	100	12.7	3	3.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	B	100	8.7	2	2.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	A + B	**200**	**10.7**	**5**	**2.5**	**4**	**2.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**2**	**1.0**	**0**	**0.0**
Positive control EMS																			
350.0 µg/mL	A	50	10.7	6	12.0	6	12.0	2	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	9.8	8	16.0	8	16.0	2	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100**	**10.3**	**14**	**14.0****	**14**	**14.0****	**4**	**4.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, ** $P \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)

Table 5.8.1-46: Chromosome aberration test with Reg.No. 413038 with metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 2

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	19.7	4	4.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	18.9	4	4.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**19.3**	**8**	**4.0**	**3**	**1.5**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Reg.No. 413038																			
700 µg/mL	A	100	10.2	1	1.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	2	2.0
	B	100	10.7	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**10.5**	**2**	**1.0**	**2**	**1.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**2**	**1.0**
750 µg/mL	A	100	15.1	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	10.1	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	A + B	**200**	**12.6**	**2**	**1.0**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**
800 µg/mL	A	100	14.9	8	8.0	6	6.0	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	12.9	2	2.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	C	100	12.1	10	10.0	9	9.0	5	5.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	D	100	13.5	11	11.0	9	9.0	5	5.0	0	0.0	0	0.0	0	0.0	1	1.0	1	1.0
	A + B	**400#**	**13.4**	**31**	**7.8**	**25**	**6.3***	**14**	**3.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.2**	**1**	**0.2**
Positive control CPP																			
0.5 µg/mL	A	50	8.9	8	16.0	8	16.0	3	6.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	8.1	8	16.0	8	16.0	5	10.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100**	**8.5**	**16**	**16.0****	**16**	**16.0****	**8**	**8.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

**: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#. Evaluation of a sample of 400 metaphases due to inhomogeneous data, when scoring a reduced sample

Report:	CA 5.8.1/37 [redacted] 2012e Reg.No. 413038 (Metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2012/1220183
Guidelines:	OECD 474 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 413038 (metabolite of BAS 500 F; batch: L83-122, purity: 99.0%) was tested for chromosomal damage (clastogenicity) and for the ability to induce spindle poison effects (aneugenic activity) in NMRI mice using the micronucleus test method. For this purpose, the test substance was administered once orally to groups of 5 male mice at dose levels of 125, 250 and 500 mg/kg body weight. The vehicle served as negative and cyclophosphamide and vincristine sulfate as positive controls. The animals were sacrificed 24 or 48 (additional high dose and vehicle group) hours after the administration and the bone marrow of the two femora was prepared and investigated for micronuclei.

The oral administration of Reg.No. 413038 did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing either small or large micronuclei. The rate of micronuclei was mostly close to the concurrent negative control and was within the range of the historical control data. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur. At the MTD (500 mg/kg body weight) lethality was observed in several animals. Due to a missing indication for a genotoxic effect induced by the test substance in any dose group this deviation from the guidelines is considered to have no detrimental impact on the validity of the study. It has to be assumed that the unexpected strong systemic toxicity in deviation to the pretest is due to the use of a highly purified batch of the test substance in the main experiment.

Both of the positive control chemicals, i.e. cyclophosphamide for clastogenic effects and vincristine for induction of spindle poison effects, led to the expected increase in the rate of polychromatic erythrocytes containing small (cyclophosphamide) or small and large (vincristine sulphate) micronuclei, thus demonstrating the sensitivity of the test system.

Reg.No. 413038 does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study, i.e. is devoid of clastogenic or aneugenic activity in vivo.

(BASF DocID 2012/1220183)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	Reg.No. 413038 (Metabolite of pyraclostrobin)
Lot/Batch #:	Solid, yellowish
Purity:	L83-122
Stability of test compound:	99.0% (tolerance ± 1.0%)
	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 Apr 2014 as indicated by the sponsor. Homogeneity of the test substance was guaranteed on account of the high purity and was ensured by mixing before test substance preparation.
Vehicle used:	DMSO and subsequent emulsion in corn oil

2. Control Materials:

Negative:	No negative control was employed in this study.
Vehicle control:	DMSO and subsequent emulsion in corn oil
Positive control:	Cyclophosphamide (CCP) 20 mg/kg for the determination of clastogenic effects Vincristine sulphate (VCR) 0.15 mg/kg for the determination aneugenic effects

3. Test animals:

Species:	Albino mice
Strain:	Crl:NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	5 - 8 weeks
Mean body weight at dosing:	30.0 g
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	Not indicated in the report
Micronucleus assay:	5 males/dose; due to the unexpected mortality at 500 mg/kg bw, each two additional animals were dosed for the 24 and 48 hour sacrifice, respectively.
Acclimation period:	At least 5 days
Diet:	Standardized pelleted feed (Maus/Ratte Haltung "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland)
Water:	Drinking water in bottles, ad libitum
Housing:	During the study the mice were housed individually in Makrolon cages, type MII

4. Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	frequency not indicated (fully air-conditioned rooms)
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	500, 750 and 1000 mg/kg (The pretests with 500 and 1000 mg/kg body weight were performed with a different batch (No. L83-78) with a reduced purity of only 92.1%.)
Micronucleus assay:	125, 250 and 500 mg/kg The test substance was administered once by oral gavage using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 30-Apr-2012 to 29-Aug-2012

2. Preliminary range finding test:

Male and female NMRI mice were treated once by oral gavage with a test substance dose of 500, 750 or 1000 mg/kg bw.

3. Micronucleus test:

Treatment and sampling:	Groups of 5 male mice were treated once with either the vehicle or 125, 250 or 500 mg test substance/kg bw by oral gavage. Additional test groups treated with the vehicle control and the high dose were used for the second sampling period. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substances CCP and VCR were administered once by oral gavage (CCP) or i.p. injection (VCR). The animals were observed for evident clinical signs of toxicity throughout the study.

Twenty-four hours after the administration the mice were sacrificed and the two femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 300xg for 5 minutes. The supernatant was discharged and the pellet resuspended in about 50 µl fresh FCS. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.

Slide preparation: One drop of the suspension was applied on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May Grünwald solution, rinsed, and finally stained with Giemsa solution (7.5%). After rinsing and clarifying in xylene, the preparations were mounted. The slides were coded prior to microscopic evaluation.

Slide evaluation: In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored.
The increase in the number of micronuclei in polychromatic erythrocytes of treated animals as compared to the vehicle control group provides an index of a chromosome-breaking (clastogenic) effect or of a spindle activity (aneugenic) of the substance tested.

In addition, the number of small micronuclei ($d < D/4$) and of large micronuclei ($d \geq D/4$) (d = diameter of micronucleus, D = cell diameter) was determined: The size of micronuclei may indicate the possible mode of action of the test substance, i.e. a clastogenic or a spindle poison effect.

The ratio of polychromatic to normochromatic erythrocytes was calculated. An alteration of this ratio indicates a toxic effect on erythropoieses and thus, that the test substance actually reached the target organ.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test (modified rank test according to Wilcoxon). Here, the relative frequencies of cells with micronuclei of each animal were used.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and significant increase in the number of micronucleated polychromatic erythrocytes was observed.
- The proportion of cells containing micronuclei exceeded both the values of the concurrent vehicle control range and the vehicle historical control range.

A test substance is generally considered negative in this test system if:

- There was no significant increase in the number of micronucleated polychromatic erythrocytes at any dose above concurrent control frequencies.
- The frequencies of cells containing micronuclei were within the historical control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle was verified analytically. The homogeneity of the test substance in the vehicle was guaranteed by constant stirring during the removal and administration of the test substance formulation and by analytical determination of 3 individual samples of each concentration. Due to a technical error in storage of the original samples, the retained samples were measured and reported. The mean test substance concentrations were determined as 13.7, 24.0, 50.2 and 51.7 mg/mL at nominal concentrations of 12.5, 25, 50 and 50 mg/mL, respectively (the latter 50 mg/mL value is for the dosing preparation used for the additional animals). These values correspond to 96% to 110% of the nominal cioncentration, which is within the expected range (90-110%). The preparations were homogenous as the relative standard deviations of the three individual samples were in the range of 1.0 to 0 6.2%. Only for the low concentration preparation the RDS was 15.1% indicating a lower homogeneity. However, as this was the low dose and no increase of micronucleated polychromatic erythrocytes was observed, this does not affect the validity of the study.

B. PRELIMINARY RANGE FINDING TEST

In the pretest in males and females, deaths were observed down to 750 mg/kg. At 500 mg/kg, all animals survived without signs of toxicity. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.

In contrast to the main study, the preliminary toxicity test was performed with batch L83-78 with a lower purity of 92.1%. The difference in toxicity observed at 500 mg/kg bw between the batches used for the pre-test and the main study may have been due to the higher purity of main study batch L83-122 (99.0% versus 92.1%).

C. MICRONUCLEUS ASSAY

Treatment of mice with Reg.No. 413038 did not lead to a biologically relevant increase in the rate of micronuclei (see Table 5.8.1-47). Micronucleus frequencies of treated animals were near to the concurrent vehicle control values and within the historical control range (0.3 to 3.0% for the vehicle olive/corn oil and 0.4 to 2.4% for the vehicle DMSO). The number of normochromatic or polychromatic erythrocytes containing small or large micronuclei did not deviate from the vehicle control value and was within the historical control range.

The PCE/NCE ratio was not affected by treatment with the test substance. Thus, there was no indication that erythropoiesis was inhibited.
The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE containing exclusively small micronuclei (16.0‰). The administration of the spindle poison vincristine resulted in an incidence of micronuclei in polychromatic erythrocytes of 30.7‰. This included 9.8‰ of PCE with large micronuclei. The positive controls thus demonstrated the sensitivity of the test system.

The administration of the test substance led to distinct clinical signs of toxicity. At 500 mg/kg body weight, three out of the ten animals died within 4 hours after test substance administration (2 animals of the 24 hour and 1 animal of the 48 hour sacrifice group). Therefore, additional four animals were treated with the top dose. However, further two animals died before reaching the respective sacrifice interval. As a consequence, there were only 4 instead of 5 animals for the determination of micronuclei for the 48 hour sacrifice group. In absence of an indication for a genotoxic effect in any dose group this deviation from the guideline is not considered to affect the validity of the study.

No signs of systemic toxicity were observed in any of the animals treated with the positive control substances or the vehicle.

The bioavailability of the test-item was determined by the analysis of blood samples withdrawn at sacrifice. In the 24 hours plasma samples the test item was quantifiable in 1/5 samples of the 250 mg/kg bw dose and in 5/5 samples of the 500 mg/kg bw dose group. In 4/5 samples of the 250 mg/kg bw group the test-item was detected, but the quantity was below the limit of quantification. Reg.No. 413038 was not detectable in samples from control animals, from the 125 mg/kg bw group and the 500 mg/kg bw 48 hour sacrifice group. The aforementioned analysis of the Reg.No. 413038 plasma concentration was performed with a method which was retrospectively validated. The validation report can be found in M-CA 4 (see BASF DocID 2016/1311551). As the bone marrow is highly perfused by blood, the plasma analysis revealed proof that Reg.No. 413038 reached its target tissue.

Table 5.8.1-47: Micronucleus test in mice administered Reg.No. 413038 by oral gavage

Treatment	PCE				NCE		PCE/NCE ratio
	scored	With MN			Number	With MN	
		total [‰]	small [‰]	large [‰]		[‰]	
DMSO emulsified in corn oil	10000	0.6	0.6	0.0	4140	0.7	2.4
Reg.No. 413038							
125 mg/kg	10000	1.6	1.6	0.0	4181	0.7	2.4
250 mg/kg	10000	0.8	0.8	0.0	4266	0.2	2.3
500 mg/kg	10000	1.9	1.8	0.1	4623	0.6	2.2
Positive controls							
Cyclophosphamide	10000	**16.0****	**16.0****	0.0	3793	0.5	2.6
Vincristine	10000	**30.7****	**20.9****	**9.8****	4824	0.8	2.1
48 h preparation interval							
DMSO emulsified in corn oil	10000	0.8	0.8	0.0	4468	0.2	2.2
Reg.No. 413038							
500 mg/kg	8000	0.9	0.9	0.0	3825	0.3	2.6

** $p \leq 0.01$ (Wilcoxon Test; one sided)
PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes; MN: micronuclei

III. CONCLUSION

Based on the result of this study 500M76 (Reg.No. 413038, metabolite of pyraclostrobin) does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study, i.e. is devoid of clastogenic or aneugenic activity in vivo. The analysis of Reg.No. 413038 in rat plasma demonstrated that the test item reached the target organ (bone marrow). Thus, the clastogenicity observed in vitro is not relevant under in vivo conditions.

Overall toxicological evaluation of 500M76 (Reg.No. 413038)

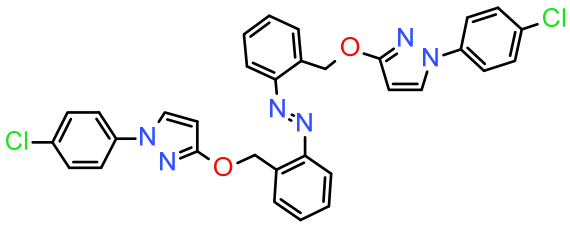
The QSAR evaluation of 500M76 is of low reliability and by weight of evidence there is no conclusive alert for genotoxicity.

By weight of evidence 500M76 is not genotoxic based on the in vitro and in vivo studies conducted. Therefore, 500M76 is considered to be **not toxicologically relevant**.

With regard to consumer exposure the TTC concept for non-genotoxic compounds was applied. Thus an ADI of 0.0015 mg/kg bw/day as well as an ARfD of 0.005 mg/kg bw/day was established. The estimated exposure (up to 15% of TTC of 0.005 mg/kg bw/day for acute and 0.2% of TTC 0.0015 mg/kg bw/day for chronic exposure) is low.

Metabolites identified in new metabolism studies

500M02 (other denominators: Reg.No. 369315, BF 500-7)



500M02 (Reg.No. 369315), a metabolite found in soil and sediment, was already evaluated during the previous Annex I listing process. It has been newly found in the comparative in vitro metabolism study (see M-CA 5.1).

A) QSAR Predicitions for 500M02 (Reg.No. 369315, BF 500-7)

OECD Toolbox (Version: 3.2) [see DocID 2014/1172955]

The OECD Toolbox revealed the alert '*AN2|AN2 >> Carbamoylation after isocyanate formation|AN2 >> Carbamoylation after isocyanate formation >> N-Hydroxylamines|Radical |Radical >> Radical mechanism via ROS formation (indirect)|Radical >> Radical mechanism via ROS formation (indirect) >> N-Hydroxylamines|SN1|SN1 >> Nucleophilic attack after metabolic nitrenium ion formation|SN1 >> Nucleophilic attack after metabolic nitrenium ion formation >> N-Hydroxylamines*' as DNA Alert for Ames, MN and CA. No protein binding alert was found.

Discussion: The identified alert is present in a number of metabolites which - like 500M02 (Reg.No. 369315) - were tested negative in Ames (Reg No 412785 - 500M62, 500M49 (Reg.No. 5916420)). In addition, 500M49 (Reg.No. 5916420) was in the mammalian gene mutation test. Therefore, the prediction does not adequately reflect the experimental database and the prediction is rejected.

OASIS TIMES (MIX V.2.27.13 TB; Mutagenicity S-9 activated v08.08) [see molecule 10 of report DocID 2014/1172952 and 2014/1172953]

The Ames mutagenicity alert for 500M02 (Reg.No. 369315) was negative (out of domain), however 1/5 in-silico generated metabolites had a positive alert (N-Hydroxylamines; in domoain). The in vitro CA alert for 500M02 (Reg.No. 369315) was negative (out of domain), however 5/6 in-silico generated metabolites had a positive alert with three different alerts (Phenols, Substituted Anilines, N-Hydroxylamines). All positive alerts were in domain.

Discussion: The alert identified for the Ames test is also present in 500M62 (Reg.No. 412785) and 500M49 (Reg.No. 5916420) which like 500M02 (Reg.No. 369315) tested negative in the Ames test. The alerts identified for the in vitro chromosome aberration were also present in pyraclostrobin (phenols) and metabolites 500M49 (Reg.No. 5916420; Substituted Anilines and N-Hydroxylamines), 500M60 (Reg.No. 411847) and 500M76 (Reg.No. 413038). The in vitro CA for pyraclostrobin and 500M49 (Reg.No.5916420) were negative. In the cases of 500M60 (Reg.No. 411847) and 500M76 (Reg.No. 413038) the positive in vitro CA was not confirmed in the negative higher tier in vivo mouse micronucleus test. Therefore, the weight of evidence indicates that 500M02 (Reg.No. 369315) does not cause chromosome aberrations. The positive alerts are therefore rejected.

VEGA: Mutagenicity model (CAESAR, version 2.1.9) [see molecule 10 of report BASF DocID 2014/1172954 for bothVEGA models]

500M02 (Reg.No. 369315) is out of model applicability domain. The prediction is ‘mutagen’ with the alert ‘Aromatic diazo’. The prediction is based on 6 molecules, of which only one was a predicted, but not an actual mutagen. All other five molecules were predicted non-mutagens, which was experimentally confirmed. The similarity of the molecules in the data set to 500M02 (Reg.No. 369315) is low as indicated by similarity factors of 0.536 to 0.593. Based on the negative Ames test (see CA 5.8.1/9) the positive prediction for genotoxicity does not reflect the experimental evidence. Therefore, the positive prediction is rejected.

VEGA: Mutagenicity model (SarPy; version 1.0.4-BETA)

500M02 (Reg.No. 369315) is out of model applicability domain. The prediction is ‘mutagen’ with the structural alert SA 96. As discussed above, this alert was also obtained for pyraclostrobin and a number of pyraclostrobin metabolites, which like 500M02 (Reg.No. 369315) were actually tested negative. The predicition is therefore rejected.

DEREK (Version 2.0.0.201011301322; Knowledge base version: 11_20_05_2010)

This metabolite was not assessed.

Genotoxicity studies on 500M02 (Reg.No. 369315, BF 500-7)

Report: CA 5.8.1/38
Engelhardt G., Hoffmann H.D., 1999a
Salmonella typhimurium/Escherichia coli reverse mutation assay (AMES standard plate test and prival preincubation test) with Reg.No. 369 315
1999/10736
Guidelines: OECD 471, EEC 92/69 B 13, EEC 92/69 B 14
GLP: no

Executive Summary

S. typhimurium and E. coli were exposed to Reg.No. 369315 (batch: 01185-022, purity: 99.9%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.

In the Ames standard plate test (SPT) Reg.No. 369315 was tested in concentrations of 20 to 5000 µg/plate with and without S9 mix (Aroclor-induced rat liver S9 mix). In the prival preincubation assay the test item was tested in concentrations of 4 to 2500 µg/plate only with metabolic activation (uninduced hamster liver S9 mix). As a deviation from the guidelines the pre-incubation test (PIT) was only performed with metabolic activation and the PIT lacked the use of E.coli WP2 uvrA.

Precipitation of the test substance was found from about 100 µg/plate onwards with and without S9 mix. A slight decrease in the number of revertants was occasionally observed in the preincubation assay depending on the strain from about 500 µg/plate onwards.

A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.

According to the results of the study, the test substance Reg.No. 369315 is not mutagenic in the Ames standard plate test and Prival preincubation test under the experimental conditions of the study.

(BASF DocID 1999/10736)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Reg.No. 369315

Description: Powder, orange

Lot/Batch #: 01185-022

Purity: 99.9%

Stability of test compound: The stability of the test substance throughout the study period has not been verified by reanalysis. The homogeneity of the test substance was guaranteed by mixing before preparation of the test substance formulations and on account of the high purity. The stability of the test substance in the vehicle DMSO has not been determined analytically.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Solvent/final concentration: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of rat metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

Positive control compounds tested with addition of hamster metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	10 µg/plate
TA 1535	2-aminoanthracene	DMSO	10 µg/plate
TA 1537	2-aminoanthracene	DMSO	10 µg/plate
TA 98	2-aminoanthracene	DMSO	10 µg/plate
	Congo red	DMSO	0.3µmol/plate
	Benzidine	DMSO	0.3µmol/plate

3. Activation:

S9 was produced from the livers of induced male Sprague-Dawley rats and from male Syrian hamsters. The rats received a single intraperitoneal injection of 500 mg/kg bw Aroclor 1254, while the hamsters remained untreated. Five days after administration the animals were sacrificed. The hamsters were not subjected to any inducing agent. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The rat liver S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

To demonstrate the efficacy of the rat liver S9 mix in this assay, the S9 batch was characterized with benzo(a)pyrene.

The hamster liver S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	20 mM
Glucose 6-phosphate dehydrogenase	2.8 units/mL
NADP	4 mM
NADH	2 mM
KCl	33 mM
$MgCl_2$	8 mM
FMN	2 mM
S9	10%

The efficacy of the hamster liver S9 mix was demonstrated by testing the two positive control articles Congo red and benzidine.

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid).
E. coli: E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay:

In the first experiment triplicate plates were prepared for each concentration (neg. control; 20, 100, 500, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without rat liver S9 mix) for all tester strains indicated above.

Prival preincubation assay:

In the second experiment the test article / vehicle / positive control substance, bacterial and hamster liver S9 mix were incubated at 30°C for the duration of about 30 minutes. Triplicate plates were prepared for each concentration (neg. control; 4, 20, 100, 500 and 2500 µg/plate and positive controls at the concentrations indicated above) for TA98, TA100, TA1535 and TA1537.

B. TEST PERFORMANCE:

1. Dates of experimental work: not given, finalization date: 09-June-1999

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates). In the experiments with E. coli the warm soft agar contains 0.5 mM tryptophan instead of histidine + biotin and the samples were poured onto minimal agar plates. After incubation at 37°C for 48 - 72 hours in the dark, the bacterial conolonies (his^+ revertants) are counted.

3. Prival preincubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix were incubated at 30°C for about 30 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37°C revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects, the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 hours at 37°C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in the vehicle DMSO and water was not verified analytically.

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

No bacteriotoxic effect was observed in the Ames standard plate test. In the Prival preincubtion assay a slight decrease in the number of revertants was occasionally observed depending on the strain from about 500 µg/plate onwards.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the Prival preincubation experiment with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested [see Table 5.8.1-48]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Test item precipitation was observed from 100 µg/plate onwards with and without S9 mix.

Table 5.8.1-48: Bacterial gene mutation assay with Reg.No. 369315- Mean number of revertants

Experiment 1: Standard plate test										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	36±5	23±2	108±7	109±6	21±2	18±2	12±2	8±2	39±1	30±3
Test item										
20 µg/plate	44±2	25±2	114±8	108±5	19±3	17±1	10±2	9±3	36±4	32±5
100 µg/plate	40±3[P]	21±2[P]	114±7[P]	99±9[P]	18±1[P]	18±2[P]	11±4[P]	8±2[P]	34±1[P]	26±2[P]
500 µg/plate	37±6[P]	19±2[P]	103±8[P]	111±9[P]	17±1[P]	18±1[P]	7±1[P]	7±1[P]	31±3[P]	26±3[P]
2500 µg/plate	31±2[P]	19±2[P]	94±10[P]	105±6[P]	17±2[P]	17±0[P]	3±1[P]	5±1[P]	27±5[P]	26±4[P]
5000 µg/plate	31±2[P]	19±2[P]	96±5[P]	96±5[P]	17±3[P]	15±3[P]	6±1[P]	6±2[P]	26±4[P]	25±2[P]
Pos. control[§]	1149±52	858±105	1205±66	1088±88	443±78	1614±170	116±12	1189±102	274±22	1225±75

Experiment 2: Prival preincubation assay				
Strain	TA 98	TA 100	TA 1535	TA 1537
Metabol. activation	+S9	+S9	+S9	+S9
Neg. control (DMSO)	45 ± 2	107 ± 3	18 ± 1	9 ± 2
Test item				
4 µg/plate	38 ± 6	114 ± 15	14 ± 3	10 ± 2
20 µg/plate	35 ± 7	112 ± 5	16 ± 1	9 ± 2
100 µg/plate	37 ± 6[P]	103 ± 8[P]	13 ± 1[P]	6 ± 2[P]
500 µg/plate	29 ± 6[P]	108 ± 6[P]	11 ± 1[P]	7 ± 1[P]
2500 µg/plate	25 ± 3[P]	109 ± 9[P]	6 ± 1[P]	4 ± 0[P]
Pos. control[§]	568 ± 56[A] 212 ± 40[B] 724 ± 32[C]	583 ± 89	187 ± 12	108 ± 22

[§] = Compound and concentrations see Material and Methods (I.A.2.) above
[A] 2-AA; [B] Congo Red; [C]Benzidine
[P] Precipitation

III. CONCLUSION

According to the results of the present study, the test substance 500M02 (Reg.No. 369315, BF 500-7) is not mutagenic in the Ames standard plate test and in the Prival preincubation test under the experimental conditions chosen here.

Based on some experimental deficits of the above study (no GLP status, no testing without metabolic activation in S. typhimurium and no testing of E.coli in the in the Prival preincubation assay), a second, fully guideline conform study was conducted.

Report:	CA 5.8.1/39 Woitkowiak C., 2016 a Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) - Salmonella typhimurium/Escherichia coli - Reverse mutation assay (prival modification) 2016/1200488
Guidelines:	OECD 471, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537, and a strain of *E. coli* WP2 uvrA were exposed with Reg.No. 369315 (metabolite of pyraclostrobin, batch: L85-192, purity: 98.3%) in the presence and absence of metabolic activation for 48 - 72 hours. Vehicle (DMSO) and positive controls were included in each experiment. In the Ames standard plate test (SPT) and the Prival pre-incubation test (pPIT), the test item was tested in triplicates of six concentrations in a range of 33 to 5000 µg/plate with and without S9 mix (phenobarbital/ß-naphthoflavone-induced rat liver S9 fraction (SPT) or uninduced hamster liver S9 fraction (pPIT)). The modified Bacterial Reverse Mutation Test according to Prival, in which flavin mononucleotide (FMN), liver S9 mix from uninduced hamsters and a pre-incubation step are used facilitates azo reduction and is therefore the most appropriate method for the investigation of azo-dyes and diazo compounds.

Test substance precipitation was found from about 333 µg/plate onward in SPT and from about 1000 µg/plate onward in pPIT with and without S9 mix.
No bacteriotoxic effect was observed up to the highest concentration tested with and without metabolic activation.
A relevant increase in the number of his^+ and trp^+ revertants was not observed in SPT and pPIT either without S9 mix or after the addition of a metabolising system. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system. The vehicle control induced number of revertants was within the range of the historical control data for each strain.

Based on the results of the present study, Reg.No. 369315 (Metabolite of pyraclostrobin) is not mutagenic in the Ames standard plate test and Prival pre-incubation test with and without metabolic activation under the experimental conditions chosen.

(BASF DocID 2016/1200488)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 369315 (metabolite of pyraclostrobin)

Description:	Solid, orange
Lot/Batch #:	L85-192
Purity:	98.3%
Stability of test compound:	The stability of the test substance is guaranteed until 01-May-2018 as indicated by the sponsor
Solvent used:	Dimethyl sulfoxide (DMSO) was used to suspend the test item. Homogeneity was ensured by shaking the preparation.

2. Control Materials:

Negative control:	In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).
Vehicle control:	The vehicle control with and without S9 mix only contained the solvent used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration:	100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Conc. [µg/plate]
TA 100	N-methyl-N'-nitroso-guanidine (MNNG)	DMSO	5
TA 1535	N-methyl-N'-nitroso-guanidine (MNNG)	DMSO	5
TA 1537	9-Aminoacridine (AAC)	DMSO	100
TA 98	4-nitro-o-phenylenediamine (NOPD)	DMSO	10
WP2 uvrA	4-Nitroguinoline-N-oxide (4-NQO)	DMSO	5

Positive control compounds tested with addition of rat metabolic activation system:

Strain	Mutagen	Solvent	Conc. [µg/plate]
TA 100	2-Aminoanthracene	DMSO	2.5
TA 1535	2-Aminoanthracene	DMSO	2.5
TA 1537	2-Aminoanthracene	DMSO	2.5
TA 98	2-Aminoanthracene	DMSO	2.5
WP2 uvrA	2-Aminoanthracene	DMSO	60

Positive control compounds tested with addition of hamster metabolic activation system:

Strain	Mutagen	Solvent	Conc. [µg/plate]
TA 100	2-Aminoanthracene	DMSO	10
TA 1535	2-Aminoanthracene	DMSO	10
TA 1537	2-Aminoanthracene	DMSO	10
TA 98	2-Aminoanthracene	DMSO	10
TA 98	Congo red	DMSO	210
WP2 uvrA	2-Aminoanthracene	DMSO	10

3. Activation:

Induced rat liver S9 fraction

S9 was produced from the livers of approximately 5 male Wistar rats (Crl:WI(Han); source: Charles River Laboratories Germany GmbH, Germany), weighting 200 – 300 g, that received 80 mg/kg bw phenobarbital i. p. and 80 mg/kg bw ß-naphthoflavone orally on 3 consecutive days.

The rat liver S9-mix was prepared freshly prior each experiment and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

Uninduced hamster liver S9 fraction

S9 was produced from the livers of approximately 10 male Syrian golden hamster (source: Charles River Laboratories Germany GmbH), 7 - 8 weeks old

The hamster liver S9-mix was prepared freshly prior each experiment and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	20 mM
Glucose 6-phosphate dehydrogenase	2.8 unit/mL
NADP	4 mM
NADH	2 mM
FMN (flavine mononucleotide)	2 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	30%

4. Test organisms: S. typhimurium strains: TA 98, TA 100, TA 1535, TA 1537
E. coli strains: WP2 uvrA
The bacterial strains are checked for the following characteristics at regular intervals: deep rough character (rfa), ampicillin resistance (R factor plasmid), and UV-light sensitivity (absence of uvrB and uvrA genes in Salmonella and E. coli strains, respectively).

Histidine and tryptophan auxotrophy was automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:
SPT/pPIT (± S9): 0, 33, 100, 333, 1000, 2500, 5000 µg/plate

B. TEST PERFORMANCE:

1. Dates of work: experimental dates: 01-Jun to 17-Jun-2016,
finalisation date: 13-Sep-2016

2. Standard Plate Test (SPT):
A mixture of 2 mL portions of warm soft agar (containing 0.5 mM L-histidine + 0.5 mM (+)-biotin (S. typhimurium) or 0.5 mM L-tryptophan (E. coli)), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL of phosphate buffer (in tests without metabolic activation) was poured onto minimal glucose agar plates. Each concentration and the controls were tested in triplicates. After incubation at 37°C for 48 - 72 hours in the dark, the bacterial colonies (his^+ or trp^+ revertants) were counted using an Image Analysis System. Colonies were counted manually, if precipitation of the test substance hindered the counting using the Image Analysis System.

3. Prival Pre-Incubation Test (pPIT):
The modified Bacterial Reverse Mutation Test according to Prival, in which flavin mononucleotide (FMN), liver S9 mix from uninduced hamsters and a pre-incubation step are used facilitates azo reduction and is therefore the most appropriate method for the investigation of azo-dyes and diazo compounds.
0.1 mL test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were incubated at 30°C for 30 minutes using a shaker. Subsequently, 2 mL soft agar (containing 0.5 mM L-histidine + 0.5 mM (+)-biotin (S. typhimurium) or 0.5 mM L-tryptophan (E. coli)) was added. After mixing, the samples were poured onto the Vogel-Bonner agar plates. Each concentration and the controls were tested in triplicates. After incubation at 37°C for 48 - 72 hours in the dark, the bacterial colonies (his^+ or trp^+ revertants) were counted as described above.

4. Statistics:
No special statistical tests were performed.

5. Acceptance and assessment criteria:

Generally, the experiment was considered valid if the following criteria were met:

- The number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain.
- The sterility controls revealed no indication of bacterial contamination.
- The positive control substances both with and without S9 mix induced a distinct increase in the number of revertant colonies within the range of the historical positive control data or above.
- Fresh bacterial culture containing approximately 10^9 cells per mL were used.

The test substance was considered positive in this assay if the following criteria were met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and E. coli WP2 uvrA) or tripling (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance was generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains were within the historical negative control data range under all experimental conditions in at least two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Stability of the test item in DMSO for a period of 4 hours at room temperature was verified analytically [BASF Study 01Y0277/15Y065].

B. TOXICITY

No bacteriotoxic effect (reduced his^- or trp^- background growth, decrease in the number of his^+ or trp^+ revertants) was observed in the SPT and in the pPIT up to the highest concentration tested.

Table 5.8.1-49: Bacterial gene mutation assay with Reg.No. 369315 - Mean number of revertants

Experiment 1: Standard plate test (SPT)										
Strain	**TA 100**		**TA 1535**		**TA 1537**		**TA 98**		**WP2 uvrA**	
Metabolic activation	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
DMSO (Vehicle)	98.0 ± 4.6	98.7 ± 6.4	11.0 ± 1.0	10.0 ± 1.0	6.0 ± 0.0	8.7 ± 1.5	21.7 ± 2.3	30.0 ± 1.7	25.7 ± 6.8	24.0 ± 4.6
Reg.No. 369315										
33 µg/plate	104.3 ± 5.5	93.0 ± 8.5	12.3 ± 2.5	10.7 ± 2.1	5.3 ± 3.2	14.7 ± 0.6	17.0 ± 7.0	29.3 ± 4.5	28.3 ± 10.0	22.3 ± 3.1
100 µg/plate	115.0 ± 21.0	91.7 ± 14.6	11.0 ± 3.0	9.0 ± 2.0	6.0 ± 2.0	10.0 ± 1.0	19.0 ± 4.6	34.7 ± 2.1	20.0 ± 1.0	26.3 ± 11.1
333 µg/plate	101.7 ± 8.1[P]	99.7 ± 10.2[P]	12.0 ± 1.7[P]	11.7 ± 2.9[P]	5.0 ± 1.0[P]	10.7 ± 0.6[P]	18.0 ± 3.0[P]	21.3 ± 0.6[P]	22.3 ± 4.5[P]	23.7 ± 5.5[P]
1000 µg/plate	107.3 ± 4.5[P]	100.0 ± 2.6[P]	10.3 ± 2.3[P]	10.7 ± 3.2[P]	5.7 ± 0.6[P]	9.0 ± 5.3[P]	19.7 ± 1.5[P]	28.3 ± 3.1[P]	25.7 ± 2.5[P]	25.0 ± 4.0[P]
2500 µg/plate	123.7 ± 6.7[P]	109.7 ± 12.0[P]	11.3 ± 1.2[P]	13.0 ± 2.0[P]	6.7 ± 0.6[P]	9.3 ± 1.2[P]	17.3 ± 3.1[P]	31.7 ± 2.9[P]	24.0 ± 3.6[P]	25.3 ± 4.2[P]
5000 µg/plate	97.7 ± 4.2[P]	78.0 ± 2.6[P]	12.0 ± 2.0[P]	10.3 ± 0.6[P]	10.3 ± 0.6[P]	8.3 ± 1.2[P]	20.3 ± 0.6[P]	28.3 ± 2.1[P]	22.7 ± 2.3[P]	24.0 ± 3.6[P]
Positive control										
Chemicals see [§]	3723.0 ± 19.7	2448.3 ± 228.3	4577.7 ± 382.2	181.7 ± 4.0	828.0 ± 24.3	181.3 ± 1.5	952.7 ± 39.6	2126.3 ± 166.2	1166.3 ± 51.6	125.7 ± 19.1
Experiment 2: Prival pre-incubation test (pPIT)										
Strain	**TA 100**		**TA 1535**		**TA 1537**		**TA 98**		**WP2 uvrA**	
Metabolic activation	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
DMSO (Vehicle)	127.7 ± 7.8	106.0 ± 12.2	10.3 ± 1.2	8.7 ± 1.2	7.7 ± 1.5	8.7 ± 2.1	17.7 ± 3.8	24.7 ± 5.7	20.3 ± 1.2	23.3 ± 4.9
Reg.No. 369315										
33 µg/plate	116.7 ± 5.8	108.7 ± 16.7	10.7 ± 4.0	11.7 ± 4.5	7.0 ± 1.0	10.0 ± 2.6	17.7 ± 2.9	28.7 ± 8.5	20.7 ± 10.6	23.0 ± 3.0
100 µg/plate	118.3 ± 10.0	111.3 ± 12.7	10.0 ± 3.6	11.0 ± 2.6	7.0 ± 1.0	8.3 ± 0.6	22.3 ± 8.3	31.0 ± 2.0	19.3 ± 5.9	18.3 ± 3.8
333 µg/plate	85.0 ± 15.5	113.7 ± 9.5	11.7 ± 3.1	13.0 ± 3.5	7.7 ± 1.5	8.3 ± 1.5	21.0 ± 9.2	25.7 ± 6.7	24.7 ± 3.5	22.0 ± 7.5
1000 µg/plate	104.0 ± 15.6[P]	114.3 ± 11.1[P]	9.0 ± 4.0[P]	9.7 ± 1.2[P]	6.3 ± 0.6[P]	6.3 ± 2.1[P]	18.7 ± 2.1[P]	31.3 ± 7.8[P]	18.7 ± 2.3[P]	25.3 ± 3.1[P]
2500 µg/plate	92.3 ± 8.5[P]	95.3 ± 11.2[P]	7.0 ± 1.0[P]	7.7 ± 1.5[P]	8.3 ± 3.1[P]	8.3 ± 1.5[P]	20.0 ± 2.6[P]	26.0 ± 6.9[P]	23.0 ± 2.0[P]	20.0 ± 2.6[P]
5000 µg/plate	97.7 ± 15.6[P]	127.7 ± 10.1[P]	9.7 ± 2.1[P]	11.0 ± 4.0[P]	6.3 ± 0.6[P]	10.0 ± 1.0[P]	14.0 ± 1.0[P]	27.0 ± 4.6[P]	17.3 ± 2.3[P]	21.7 ± 0.6[P]
Positive control										
2-Aminoanthracene [§]	1593.3 ± 91.6	765.0 ± 80.6	1633.0 ± 156.1	134.3 ± 22.9	2226.3 ± 343.6	102.7 ± 8.1	859.3 ± 105.3	830.7 ± 26.7	239.7 ± 119.7	1387.0 ± 96.8
Congo red								481.3 ± 50.9		

[§] = Compound and/or concentrations see Material and Methods (I.A.2.) above
P = precipitation

C. SOLUBILITY

Test substance precipitation was found from about 333 µg/plate onward in SPT and from about 1000 µg/plate onward in pPIT with and without S9 mix.

D. MUTATION ASSAYS

In the SPT and pPIT [see Table 5.8.1-49] experiments with and without metabolic activation no biologically relevant increase in number of his^+ or trp^+ revertants was observed in any strain tested.

III. CONCLUSION

Based on the results of the present study, Reg.No. 369315 (metabolite of pyraclostrobin) is not mutagenic in the Ames standard plate test and Prival pre-incubation test with and without metabolic activation under the experimental conditions chosen.

Report:	CA 5.8.1/40 Schulz M., Landsiedel R., 2016 a Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells (TK Locus assay, microwell version) 2016/1225056
Guidelines:	OECD 490 (2015), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17 No. L 142, EPA 870.5300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany

Executive Summary

Reg.No. 369315 (Batch: L85-192, purity: 98.3%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the TK locus in Mouse Lymphoma L5178Y cells. Two independent experiments were conducted in the presence 4-h incubation) or absence (4-h and 24-h incubation) of metabolic activation. In all cases two cultures were evaluated in parallel. Based on the results of a preliminary cytotoxicity assay concentrations of up to 150 µg/mL were used in the main experiment. Methylmethanesulfonate (MMS), 7,12-dimethylbenz[a]anthracene (DMBA) and Cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively.

No biologically relevant increase in the number of mutant colonies was observed in both experiments with and without metabolic activation. No relevant cytotoxicity as indicated by reduced relative total growth (RTG) of below 20% of control was observed in both experiments up to the highest concentration tested, which showed clear test substance precipitates in culture medium. The positive control mutagens (MMS, DMBA, and CPA) induced a distinct increase in mutant frequency, indicating that the tests were sensitive and valid.

Based on the results of the study it is concluded that under the conditions of this test Reg.No. 369315 (500M02; metabolite of pyraclostrobin) did not induce forward mutations and/or chromosomal aberrations in vitro in the mouse lymphoma assay with L5178Y $TK^{+/-}$ cells in the absence and presence of metabolic activation.

(BASF DocID 2016/1225056)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg. No. 369315 (Metabolite of BAS 500 F, Pyraclostrobin)

Description:	solid / orange
Lot/Batch #:	L85-192
Purity/content:	98.3% (tolerance ± 1.0%)
Stability of test compound:	The stability of the test item under storage conditions over the study period was guaranteed by the sponsor (expiry date May 01, 2018).
Vehicle:	DMSO (1% final concentration)

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	1% (v/v) DMSO in culture medium
Positive control -S9:	Methyl methanesulfonate (MMS) 15 and 5 µg/mL during the 4-h and 24-h exposure (dissolved in medium), respectively.
Positive control +S9:	Cyclophosphamide (CPP) 2.5 µg/mL (dissolved in medium); 7,12-dimethylbenz[a]anthracene (DMBA) 1 µg/mL (dissolved in DMSO)

3. Activation: S9 was produced from the livers of phenobarbital/β-naphthoflavone induced rats. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and an appropriate amount of S9-fraction is mixed with an equal volume of S9-cofactor solution. The co-factors in the S9-mix had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

4. Test organism:	L5178Y mouse lymphoma cells were used. They have a high proliferation rate (doubling time about 9-10 h), a high plating efficiency (~90%) and a stable karyotype with a near diploid (40 ± 1) chromosome number. Stocks of the L5178Y cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination, karyotype stability, and spontaneous mutant frequency. Prior to treatment, spontaneous TK-deficient mutants were eliminated from the stock cultures by growing the cells for 1 day in pre-treatment medium A and 3 days in pre-treatment medium B (see below).
5. Culture media:	
Culture medium (basis):	RPMI-0 (RPMI 1640 medium with 1% of 10000 IU/10000 µg/mL Penicillin/Streptomycin, 1% Sodium Pyruvate (10 mM))
Medium (treatment +S9):	RPMI-0 + 5% FCS (RPMI-5)
Medium (treatment -S9):	RPMI-0 + 10% FCS (RPMI-10)
Medium (CE/selection):	RPMI-0 + 20% FCS (RPMI-20)
Pre-treatment medium A:	("THMG" medium): RPMI-10 medium containing hypoxanthine (5 µg/mL), thymidine (3 µg/mL), methotrexate (0.1 µg/mL), and glycine (7.5 µg/mL).
Pre-treatment medium B:	("THG" medium): RPMI-10 medium containing hypoxanthine (5 µg/mL), thymidine (3 µg/mL), and glycine (7.5 µg/mL).
Selection medium:	RPMI-20 supplemented with 4 µg/mL trifluorothymidine (TFT).
6. Locus examined:	thymidine kinase (TK)
7. Test concentrations:	
a) Preliminary toxicity assay:	Nine concentrations ranging from 7.81 to 2000 µg/mL (±S9)
b) Mutation assay:	
1st experiment:	4.69, 9.38, 18.75, 37.5, 75, 150 µg/mL with and without metabolic activation (4-h exposure)
2nd experiment:	1.56, 3.13, 6.25, 12.5, 25, 50, 100 µg/mL without metabolic activation (24-h exposure)
2nd experiment:	3.13, 6.25, 12.5, 25, 50, 100 µg/mL with metabolic activation (4-h exposure)

B. TEST PERFORMANCE:

1. Dates of experimental work: 06-June-2016 to 16-Sep-2016

2. Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. Both, pH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation. Cells were treated for 4 h in the presence and absence of S9 mix with the test substance at concentrations from 7.81 to 2000 µg/mL. The relative suspension growth was determined.

3. Mutation Assay:

Pre-treatment of cells:
During the week prior to treatment $3x10^5$ cells were seeded into 75 cm^2-flasks and incubated for 1 day with "THMG" medium and for the following 3 days in "THG" medium to eliminate spontaneous TK-deficient mutants.

Cell treatment and expression period:
The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4 hours. The second experiment was performed with a treatment period of 24 hours in the absence of metabolic activation and a treatment period of 4 hours in the presence of metabolic activation.
For each test group, $1.5x10^7$ ($1x10^7$ during 24 h exposure) cells per flask (75 cm^2 flasks) suspended in 20 mL RPMI medium were exposed to various concentrations of the test item either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment), the test item was removed by centrifugation (173 x g, 5 min) and the cells were washed twice in RPMI-5 medium. From each culture a sample of treated cells (2 x 10^5 cells/mL or 6 x 10^6 cells/flask) was pipetted in 75 cm^2 flasks and was incubated for a 2-day expression period. To maintain exponential growth during this phase, each culture was counted daily and the cell numbers were adjusted at each day to 2 x 10^5 cells/mL in 30 mL RPMI-10 medium. The cell numbers were determined using a cell counter.

Seeding for selection
For the selection of the mutants, the cells were centrifuged and resuspended in 50 mL selection medium. Per culture 200 µL were dispensed in each well of two 96-well plates (2000 cells/well). After incubation for at least 9 days, both the number of negative wells and the number of wells containing small or large colonies were scored for calculation of the mutant frequency (MF).

Cloning efficiency

For determination of cytotoxicity, at the end of the treatment (Cloning efficiency 1 (survival)) and expression period (Cloning efficiency 2 (viability)) the cells were centrifuged resuspended. Per culture 200 µL were dispensed in each well of two 96-well plates (1.6 cells/well). After incubation for 9 - 11 days the plates were scored for empty wells.

For calculation of the relative suspension growth (RSG) and the relative total growth (RTG) the cell counts determined within the expression period at 2nd and 3rd passage after exposure in the case of 4-hour exposure and 1st, 2nd and 3rd passage after exposure in the case of 24-hour exposure were used.

Colony counting

The number of empty wells and the number of wells containing colonies were scored and reported. The colonies are classified into large colonies (indication of gene mutation) and small colonies (indication of chromosome breakage). Small colonies are defined as less than 1/4 of the diameter of the well. Size is the key factor and morphology (the optical density of the small colonies is considerably higher) should be secondary.

Calculations and processing of the data:

Cloning efficiency 1 (survival) and 2 (viability) was determined for each test group and given as absolute and relative cloning efficiency (CE_x and RCE_x, respectively).

$$CE_x = \frac{-\ln(\text{total number of empty wells} \div \text{total number of seeded wells (96)})}{\text{number of seeded cells per well (1.6)}} \times 100$$

$$RCE_x = \frac{CE_x \text{ of the test group}}{CE_x \text{ of the negative / vehicle control}} \times 100$$

The RTG is the standard measure of cytotoxicity. This measure includes the relative growth in suspension (RSG) during the expression period and the relative cloning efficiency (RCE_2; viability) at the time the mutants are selected.

For taking into account any loss of cells during the 4-hour treatment period a relative growth during treatment factor (RGDT, %) was calculated by comparing the growth of each treated culture relative to the control:

$$\text{RGDT} = \frac{\text{Cell count after 4 h of the test group}}{\text{Cell count after 4 h of the negative/v ehicle control}} \times 100$$

The total suspension growth (SG) and the RSG (in %) were calculated for each test group as follows:

Total suspension growth after 4 hour-exposure

$$SG = \frac{\text{Cell count after 24 h}}{2 \text{ x } 10^5 \text{ cells per mL}^{1,2}} \times \frac{\text{Cell count after 48 h}}{2 \text{ x } 10^5 \text{ cells per mL}^2} \times \frac{\text{RGDT}}{100}$$

Total suspension growth (SG) after 24 hour-exposure:

$$SG = \frac{\text{Cell count after 24 h}}{2 \times 10^5 \text{ cells per mL}} \times \frac{\text{Cell count after 48 h}}{2 \times 10^5 \text{ cells per mL}^2} \times \frac{\text{Cell count after 72 h}}{2 \times 10^5 \text{ cells per mL}^2}$$

$$\text{RSG} = \frac{\text{SG of the test group}}{\text{SG of the negative/vehicle control}} \times 100$$

$$RTG = \frac{RSG \times RCE_2}{100}$$

[1] Cell number seeded following 4-hour treatment
[2] If cell number was lower than 2 x 10^5 cells per mL all remaining cells were seeded
The uncorrected mutant frequency per 10^6 cells ($MF_{uncorr.}$) was calculated for each test group as follows:

$$MF_{uncorr} = \frac{-\ln(\text{total number of empty well s} \div \text{total number of seeded wells (96)})}{\text{number of seeded cells per well (2000)}} \times 10^6$$

The corrected mutant frequency (MF_{corr}) was calculated regarding the values of CE_2:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_2} \times 100$$

Determination of borderline mutant frequency based on GEF

The GEF (global evaluation factor) evaluation method requires that the MF exceeds a value based on the global distribution of the background MF of the test method. This value is defined as the mean of the negative/vehicle MF distribution plus one standard deviation. Based on a large data base (n = 493 experiments) from six laboratories a GEF of 126 mutant colonies per 10^6 cells (mean MF_{corr} = 99 x10^{-6} colonies; standard deviation = 27 x10^{-6} colonies) was calculated for the microwell method. To be judged positive the mutation frequency has to exceed a threshold of 126 colonies per 10^6 cells (GEF) above the concurrent negative/vehicle control value.

4. Statistics

An appropriate statistical method to test for linear trend (MS EXCEL function RGP; 10) was performed to assess a possible linear dose-relation in mutant frequencies. The dependent variable was the corrected mutant frequency and the independent variable was the concentration. A trend was judged as statistically significant whenever the one-sided p-value (probability value) was below 0.05 and the slope was greater than 0. However, both, biological and statistical significance has been considered together.

5. Acceptability criteria:

A mutation assay is considered acceptable if it meets the following criteria (the current recommendations of the IWGT are considered):

- The absolute cloning efficiency at the time of mutant selection (CE_2) of the solvent controls is 65 – 120%.
- The suspension growth (SG) of the negative/vehicle controls referring to the expression period following treatment should fall in the range of 8 - 32 for 4-hour exposure and 32 - 180 for 24-hour exposure.
- The range of the solvent control mutant frequency is in the range of 50 – 170 x 10^{-6} cells.
- The positive controls should yield an absolute increase in total mutant frequency (MF) that is an increase above the spontaneous background MF (an induced MF [IMF]) of at least 300 x 10^{-6} colonies. The small colony MF should account for at least 40% of that IMF, means a small colony IMF of at least 120 x 10^{-6} colonies. Alternatively, the positive controls should induce at least 150 x 10^{-6} small colonies above the spontaneous background MF. The upper limit of cytotoxicity observed in the positive controls should have a RTG that is greater than 10%.
- The highest applied concentration of the test substance should be 2 mg/mL, 2 µL/mL or 10 mM, unless limited by cytotoxicity or solubility of the test substance. If toxicity occurs, the highest concentration should lower the RTG to 10 to 20% of survival. If precipitation occurs, the highest evaluated concentration should be the lowest concentration where precipitation is observed by the unaided eye.

6. Evaluation criteria:

A test item is classified as mutagenic **if all** of the following criteria are met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells above the corresponding solvent control.
- Evidence of reproducibility of any increase in mutant frequencies, means the mutagenic response occurs at least in both parallel cultures of one experiment.
- A statistically significant dose-related increase in mutant frequencies using an appropriate tatistical trend test.

The test substance is considered non-mutagenic **if at least one** of the following criteria are met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells above the concurrent solvent control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistically significant dose-related increase in mutant frequencies using an appropriate statistical trend test.

However, in the evaluation of the test results the historical variability of the mutation rates in negative and vehicle controls (95% control limit) and the mutation rates of all negative and vehicle controls of this study were taken into consideration.
Results of test groups are generally rejected if the relative total growth is less than 10% of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects are indicated.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY

In the pretest the pH value was not influenced by the addition of the test substance preparation to the culture medium at the concentrations tested. Precipitation occurred at ≥ 62.5 µg/mL with and without metabolic activation. Neither with or without S9 a reduction of the relative suspension growth (RSG) below 20% was noted.

B. TREATMENT CONDITIONS

Osmolarity and pH values were not influenced by test substance treatment. The test substance was poorly soluble in the commonly used vehicles. DMSO was the most suitable one. Homogeneous suspensions were obtained at concentrations ≥ 37.5 µg/ml, while the test item was soluble in DMSA at ≤ 25 µg/ml. In the 1st Experiment, test substance precipitation in culture medium at the end of treatment was observed at 18.75 µg/mL and above with and without S9 mix. In the 2nd Experiment, test substance precipitation occurred at ≥ 25.0 µg/mL at all experimental conditions at the end of treatment.

C. CYTOTOXICITY

In both experiments the relative total growth (RTG) was above 20% and thus no considerable toxicity was noted. This is in agreement with the data of the preliminary toxicity test.

D. MUTAGENICITY ASSAYS

In this study, no relevant increase in the number of mutant colonies was observed either without S9 mix or after the addition of a metabolizing system [see Table 5.8.1-50 and Table 5.8.1-51]. The corrected mutant frequencies obtained for both experiments were always close to the respective vehicle control values and below the calculated threshold taking in consideration the Global Evaluation Factor (GEF).

The statistical analyses by testing for linear trend led to clearly negative findings for both experiments with and without S9 mix.

As expected, both positive control substances known to induce gene mutations, MMS (without S9 mix) and DMBA (with S9 mix), and the well-known clastogen CPP (with S9 mix) led to clearly increased mutant frequencies. The values of the corrected mutant frequencies exceeded the respective calculated thresholds for a mutagenic effect based on the GEF (126 plus the mutant frequency of the respective negative control). In addition, the corrected mutant frequencies were within the historical positive control data range (222.2 – 1490.7 per 10^6 cells; see Appendix 7 of the report).

Table 5.8.1-50: Gene mutation in mammalian cells - 1st experiment

Test group	MF_{corr} (per 10^6 cells)	Mean colony counts (%)		Toxicity data			Cloning efficiency (viability)	
		small	large	SG	RSG	RTG	absolute	relative
Without metabolic activation; 4-hour exposure period								
Vehicle (DMSO)	62.8	92	8	25.2	100.0	100	106.4	100
4.69	37.6	88	13	23.8	94.2	95.8	108.2	101.7
9.38	54.9	92	8	22.8	90.4	87.5	102.9	96.7
18.75	43.0	78	22	23.5	93.2	97.2	111.0	104.4
37.50	53.0	82	18	22.8	90.2	87.9	103.8	97.5
75.00	33.8	75	25	21.8	86.5	97.9	120.3	113.1
150.00	38.4	67	33	22.4	88.7	97.6	117.1	110.0
MMS 15 µg/mL	597.5	90	10	13.2	52.3	33.3	67.7	63.6
With metabolic activation; 4-hour exposure period								
Vehicle (DMSO)	54.8	92	8	9.2	100.0	100.0	119.2	100.0
4.69	51.5	82	18	7.6	82.7	70.2	101.2	84.9
9.38	40.4	89	11	8.1	87.6	86.8	118.1	99.1
18.75	52.2	85	15	7.9	85.7	89.8	125.0	104.8
37.50	40.9	89	11	7.6	82.0	82.7	120.3	100.9
75.00	51.6	73	27	7.4	80.7	77.9	115.0	96.4
150.00	57.6	86	14	8.8	94.9	98.5	123.8	103.8
CPP 2.5 µg/mL	393.4	95	5	7.2	78.4	53.7	81.6	68.5
DMBA 1 µg/mL	1089.1	77	23	4.4	47.6	16.3	40.8	34.2

Table 5.8.1-51: Gene mutation in mammalian cells – 2nd experiment

Test group	$MF_{corr.}$ (per 10^6 cells)	Mean colony counts (%)		Toxicity data			Cloning efficiency (viability)	
		small	large	SG	RSG	RTG	absolute	relative
Without metabolic activation; 24-hour exposure period								
Vehicle (DMSO)	54.2	91	9	68.7	100.0	100.0	98.8	100.0
1.56	59.6	92	8	68.9	100.3	103.6	102.1	103.3
3.13	51.7	92	8	64.4	93.8	103.5	109.1	110.4
6.25	29.7	88	13	63.7	92.7	119.6	127.4	128.9
12.5	46.9	90	10	62.0	90.2	101.3	111.0	112.3
25	38.7	78	22	62.4	90.8	106.6	116.0	117.4
50	36.3	75	25	56.5	82.3	93.2	112.0	113.3
100	52.9	92	8	56.8	82.7	96.2	115.0	116.4
MMS 5 µg/mL	740.0	92	8	44.4	64.7	44.3	67.7	68.5
With metabolic activation; 4-hour exposure period								
Vehicle (DMSO)	70.7	86	14	22.0	100.0	100.0	102.9	100.0
3.13	54.9	92	8	20.5	93.0	95.3	105.5	102.5
6.25	63.1	92	8	21.4	97.2	106.7	113.0	109.8
12.5	59.6	100	0	17.6	79.8	77.3	99.6	96.8
25	68.0	89	11	18.8	85.2	110.9	134.0	130.2
50	57.5	92	8	18.0	81.7	92.1	116.0	112.7
100	66.6	90	10	20.1	91.4	75.3	84.7	82.3
CPP 2.5 µg/mL	351.5	95	5	18.3	83.1	73.2	90.7	88.1
DMBA 1 µg/mL	745.3	91	9	12.8	58.1	38.2	67.7	65.8

III. CONCLUSION

Under the experimental conditions chosen here, Reg.No. 369315 (500M02; metabolite of pyraclostrobin) did not induce forward mutations and/or chromosomal aberrations in vitro in the mouse lymphoma assay with L5178Y $TK^{+/-}$ cells in the absence and presence of metabolic activation.

Report:	CA 5.8.1/41 Chang S., 2016 a Reg.No. 369315 (Metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in human lymphocytes in vitro 2016/1135652
Guidelines:	OECD 487 (2014), Commission Regulation (EU) No 640/2012
GLP:	yes (Laboratory certified by Hess. Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany)

Executive Summary

Reg.No. 369315 (metabolite of pyraclostrobin; batch: L85-192; purity: 98.3%) was tested for its potential to induce micronuclei in human lymphocytes *in vitro* in the absence and presence of metabolic activation by S9 mix. Two independent experiments were performed. In duplicate cultures the cells were incubated for 4 (±S9 mix) or 20 hours (-S9 mix) with the test substance at concentrations in the range of 2.9 to 2035 µg/mL. Three concentrations were evaluated, which were limited by the precipitation of the test item in the media. The vehicle DMSO (1.0% in culture medium) served as negative control, Mitomycin C (4 h) and Demecolcin (20 h) as positive controls in the absence of metabolic activation and Cyclophosphamide as positive control in the presence of metabolic activation. Treatments started after a 48-hour stimulation period with phytohemeagglutinine. Thereafter Cytochalasin B was added and the cultures were fixed and stained finally after another 20 hours. Cytokinesis-block proliferation index (CBPI) and cytostasis were determined in 1000 binucleated cells as cytotoxicity parameters. The number of micronucleated cells were determined in 2000 binucleated cells for evaluation of mutagenicity.

No cytotoxicity was observed up to the highest evaluated concentration, which showed precipitation in Experiment I at 13.2 µg/mL in the presence and absence of S9 mix and in Experiment II at 100 µg/mL in the absence of S9 mix and at 14.8 µg/mL and above in the presence of the S9 mix.
Independent of metabolic activation, no increase in the number of cells carrying micronuclei was observed in both experiments. The solvent control revealed values that were within the range of the laboratory historical negative control data. The positive control chemicals led to the expected increase in cells containing micronuclei that was within the range of the historical positive control data, thus demonstrating the sensitivity of the test system and the validity of the study.

In conclusion, under the experimental conditions Reg.No. 369315 (M50002; Metabolite of BAS 500 F (Pyraclostrobin)) did not induce the formation of micronuclei in human lymphocytes under in vitro conditions in presence and absence of metabolic activation.
(BASF DocID 2016/1135652)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 369315 (metabolite of pyraclostrobin)

Description:	Solid, orange
Lot/Batch #:	L85-192
Purity:	98.3%
Stability of test compound:	The stability of the test substance is guaranteed (expiry date 01-May-2018)
Solvent used:	DMSO

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	DMSO (1.0% final concentration in culture medium)
Positive control:	Without metabolic activation: Mitomycin C (MMC, 1.5 µg/mL; pulse treatment) dissolved in deionized water; Demecolcin (150 ng/mL; continuous treatment) dissolved in deionized water With metabolic activation: Cyclophosphamide (CPA, 17.5 µg/mL (Exp. I) or 15.0 µg/mL (Exp. II) dissolved in saline (0.9% NaCl))

3. Activation: S9 was produced from the livers of rats pretreated with β-naphthoflavone/phenobarbital. For testing, a sufficient amount of S9-fraction (protein content: 29.8 mg/mL) was thawed at room temperature and mixed with an appropriate volume of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

50 µL S9 mix per mL culture medium were used, yielding a 2.5% S9 fraction or final protein concentration of 0.75 mg/mL in each culture.

4. Test organism: Human peripheral blood lymphocytes

Donor(s):	Experiment I: 22 years old male, non-smoking, not medicated Experiment II: 26 years old male, non-smoking, not medicated

5. Culture media:

Culture medium:	Dulbecco´s Modified Eagles medium/Ham´s F12 (1:1) with GlutaMAX™ (200 mM) supplemented with 10% (v/v) fetal bovine serum (FBS), Pen/Strep (100 U/mL / 100 µg/mL), HEPES (10 mM), heparin (125 U.S.P.-U/mL), and phytohemeagglutinine (PHA, 3 µg/mL).

6. Test concentrations:

Micronucleus assay

Experiment I	
(4-h exposure, ±S9):	**4.3, 7.6, 13.2**, 23.1, 40.5, 70.8, 124, 217, 379.7, 664.5, 1163 and 2035 µg/mL; precipitation at ≥ 13.2 µg/ml (evaluated concentrations are indicated in bold)
Experiment II	
(4-h exposure, +S9):	2.9, 4.4, **6.6, 9.9, 14.8**, 22.2, 33.3, 100 µg/mL; precipitation at ≥ 14.8 µg/ml (evaluated concentrations are indicated in bold)
(20-h exposure, -S9):	2.9, 4.4, 6.6, 9.9, 14.8, **22.2, 33.3, 100** µg/mL; precipitation at 100 µg/ml (evaluated concentrations are indicated in bold)

B. TEST PERFORMANCE

1. Dates of experimental work: 08-Jun-2016 to 27-Jul-2016

2. Dose selection:

Dose selection was performed according to the current OECD Guideline for the in vitro micronucleus test. The highest test item concentration should be 2000 µg/mL, 2 µL/mL or 10 mM, whichever is the lowest. At least three test item concentrations should be evaluated for cytogenetic damage.

A preliminary cytotoxicity test was performed to determine the concentrations to be used in the main experiment. Cytotoxicity is characterized by the percentages of reduction in the CBPI in comparison with the controls (% cytostasis) by counting 500 cells per culture in duplicate. The experimental conditions in this pre-experimental phase were identical to those required and described below for the mutagenicity assay. The pre-test was performed with 12 concentrations of the test item separated by no more than a factor of √10 and a solvent and positive control. All cell cultures were set up in duplicate. Exposure time was 4 hours (± S9 mix). The preparation interval was 40 hours after start of the exposure.

With regard to the purity (98.3%) of the test item, 2035 µg/mL were applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations ranging from 4.3 to 2035 µg/mL (± S9 mix) were chosen for the evaluation of cytotoxicity. In the pre-test for toxicity, precipitation of the test item was observed at the end of treatment at 13.2 µg/mL and above in the absence and presence of S9 mix. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.

No cytotoxicity was observed in Experiment I after 4 hours treatment in the absence and presence of S9 mix. 100 µg/mL was chosen as top treatment concentration for Experiment II.

3. Micronucleus test:

Pulse exposure (±S9)

About 48 h after seeding, 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL culture medium was added. After 4 h the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were resuspended in and washed with saline. The washing procedure was repeated once as described. The cells were resuspended in complete culture medium with 10 % FBS (v/v) and cultured for a 16-hour recovery period. After this period Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Continuous exposure (-S9)

About 48 h after seeding 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with complete medium (with 10 % FBS) containing the test item. After 20 hours the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in and washed with saline. The washing procedure was repeated once as described. After washing the cells were re-suspended in complete culture medium containing 10 % FBS (v/v). Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Preparation of cells

The cultures were harvested by centrifugation 40 h after beginning of treatment. The cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in approximately 5 mL saline and spun down once again by centrifugation for 5 minutes. Then the cells were resuspended in 5 mL KCl solution (0.0375 M) and incubated at 37°C for 20 minutes. 1 mL of ice-cold fixative mixture of methanol and glacial acetic acid (19 parts plus 1 part, respectively) was added to the hypotonic solution and the cells were resuspended carefully. After removal of the solution by centrifugation the cells were resuspended for 2 x 20 minutes in fixative and kept cold. The slides were prepared by dropping the cell suspension in fresh fixative onto a clean microscope slide. The cells were stained with Giemsa.

4. Statistics:

Statistical significance was confirmed by using the Chi-squared test ($\alpha < 0.05$) using the validated R Script CHI2.Rnw for those values that indicated an increase in the number of cells with micronuclei compared to the concurrent solvent control.

5. Cytotoxicity evaluation:

Evaluation of cytotoxicity and cytogenetic damage

Evaluation of the slides was performed using microscopes with 40 x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The micronuclei had to be stained in the same way as the main nucleus. The area of the micronucleus should not extend the third part of the area of the main nucleus. At least 1000 binucleated cells per culture were evaluated for cytogenetic damage on coded slides. The frequency of micronucleated cells was reported as % micronucleated cells. To describe a cytotoxic effect, the CBPI (Cytokinesis-block proliferation index) was determined in 500 cells per culture and cytotoxicity is expressed as % cytostasis. A CBPI of 1 (all cells are mononucleate) is equivalent to 100 % cytostasis.

The CBPI was calculated as follows:

$$CBPI = \frac{(MONC \times 1) + (BINC \times 2) + (MUNC \times 3)}{n}$$

CBPI	Cytokinesis-block proliferation index
n	Total number of cells
MONC	Mononucleate cells
BINC	Binucleate cells
MUNC	Multinucleate cells

$$\text{Cytostasis \%} = 100 - 100\,[(CBPI_T - 1) / (CBPI_C - 1)]$$

T	Test item
C	Solvent control

6. Evaluation criteria:

Acceptability criteria:

- The rate of micronuclei in the solvent controls falls within the historical laboratory control data range.
- The rate of micronuclei in the positive controls is statistically significant increased.
- The quality of the slides must allow the evaluation of a sufficient number of analysable cells.

Evaluation criteria:

A test item can be classified as non-clastogenic and non-aneugenic if:

- the number of micronucleated cells in all evaluated dose groups is in the range of the historical laboratory control data and
- no statistically significant or concentration-related increase of the number of micronucleated cells is observed in comparison to the respective solvent control.

A test item can be classified as clastogenic and aneugenic if:

- the number of micronucleated cells is not in the range of the historical laboratory control data and
- either a concentration-related increase in three test groups or a statistically significant increase in the number of micronucleated cells is observed.

If the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

An increase in the number of micronucleated mononucleate cells may indicate that the test item has aneugenic potential.

II. RESULTS AND DISCUSSION

A. ANALYTICS

Stability of the test item in DMSO for a period of 4 hours at room temperature was verified analytically [see separate report 01Y0701/14Y117].

B. CYTOTOXICITY, PRECIPITATION AND OSMOLARITY

Precipitation of the test item in the culture medium was observed in Experiment I at 13.2 µg/mL and above in the absence and presence of S9 mix and in Experiment II at 100 µg/mL in the absence of S9 mix and at 14.8 µg/mL and above in the presence of S9 mix at the end of treatment.
No relevant influence on osmolarity or pH was observed. When using 1% DMSO as solvent the osmolarity is generally high, if compared to the physiological level of approximately 300 mOsm. This effect, however, is based on a final concentration of 1% DMSO in medium. As the osmolarity is measured by freezing point reduction, 1% of DMSO has a substantial impact on the determination of osmolarity. The outcome of the study is not affected by this impact.
In the absence and presence of S9 mix, no cytotoxicity was observed up to the highest evaluated concentration, which showed precipitation.

C. MICRONUCLEUS ASSAY

In this study, neither a statistically significant nor a biologically relevant increase in the number of micronucleated cells was observed after treatment with the test item [see Table 5.8.1-52].
The micronucleus rates of the cells after treatment with the test item were close to the range of the respective solvent control values and within the range of the laboratory historical control data (HCD). The HCD in the absence of S9 mix were 0.15-1.25% (95% control limits 0.02 to 1.15%) for (4h) pulse treatment and 0.05-1.43% (95% limits: 0.05 – 1.05%) for continuous (20h) treatment. The HCD in the presence of S9 mix were 0.15-1.35% (95% limits: 0.08 – 1.20%) for pulse treatment.

All positive controls used, i.e. Demecolcin (150 ng/mL), MMC (1.5 µg/mL) or CPA (17.5 or 15.0 µg/mL), resulted in distinct increases in cells with micronuclei (3.3 to 9.25%). These responses were within the range of the laboratory historical positive control data thus demonstrating the sensitivity and validity of the test system.

Table 5.8.1-52 Summary of results of the in vitro micronucleus test in human lymphocytes with Reg.No. 369315

Exp.	Exposure period [h]	Test item concentration [µg/mL]	Proliferation index CBPI	Cytostasis in %[a]	Micronucleated cells in %[b]
Without S9 mix					
I	4	Solvent control[1]	1.82		0.60
		Positive control[2]	1.84	n.c.	**9.25***
		4.3	1.79	4.0	0.75
		7.6	1.76	7.8	0.55
		13.2[P]	1.81	0.9	0.50
II	20	Solvent control[1]	1.91		0.65
		Positive control[3]	1.55	40.0	**5.15***
		22.2	1.80	12.4	0.30
		33.3	1.75	17.5	0.75
		100 [P]	1.70	23.0	0.70
With S9 mix					
I	4	Solvent control[1]	1.87		0.95
		Positive control[4]	1.61	29.7	**3.30***
		4.3	1.80	8.5	0.65
		7.6	1.83	5.4	0.65
		13.2 [P]	1.89	n.c.	1.08
II	4	Solvent control[1]	1.74		0.40
		Positive control[4]	1.44	40.2	**4.15***
		6.6	1.78	n.c	0.45
		9.9	1.84	n.c	0.45
		14.8 [P]	1.86	n.c	0.65

a: For the positive control groups and the test item treatment groups the values are related to the solvent controls
b: The number of micronucleated cells was determined in a sample of 2000 binucleated cells
c: The number of micronucleated cells was determined in a sample of 4000 binucleated cells
P: Precipitation occurred at the end of treatment by the unaided eye
CBPI: cytokinesis-block proliferation index
n. c. Not calculated as the CBPI is equal or higher than the solvent control value
*: The number of micronucleated cells is statistically significantly higher than corresponding control values ($p \leq 0.05$, Chi-squared test)
[1] DMSO 1.0% (v/v)
[2] MMC 1.5 µg/mL
[3] Demecolcin 150.0 ng/mL
[4] CPA 17.5 µg/mL
[5] CPA 15.0 µg/mL

III. CONCLUSION

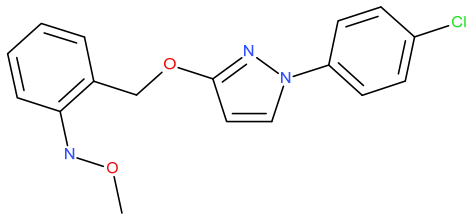
Under the experimental conditions of this study Reg.No. 369315 (500M02; metabolite of pyraclostrobin) did not induce micronuclei as determined by the in vitro micronucleus test in human lymphocytes when tested up to precipitating concentrations with and without metabolic activation.

Toxicological evaluation of 500M02 (Reg.No. 369315, BF 500-7)

By weight of evidence 500M02 is considered to be not genotoxic based on a set of three negative in vitro assays assessing the gene mutation in bacterial and mammalian cells as well as the effects on chromosomes. In conclusion 500M02 is considered to be **not toxicologically relevant**.

Based on the results of the in vitro comparison study, it cannot be excluded that metabolite 500M02 might be also formed in humans under in vivo conditions. Therefore, dietary exposure assessments have been performed (see M-CA 6.9).

500M106 (other denominator: Reg.No. 399379)



500M106 (Reg.No. 399379) is a metabolite newly found in the comparative in vitro metabolism study as well as in a new rat plasma study.

A) QSAR prediction for 500M106 (Reg.No. 399379)

OECD Toolbox (Version: 3.2) [see BASF DocID 2014/1172955]

In the OECD toolbox no DNA alerts for genotoxicity (Ames, MN, CA) or alerts for DNA or protein binding were observed for 500M106 (Reg.No. 399379). However, one of 3 in-silico generated metabolites generated two alerts: '*AN2|AN2 >> Michael-type addition, quinoid structures|AN2 >> Michael-type addition, quinoid structures >> Quinoneimines|Non-specific|Non-specific >> Incorporation into DNA/RNA, due to structural analogy with nucleoside bases |Non-specific >> Incorporation into DNA/RNA, due to structural analogy with nucleoside bases >> Specific Imine and Thione Derivatives|Radical|Radical >> Radical mechanism via ROS formation (indirect)|Radical >> Radical mechanism via ROS formation (indirect) >> Specific Imine and Thione Derivatives|Radical >> ROS formation after GSH depletion (indirect)|Radical >> ROS formation after GSH depletion (indirect) >> Quinoneimines|SN2|SN2 >> Nucleophilic substitution on diazonium ion|SN2 >> Nucleophilic substitution on diazonium ion >> Specific Imine and Thione Derivatives*' for genotoxicity (Ames, MN, CA) and DNA binding. For protein binding the alert was '*Michael Addition|Michael Addition >> Quinoide type compounds|Michael Addition >> Quinoide type compounds >> Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphthoquinone(s)/imines |Nucleophilic addition|Nucleophilic addition >> Addition to carbon-hetero double bonds|Nucleophilic addition >> Addition to carbon-hetero double bonds >> Ketones'*

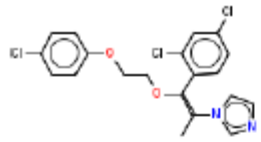
OASIS TIMES (MIX V.2.27.13 TB; Mutagenicity S-9 activated v08.08) [see molecule 9 of report BASF DocID 2014/1172952 and 2014/1172953]

The alert for Ames mutagenicity of 500M106 (Reg.No. 399379) was negative (out of domain). However, one out of 7 in-silico generated metabolites displayed a positive alert (Quinoneimines, specific imine and thione derivatives). It is interesting to note that in contrast to the vast majority of positive alerts observed in the course of the QSAR evaluation of pyraclostrobin and its metabolites, the positive metabolite was out of domain!

For in vitro chromosome aberration the situation was similar. The prediction for 500M106 (Reg.No. 399379) was negative (out of domain). This time 3 out of 7 in-silico metabolites were predicted positive - 2 metabolites with the alert 'phenols' (in domain!) and one metabolite (the same as above for the Ames prediction) with the alert 'Quinoneimines, specific imine and thione derivatives, alpha, beta-unsaturated' (Out of domain!). Whereas the latter alert was unique in the set of molecules evaluated here, the alert 'phenols' was also present for pyraclostrobin and metabolites 500M76 (Reg.No. 413038) and 500M60 (Reg.No. 411847). Whereas the in vitro study with pyraclostrobin was negative, the in vitro study with 500M76 (Reg.No. 413038) and 500M60 (Reg.No. 411847) was positive. However, the in vitro results were not confirmed in the negative higher tier in vivo mouse micronucleus studies. Therefore, the weight of evidence at least for this alert indicates that 500M106 (Reg.No. 399379) is not genotoxic.

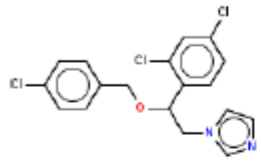
VEGA: Mutagenicity model (CAESAR, version 2.1.9) [see molecule 9 of report BASF DocID 2014/1172954 for both VEGA models]

The prediction for 500M106 (Reg.No. 399379) is mutagen (out of domain) with no specific alert information. Of the 6 molecules with the highest structural similarity (0.561 to 0.657) the predicted mutagenicity was only confirmed for the two least similar molecules. As visible from the structures depicted below, the similarity to 500M106 (Reg.No. 399379) is low.



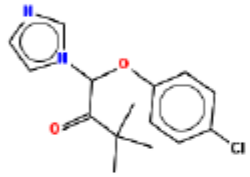
CAS: 74512-12-2
Dataset id: 1714 (training set)
SMILES: n1ccn(c1)C(=C(OCCOc2ccc(cc2)Cl)c3ccc(cc3Cl)Cl)C
Similarity: 0.657

Experimental value: NON-Mutagen
Predicted value: NON-Mutagen



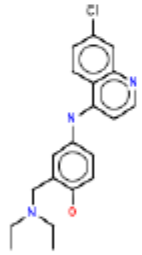
CAS: 24169-02-6
Dataset id: 4222 (test set)
SMILES: n1ccn(c1)CC(OCc2ccc(cc2)Cl)c3ccc(cc3Cl)Cl
Similarity: 0.606

Experimental value: NON-Mutagen
Predicted value: NON-Mutagen



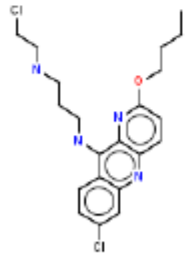
CAS: 38083-17-9
Dataset id: 1209 (training set)
SMILES: O=C(C(Oc1ccc(cc1)Cl)n2cncc2)C(C)(C)C
Similarity: 0.571

Experimental value: NON-Mutagen
Predicted value: NON-Mutagen



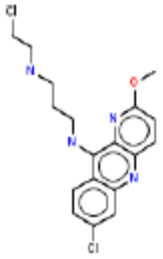
CAS: 86-42-0
Dataset id: 3822 (test set)
SMILES: Oc1ccc(cc1CN(CC)CC)Nc3ccnc2cc(ccc23)Cl
Similarity: 0.567

Experimental value: NON-Mutagen
Predicted value: Mutagen



CAS: 38915-38-7
Dataset id: 2484 (training set)
SMILES: n2c1ccc(nc1c(NCCCNCCCl)c3ccc(cc23)Cl)OCCCC
Similarity: 0.563

Experimental value: Mutagen
Predicted value: Mutagen



CAS: 36167-69-8
Dataset id: 2953 (training set)
SMILES: n2c1ccc(nc1c(NCCCNCCCl)c3ccc(cc23)Cl)OC
Similarity: 0.561

Experimental value: Mutagen
Predicted value: Mutagen

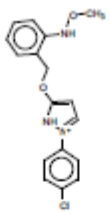
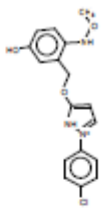
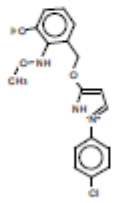
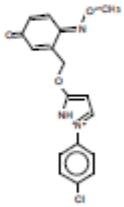
VEGA: Mutagenicity model (SarPy; version 1.0.4-BETA)

The prediction for 500M106 (Reg.No. 399379) is 'mutagen' with the structural alert SA 96. The prediction is based on the same molecules as described above for the CAESAR prediction model.
As discussed above, this alert was also obtained for pyraclostrobin and a number of pyraclostrobin metabolites, which were actually tested negative.

DEREK (Version 2.0.0.201011301322; Knowledge base version: 11_20_05_2010)

The molecule was not evaluated in this model.

Discussion: The prediction of genotoxicity in the OECD Toolbox and in OASIS Times is solely based on the same in-silico predicted metabolite (see 9.3 below) with well comparable alerts in both QSAR models. This is not surprising as the underlying algorithms were developed by the same scientists. The additional 4 metabolites in OASIS Times are simply glucuronic and gluthatione conjugates of metabolites 9.1 and 9.2. Obviously, the Phase II metabolite reactions are not implemented in the OECD Toolbox.

9.0 Parent 	9.1 Metabolite 
Predicted Ames Mutagenicity in vitro Ames negative **Alert info** **ModelReliability** **Total Domain** Out of Domain	**Predicted Ames Mutagenicity** in vitro Ames negative **Alert info** **ModelReliability** **Total Domain** N/A
9.2 Metabolite 	9.3 Metabolite 
Predicted Ames Mutagenicity in vitro Ames negative **Alert info** **ModelReliability** **Total Domain** N/A	**Predicted Ames Mutagenicity** in vitro Ames positive **Alert info** Quinoneimines,Specific Imine and Thione Derivatives **ModelReliability** High, >= 60% (n>=10) **Total Domain** Out of Domain

It is worth to note that it was impossible to actually find any half way similar reference compound with the respective structure in the OECD Toolbox, which has experimental data underpinning the alert. The only reference compound with experimental data was pyraclostrobin itself. The value of this alert is therefore highly questionable.

B) Subchronic toxicity of 500M106 (Reg.No. 399379)

Report:	CA 5.8.1/42 [redacted] 2017 a Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Repeated-dose 28-day oral toxicity study in Wistar rats - Administration by gavage 2016/1288407
Guidelines:	OECD 407, OECD 408, EPA 870.3100, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.26
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Please note: This study was essentially conducted according to OECD 408, but the treatment period was only 28-days. Thus, it is an OECD 407 study with 10 animals/sex/dose enhanced by the additional parameters investigated in OECD 408.

Executive Summary

Administration of Reg.No. 399379 (metabolite of pyraclostrobin; batch L87-218; purity: 97.7%) to Wistar rats at dose levels of 0, 100, 300 and 1000 mg/kg bw/day for 28-days resulted in adverse signs of systemic toxicity at 1000 mg/kg bw/day in both sexes.

Treatment with Reg.No. 399379 did not affect the survival, body weight development or food consumption, FOB, clinical chemistry or urinalysis parameters. Treatment related effects at the high dose (1000 mg/kg) consisted of a regenerative normochromic-normocytic anemia characterized by decreased erythrocyte counts (RBC), hemoglobin (HGB) and hematocrit (HCT) in males and increased absolute reticulocyte counts in both sexes, increased duodenum weights in males without a histopathological correlate, increased absolute and relative liver weights in males accompanied by a minimal centrilobular hypertrophy of hepatocytes and extramedullary hematopoiesis in the spleen of both sexes. Only the anemia and the accompanying extramedullary hematopoiesis were considered to represent adverse findings.

In high dose males a slight regenerative normochromic-macrocytic anemia was noted. This was accompanied by an adaptive increase of extramedullary hematopoiesis in the spleen of high dose males. Finally, high dose males displayed some changes of clinical chemistry parameters, i.e. decreased total protein and globulin as well as increased triglyceride levels.

Under the conditions of the present study the no observed adverse effect level (NOAEL) in both sexes was 300 mg/kg bw/day.

(BASF DocID 2016/1288407)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	Reg.No. 399379 (metabolite of pyraclostrobin)
	Description:	Solid, orange
	Lot/Batch #:	L85-218
	Purity:	97.7% (tolerance ± 1.0%)
	Stability of test compound:	The test substance was stable over the study period until 01 Aug 2018
2.	**Vehicle and/or positive control:**	0.5% aqueous carboxymethylcellulose
3.	**Test animals:**	
	Species:	Rat
	Strain:	Crl:WI (Han)
	Sex:	Male and female
	Age:	35 ± 1 day at delivery
	Weight at dosing:	means: males: 159.6 ± 7.1 g, females: 127.7 ± 6.4 g
	Source:	Charles River Laboratories, Sulzfeld, Germany
	Acclimation period:	7 days
	Diet:	Kliba maintenance diet mouse/rats "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
	Water:	Drinking water from water bottles, ad libitum
	Housing:	Group housing (5 animals/cage) in type 2000P poly-sulfonate cages (TECNIPLAST, Hohenpeißenberg, Germany)
		floor area about 2065°cm²; bedding: wooden dust free bedding; enrichment: wooden gnawing blocks type NGM E-022 (Abedd® Lab. and Vet. Service GmbH, Austria)
	Environmental conditions:	
	Temperature:	20 - 24°C (central air-conditioning)
	Humidity:	30 - 70% (central air-conditioning)
	Air changes:	15 per hour
	Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

7. Dates of experimental work: 16-Aug-2016 to 21-Dec-2016
Inlife dates:
23-Aug-2016 (start of administration) to
22-Sep-2016 (necropsy of last female animals)

8. Animal assignment and treatment:

Reg.No. 399379 was administered to groups of 10 male and 10 female Wistar rats by gavage at target concentrations of 0, 100, 300 and 1000 mg/kg bw/day for 28 days.

The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights. At the end of the administration period the animals were sacrificed after a fasting period of at least 16 hours.

9. Test item preparation and analysis:

The test item was administered as suspension in 0.5% aqueous CMC. Appropriate amounts of the test item were weighed and subsequently filled up with the vehicle. The suspension was prepared by mixing with an Ultraturrax and was kept homogenous by stirring with a magnetic stirrer. Suspensions were prepared weekly.

The stability of Reg.No. 399379 in suspension at room temperature for a period of 7 days was demonstrated before the start of the study (BASF project No. 01Y0701/14Y119; copy of the report included in Volume III of the report).

The homogenous distribution of Reg.No. 399379 in the diet was verified for the highest and lowest concentration (see below) using the first suspension preparation. Additionally, the test-item concentration was determined at the mid dose. These analyses were performed with a validated method (see M-CA 4; BASF DocID 2016/1230128).

Analysis of diet preparations for homogeneity and test-item content

Nominal concentration [g/100 ml]	Sampling	Concentration measured [g/100 ml] Mean ± SD#	Relative standard deviation [%]	% of nominal concentration
1.0	23.08.2016	0.895 ±0.031	3.4	89.5
3.0	23.08.2016	2.516		83.9
		2.583 (R)		86.1
10.0	23.08.2016	9.293 ±0.135	1.4	92.9

(R) Reserve sample
Values may not calculate exactly due to rounding of values

As indicated by relative standard deviations of 1.4 to 3.4%, the prepared dosing suspensions were homogenous. The test-item content at the low and high concentration was within the acceptable range of 90 to 110%. The concentration at the mid dose was slightly below this range.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
Body weight, body weight change	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
Rearing, grip strength forelimbs, grip strength hind limbs, foots play test, motor activity	Non-parametric one-way analysis using KRUSKALWALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON test (two-sided) for the equal medians

Statistics of clinical pathology

Parameter	Statistical test
Blood parameters	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians
Urinalysis parameters (apart from pH, volume, specific gravity, color and turbidity)	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians. In case of exactly the same numbers of the dose group and the control, no statistical test is performed.
Urine pH, volume, specific gravity	Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians.
Urine color and turbidity	Urine color and turbidity are not evaluated statistically.

Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test (two-sided) for the equal medians

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. If animals were in a moribund state, they were sacrificed and necropsied.
All animals were checked daily for any abnormal clinically signs before the administration as well as within 2 hours and within 5 hours after the administration.
Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this, the animals were transferred to a standard arena (50 x 37.5 cm; sides 25 cm high). The findings were ranked according to the degree of severity, if applicable. The following parameters were examined:

1. abnormal behavior during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. feces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

2. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0) and at weekly intervals thereafter. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.

3. Food consumption

Food consumption was determined weekly and calculated as mean food consumption in grams per animal and day.

4. Water consumption:

Drinking water consumption was monitored by daily visual inspection of the water bottles for any changes in volume.

5. Ophthalmoscopy:

The eyes of all animals were examined prior to the start of the administration period. At the end of the administration period, i.e. study day 27, the eyes of animals in test groups 0 (control) and 3 (1000 mg/kg bw/d) were examined for any changes using an ophthalmoscope (HEINE OPTOTECHNIK, Herrsching, Germany) after application of a mydriatic (Mydrum, Chauvin ankerpharm GmbH, Rudolstadt, Germany).

6. Functional observation battery (FOB):

A functional observational battery was performed in all animals at the end of the administration period starting at about 10:00 a.m. At least one hour before the start of the FOX the animals were transferred into single-animal polycarbonate cages. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians not being aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation special attention was paid to posture, tremors and convulsions, abnormal movements and gait abnormalities.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements/stereotypes
5. nose discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces (number of fecal pellets/appearance/ consistency) within two minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes
	19. other findings

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1. approach response	8. behavior during "handling"
2. touch response	9. vocalization
3. vision ("visual placing response")	10. pain perception ("tail pinch")
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hind limbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

7. Motor activity measurement:

Motor activity was measured at the same day when the FOB was performed in a randomized order. The measurement was performed in the dark using the TSE Labmaster System (TSE Systems GmbH, Bad Homburg, Germany) with 18 beams per cage. During the measurement the animals were kept in clean polycarbonate cages with absorbent material. Motor activity measurements were conducted from 2:00 p.m. onwards. The number of beam interrupts was counted over twelve 5 minute intervals. Measurement started when the first beam was interrupted by pushing the cage into the rack (staggered start). Measurements ended exactly 60 minutes thereafter. During the measurements the animals received no food and no water.

8. Hematology and clinical chemistry:

Blood was taken in the morning from fasted, isoflurane anesthetized animals from the retro-orbital venous plexus. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.

The following hematological and clinical chemistry parameters were determined for 10 animals per test group and sex:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Platelet count
✓	Hemoglobin (Hb)	✓	Differential blood count	✓	Prothrombin time
✓	Hematocrit (Hct)				
✓	Mean corp. volume (MCV)				
✓	Mean corp. hemoglobin (MCH)				
✓	Mean corp. Hb. conc. (MCHC)				
✓	Reticulocytes				
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bile acids	✓	Aspartate aminotransferase (AST)
✓	Phosphorus (inorganic)	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Potassium	✓	Cholesterol	✓	γ-glutamyl transpeptidase (γ-GT)
✓	Sodium	✓	Creatinine		
		✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

9. Urinalysis:

The dry chemical reactions on test strips (Combur-10-test M, Roche, Mannheim, Germany) used to determine urine constituents semiquantitatively were evaluated with a reflection photometer (Miditron M; Roche, Mannheim, Germany). The following parameters were determined in all animals:

Urinalysis					
	Quantitative parameters:		*Semiquantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilinogen
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

10. Sacrifice and pathology:

All animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. Special attention was given to the gastrointestinal tract (duodenum). The organs were sampled, weighed, and examined histopathologically as indicated in the table below.

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓		#	larynx	✓		#	skin
✓		#	aorta	✓	✓	✓	liver	✓		#	spinal cord (3 levels)[@]
✓		#	bone marrow[§]	✓		#	lung	✓	✓	✓	spleen
✓	✓	#	brain	✓		#	lymph nodes[#]	✓		#	sternum w. marrow
✓		#	caecum	✓		#	mammary gland (♀[+]&♂)	✓		#	stomach (fore- & glandular)
✓		#	colon	✓		#	muscle, skeletal	✓	✓	#	testes
✓	✓	✓	duodenum	✓		#	nerve, peripheral (sciatic n.)	✓	✓	#	thymus
✓	✓	#	epididymides	✓		#	nose/nasal cavity[‡]	✓	✓	✓	thyroid glands
✓		#	esophagus	✓	✓	#	ovaries and oviduct**	✓		#	trachea
✓		#	eyes (with optic nerve)	✓		#	pancreas	✓		#	urinary bladder
✓		#	femur (with knee joint)	✓		#	parathyroid glands	✓	✓	#	uterus with cervix
✓		✓	gross lesions			#	Peyer's patches	✓		#	vagina
✓		#	Harderian gland	✓		#	pharynx				
✓	✓	#	heart	✓		#	pituitary		✓		body (anesthetized animals)
✓		#	ileum	✓	✓	#	prostate				
✓		#	jejunum (w. Payer's plaque)	✓		#	rectum				
✓	✓	✓	kidneys	✓		#	salivary glands*				
✓			lacrimal glands[%]	✓	✓	#	seminal vesicles~				

[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed or histopathologically examined; [%] extraorbital, [‡] histopathology at level III, ~ with coagulation glands; [+] histopathology in females only

11. Plasma kinetics

On study day 15, EDTA blood samples (~ 200µL) were obtained from non-fasted animals by puncturing the retro-orbital plexus under isoflurane anesthesia. The first 5 animals of each group were bled 2 hours after the administration of the test substance while the last 5 animals of each group were bled 4 hours after administration. The plasma was analyzed for the test item concentration, i.e. Reg. No 399379 (500M106) and for its presumed metabolite Reg. No 298327 (500M04) as this metabolite was identified in urine of the ^{14}C-Reg.No. 339379 rat ADME study (see M-CA 5.1).

II. RESULTS AND DISCUSSION

A. Observations

1. Clinical signs of toxicity

No test substance-related effects were observed.

2. Mortality

No animal died prematurely in this study.

3. Ophthalmoscopy

No test substance-related effects were observed. The incidence and distribution of findings was comparable between control and high dose (1000 mg/kg bw/day) animals and typical for the strain and age of animals used.

B. Body weight and body weight gain

No test substance-related changes in body weight parameters of both sexes were observed in treated animals when compared to controls. All values represent the normal biological variability typical for animals of this strain and age [see Figure 5.8.1-4 and Table 5.8.1-53].

Figure 5.8.1-4: Body weight development of male and female rats administered Reg.No. 399379 for 28 days

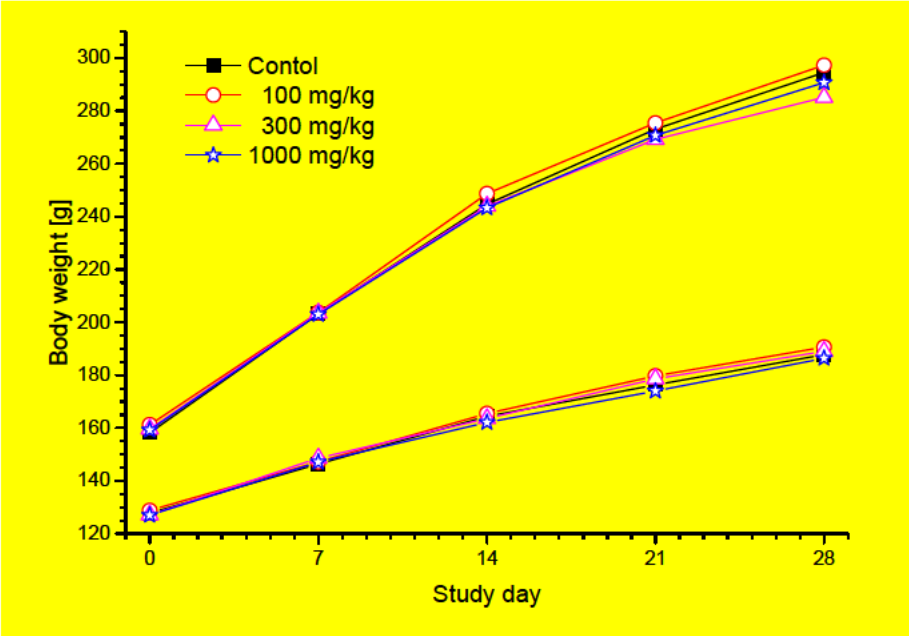


Table 5.8.1-53: Body weight development of rats administered Reg.No. 399379 for 28 days

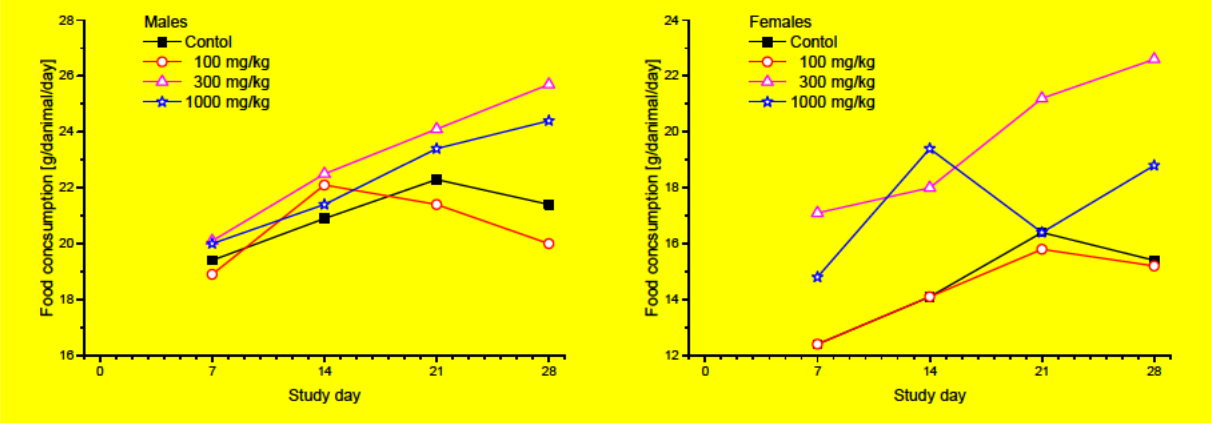
Dose level [mg/kg]	0	100	300	1000
		Males		
Body weight [g]				
- Day 0	158.2 ± 6.6	162.1 ± 7.6	159.6 ± 7.7	159.2 ± 7.3
- Day 28	294.5 ± 16.6	297.3 ± 14.3	285.1 ± 12.1	290.7 ± 11.0
Δ% (compared to control) #		1.0	-3.2	-1.3
Overall body weight gain [g]	136.2 ± 13.8	136.1 ± 13.0	125.5 ± 9.4	131.5 ± 12.3
Δ% (compared to control) #		-0.1	-7.9	-3.5
		Females		
Body weight [g]				
- Day 0	127.8 ± 7.5	128.8 ± 7.1	127.1 ± 5.5	127.0 ± 6.2
- Day 28	187.7 ± 8.2	190.6 ± 13.5	189.0 ± 6.7	186.4 ± 9.4
Δ% (compared to control) #		1.5	0.7	-0.7
Overall body weight gain [g]	60.0 ± 5.4	61.8 ± 9.7	61.9 ± 5.1	59.4 ± 5.3
Δ% (compared to control) #		3.0	3.1	-1.0

Values may not calculate exactly due to rounding of figures

D. Food and water consumption

Food spillage was observed in all treated groups which led to the exclusion of some values in low (100 mg/kg bw/day) and mid dose (300 mg/kg bw/day) males. Even though there was a trend towards higher food consumption at dose levels ≥ 300 mg/kg bw/day, this increase was not strictly dose dependent and considered to be affected by the observed food spillage [see Figure 5.8.1-5].

Figure 5.8.1-5: Mean daily food consumption in rats administered Reg.No 399379 for 28 days



No treatment-related effects on food consumption were noted.

E. Functional observation battery and motor activity

1. Functional observational battery

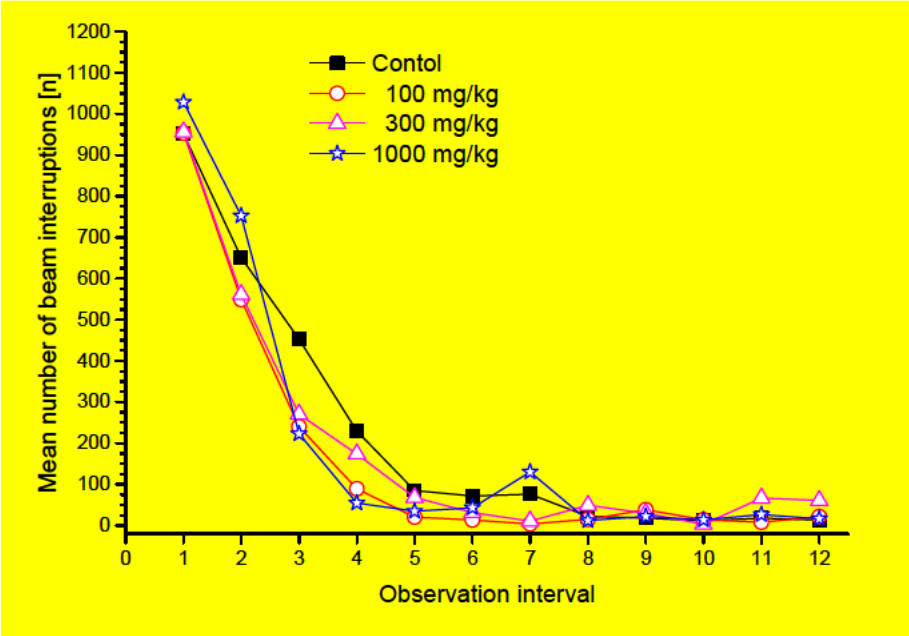
No treatment-related effects were observed during the FOB examinations. There were a few findings, which however were either equally distributed between treated groups and controls, were without a dose response relationship or occurred in single rats only. These observations were considered to be of incidental nature rather than related to treatment.

At the home cage observations and sensorimotor tests/reflexes the animals showed no test substance-related effects. Hind limb grip strength was significantly lower in low dose females. In absence of a dose-response relationship this was considered to be of incidental nature.

2. Motor activity measurement

No test substance-related effects were observed. In low dose males in the 7th observation interval the number of beam interruptions was statistically lower when compared to the control [see Figure 5.8.1-6]. In absence of a dose-response relationship this was considered to be unrelated to treatment. Furthermore, the total number of beam interruptions was comparable between all groups.

Figure 5.8.1-6: Motor activity of male rats administered Reg.No. 399379 for 28 days



F. Clinical biochemistry

1. Hematological findings

Treatment-related and adverse hematology changes were restricted to high dose males and females and consisted of a slight regenerative normochromic-normocytic anemia indicated by decreased erythrocyte counts (RBC), hemoglobin (HGB) and hematocrit (HCT) in males and increased absolute reticulocyte counts in both sexes [see Table 5.8.1-54].

Platelet counts were significantly higher in all treated male groups. However, the values were within the range observed in control animals of this strain and age (683-973 $x10^9$/L).
A statistically significant increase of the number of reticulocytes was noted in both sexes at the low dose level. Again, the numbers were within the historical control range (males: 102.1-184.6 $x10^9$/L; females: 102.2-189.8 $x10^9$/L) and not dose-related. The apparently dose related increase of reticulocytes in mid dose females appeared due to animal #64, which displayed a spontaneous malignant lymphoma resulting in a marked increase of reticulocyte counts (405.7 $x10^9$/L]. In addition, red blood cell counts (5.88 $x10^{12}$/L), hemoglobin (7.2 mmol/L) and hematocrit (0.332 L/L) indicated an anemic condition of this animal. If this animal is for good reasons excluded from calculation, the mean reticulocyte counts are well within the historical control range (see separate row at the end of Table 5.8.1-54).

A non-parametic trend test according to Jonkheere-Terpstra (Jonckheere A. R. (1954): A distribution-free k-sample test again ordered alternatives. Biometrika 41, 133–145; Terpstra T. J. (1952): The asymptotic normality and consistency of Kendall's test against trend, when ties are present in one ranking. Indagationes Mathematicae 14, 327–333) was performed using SAS as a step-down test to identify the minimum effective dose for effecs on reticulocyte counts. Statistical significance was only observed for the high dose of 1000 mg/kg (see BASF DocID 2017/1025730, available on request), confirming the view that values at the mid and low dose are not indicative for an effect of treatment.
A significantly prolonged prothrombin time (HepatoQuick) was noted in mid dose males, which was not dose-dependent and thus - like the other findings above – considered incidental and not related to treatment.

Table 5.8.1-54: Selected hematological parameters of rats administered Reg.No. 399379 for 28 days (Group means ± SD)

Parameter/Dose level [mg/kg]	0	100	300	1000
Males				
- RBC [1012/L]	7.86 ± 0.31	7.67 ± 0.38	7.88 ± 0.27	**7.35** ± **0.32**
- HGB [mmol/L]	8.8 ± 0.2	8.8 ± 0.2	8.8 ± 0.3	**8.4** ± **0.3**
- HCT [1/1]	0.417 ± 0.006	0.415 ± 0.015	0.414 ± 0.012	**0.394** ± **0.013**
- Platelets [109/L]	712 ± 60	772* ± 34	775* ± 55	817** ± 64
- Reticulocytes [109/L]	141.2 ± 26.6	172.4** ± 20.2	151.7 ± 29.6	**248.1** ± **57.7**
- HepatoQuick [sec]	36.0 ± 1.8	36.6 ± 2.4	38.4** ± 1.7	35.4 ± 2.1
Females				
- RBC [1012/L]	7.15 ± 0.31	7.04 ± 0.33	6.88 ± 0.47	6.9 ± 0.41
- HGB [mmol/L]	8.2 ± 0.3	8.2 ± 0.2	8.0 ± 0.3	8.3 ± 0.3
- HCT [1/1]	0.375 ± 0.017	0.373 ± 0.013	0.368 ± 0.016	0.368 ± 0.016
- Platelets [109/L]	756 ± 71	676 ± 71	711 ± 107	733 ± 62
- Reticulocytes [109/L]	146.1 ± 26.4	173.7* ± 23.7	191.5 ± 85.9	**211.7** ± **30.9**
- HepatoQuick [sec]	34.2 ± 1.9	35.8 ± 1.9	35.7 ± 1.7	34.7 ± 1.4
Excluding mid dose female #64 (spontaneous malignant lymphoma)				
- Reticulocytes [0/00]	146.1 ± 26.4	173.7* ± 23.7	167.7 ± 44.0	**211.7** ± **30.9**

* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis + Wilcoxon test (two-sided))
HCD Reticulocytes: males: 102.1-184.6 x10^9/L; females: 102.2-189.8 x10^9/L
HCD Platelets: males: 683-973 x10^9/L

2. Clinical chemistry findings

There were no treatment-related changes of clinical chemistry parameters. The only statistically significant difference consisted of a lower alanine amino transferase (ALT) activity in mid dose females (0.54 ± 0.11, 0.55 ± 0.08, 0.42* ± 0.08 and 0.51 ± 011 µkat/L at 0, 100, 300, 1000 mg/kg bw/day, respectively).

3. Urinalysis

No treatment-related changes of urinalysis parameters were noted.

4. Plasma kinetics

The concentrations of Reg.No. 399379 and its metabolite Reg.No. 298327 in plasma were determined using a validated analytical method (see M-CA 4, BASF DocID 2016/1333356). No detectable concentrations of Reg.No. 298327 were noted at any sampling time or administered dose. A number of Reg.No. 399379 concentrations were below the working Limit of Quantification (wLOQ) of 50 ng/ml plasma. This was especially the case at the low dose (all sampling times) and the mid and high dose in samples of the 4 hour sampling time. Thus, these values are of limited precision [see Table 5.8.1-55].

Table 5.8.1-55: Reg.No 399379 plasma levels 2 and 4 hours after administration [ng/ml]

Sex	Males		Females	
Sampling time [h]	2	4	2	4
0 mg/kg	n.d.	n.d.	n.d.	n.d
100 mg/kg	40.3 ±14.6	16.7 ± 5.7	15.8 ± 4.9	11.3 ± 7.9
300 mg/kg	70.4 ±28.9	48.1 ±34.1	64.1 ±31.8	15.9 ± 4.8
1000 mg/kg	143.3 ±39.7	118.5 ±37.4	132.0 ±18.4	47.6 ±15.7

Figures calculated based on unrounded values

The measurements were additionally affected by the low recovery observed in the stability samples. An initial investigation of Reg. No. 399379 stability samples (950 ng/mL) in plasma revealed a recovery of about 65% of the spiked concentration. These samples were generated at the day the plasma samples were obtained from the treated animals (07 September 2016) and stored frozen with the study plasma samples until analysis beginning of January 2017.
As the highest measured plasma concentrations at maximum reached about a fifth of the stability sample concentration, the recovery analysis of Reg. No. 399379 from spiked plasma samples was repeated at 5 concentrations ranging from about 50 (LOQ) to 250 ng/mL. The spiked plasma samples were frozen for about 48 hours prior to extraction and analysis. In order to investigate the influence of the extraction media on recovery, the plasma samples were either extracted with acetonitrile, which was used in the previous analyses, acetonitrile + acetone (4 + 1) or acetone. The samples were extracted and analyzed after stored frozen for about 48 hours. The analyses revealed a concentration dependent decrease of the recovery ranging from about 30% at 50 mg/mL to about 60% at 250 ng/ml [see Table 5.8.1-56]. The different extraction media did not have a substantial effect on the recovery.

Table 5.8.1-56: Recovery of spiked Reg.No 399379 from rat plasma

Samples	Determined concentration [ng/ml]	Nominal concentration [ng/ml]	Nominal concentration [% of nominal]
LS 1A	15.4	50.9	30.2
LS 2A	38.3	101.8	37.6
LS 3A	70.3	152.7	46.1
LS 4A	103.8	203.6	51.0
LS 5A	154.5	254.5	60.7
LS 5A Mix	161.9	254.5	63.6
LS 5A Acetone	144.5	254.5	56.8

Samples LS 1A – LS 5A: Standard extraction (100 % acetonitrile)
Sample LS 5A Mix: Extracted with acetonitrile + acetone (4 + 1)
Sample LS 5A Acetone: Extracted with 100 % acetone

The above investigation was likewise performed for Reg.No. 298327, as no 4-month stability samples were prepared for this metabolite. This was due to the fact that the decision to determine the plasma concentrations of Reg.No. 298327 was taken only in November 2016, i.e. about two months after blood sampling for plasma analysis (Amendment No. 6 to the study plan dated November 15, 2016). In contrast to Reg. No. 399379, recovery of Reg. No. 298327 was about 100% independent on the spiked concentration and the extraction media used [see Table 5.8.1-57.]

Table 5.8.1-57: Recovery of spiked Reg.No 298327 from rat plasma

Samples	Determined concentration [ng/ml]	Nominal concentration [ng/ml]	Nominal concentration [% of nominal]
LS 1B	56.4	55.4	101.8
LS 2B	107.9	110.8	97.4
LS 3B	166.9	166.2	100.4
LS 4B	218.0	221.6	98.4
LS 5B	275.3	277.0	99.4
LS 5B Mix	275.7	277.0	99.5
LS 5B Acetone	277.5	277.0	100.2

Samples LS 1B – LS 5B: Standard extraction (100 % acetonitrile)
Sample LS 5B Mix: Extracted with acetonitrile + acetone (4 + 1)
Sample LS 5B Acetone: Extracted with 100 % acetone

The Quality Control samples used in the initial plasma analysis for Reg.No. 399379 consistently revealed values within ± 10% of the nominal concentrations. As these samples were immediately extracted after addition of Reg. No. 399379, it was speculated that the decreased recovery is due to the increased storage time and/or freezing procedure. In order to test this hypothesis, 5 replicate spiked plasma samples were extracted and analyzed immediately after preparation and after freezing for 2, 5 and 12 days.

Table 5.8.1-58: Time dependence of the recovery of spiked Reg.No 298327 from rat plasma

Samples	Determined concentration [ng/ml]	Nominal concentration [ng/ml]	Nominal concentration [% of nominal]
Day 0, non frozen samples			
LS 1a	49.0	50.1	97.8
LS 2a	49.4	50.1	98.6
LS 3a	50.8	50.1	101.4
LS 4a	52.1	50.1	104.0
LS 5a	52.2	50.1	104.1
Mean ± SD			**101.2 ± 2.9**
LS 1b	20.9	52.5	39.7
LS 2b	36.1	52.5	68.8
LS 3b	24.9	52.5	47.5
LS 4b	26.4	52.5	50.2
LS 5b	27.5	52.5	52.3
Mean ± SD			**51.7 ± 10,7**
LS 1c	17.0	52.5	32.4
LS 2c	17.1	52.5	32.5
LS 3c	15.4	52.5	29.3
LS 4c	17.1	52.5	32.6
LS 5c	15.0	52.5	28.5
Mean ± SD			**31.1 ± 2.0**
LS 1d	13.8	52.5	26.4
LS 2d	13.1	52.5	25.0
LS 3d	14.0	52.5	26.6
LS 4d	14.2	52.5	27.1
LS 5d	13.0	52.5	24.7
Mean ± SD			**26.0 ± 1.1**

Samples LS 1x – LS 5x: 5 Replicates spiked with about 50 ng/mL
a to d indicated the storage duration (a = no storage at -80°C, b, c, d = frozen storage for 2, 5, and 12 days, respectively)

The results this analysis confirmed the hypothesis as the recovery of spiked Reg.No. 399379 was 100% when the plasma was extracted at the day of preparation without freezing. In contrast, a time dependent decrease of the recovery of spiked Reg.No. 399379 was noted, decreasing from about 52% after 2 days freezing to about 26% after 12 days freezing [see Table 5.8.1-58]. Due to time constraints no further time points could be evaluated. But as the decline of recovery diminished with time, it is to assumed that at a certain storage time the maximum decrease of the recovery is reached. This time is probably dependent of the amount of spiked Reg.No. 299378.

However, irrespective of the analytical difficulties to correctly determine the Reg. No. 399379 plasma concentrations, the concentrations are relatively low. The decrease of plasma concentrations in samples taken 2 and 4 hours after gavage application indicate that the peak plasma concentrations were probably reached prior to the 2 hour sampling time.

The low Reg.No. 399379 concentrations in plasma are not necessarily indicating a low systemic bioavailability of the test item and its metabolites. This is indicated by the fact that in the absorption and distribution study with Reg.No. 399379 in mice (see BASF DocID 2016/1225930), total radioactivity levels in plasma equivalent to about 110 µg/mL were determined 4 hours after single gavage administration of 500 mg/kg bw. It is important to note that the vehicle and the route of application in both studies, i.e. the 28-day rat and the mouse study were identical. This plasma concentration of Reg.No. 399379 and its metabolites in mice is 3 orders of magnitude higher than the concentration of Reg.No. 399379 in rats.

Furthermore, the ^{14}C-Reg.No. 399379 metabolism study in rats revealed a systemic bioavailability of at least 17% as shown by the amount of radioactivity excreted in the urine after administration of a single dose of 10 mg/kg bw by gavage as suspension in 0.5% aqueous CMC (see BASF DocID 2015/1198482).

G. Necropsy

1. Organ weight

Changes of absolute and or relative organ weights were noted in high dose males and consisted of increased liver and duodenum weights. In addition, relative liver weights significantly increased in high dose females [see Table 5.8.1-59].
While the increased liver weights in males were accompanied by histopathological changes, no corroborative changes were noted in the duodenum in males. Despite the lack of a histopathological correlate, the weight changes in the duodenum were – like those in the liver of males – considered to be related to treatment.
In contrast, the marginal, but statistically significant increase of relative liver weights in high dose females was within the historical control range (2.525 - 2.918%) and not corroborated by histopathological changes. Thus, this change was considered to be of incidental nature.

The liver (8.15 g) and especially spleen weights (3.31 g) of mid dose female #64, which displayed a spontaneous malignant lymphoma, increased because of infiltrations with this tumor. This considerably affected the mean weights for these organs. Therefore, these organs were excluded from the calculation of means.

Table 5.8.1-59: Selected absolute and relative weights of rats administered Reg. No. 399379 for 28 days§

Sex		Males				Females			
Organ weight	Dose [mg/kg]	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ% #	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ% #
Terminal weight [g]	0	272.12				172.94			
	100	275.25	(1.2)			176.71	(2.2)		
	300	264.29	(-2.9)			176.15	(1.9)		
	1000	266.28	(-2.1)			174.08	(0.7)		
Duodenum [g]	0	0.477		0.176		0.442		0.256	
	100	0.508	(6.5)	0.185	(5.1)	0.432	(-2.3)	0.245	(-4.3)
	300	0.485	(1.7)	0.184	(4.5)	0.425	(-3.8)	0.241	(-5.9)
	1000	**0.532***	**(11.5)**	**0.200***	**(13.6)**	0.456	(3.2)	0.263	(2.7)
Liver [g]	0	7.321		2.689		4.700		2.716	
	100	7.567	(3.4)	2.749	(2.2)	4.678	(-0.5)	2.647	(-2.5)
	300	7.225	(-1.3)	2.732	(1.6)	4.833	(2.8)	2.741	(0.9)
	1000	**7.997***	**(9.2)**	**3.003****	**(11.7)**	4.945	(5.2)	2.840*	(4.6)
Spleen [g]	0	0.583		0.214		0.462		0.266	
	100	0.531	(-8.9)	0.193	(-9.8)	0.373	(-19.3)	0.211	(-20.7)
	300	0.549	(-5.8)	0.207	(-3.3)	0.407	(-11.9)	0.231	(-13.5)
	1000	0.578	(-0.9)	0.217	(1.4)	0.419	(-9.3)	0.240	(-9.8)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test. two sided)
# Values may not calculate exactly due to rounding of figures
§ Female animal #64 excluded from calculation of liver and spleen group means

2. Gross and histopathology

There were no treatment-related gross necropsy findings. All findings were either observed in single incidences only and/or without a dose response.

Treatment-related histopathological findings were restricted to the high dose level and noted in the liver of high dose males and the spleen of high dose males and females [see Table 5.8.1-60]. In the liver of high dose males a minimal centrilobular hepatocellular hypertrophy was noted, which correlated to the increased liver weights. In the spleen an increased incidence and severity of extramedullary hematopoiesis was noted, which correlated to the slight anemia observed at this dose level. There was no histopathological correlate to the slightly increased absolute and relative duodenum weights.
The extramedullary hematopoiesis observed in low and mid dose animals was minimal only. Minimal extramedullary hematopoiesis is occasionally observed in control animals at incidences up to 100% (HCD: >47 studies conducted between 2011 and 2015). Thus the slightly increased incidence of this finding in mid dose females is considered incidental and unrelated to treatment.

Table 5.8.1-60: Selected histopathological findings in rats administered Reg.No 399379 for 28 days

Sex	Males				Females			
Dose [mg/kg]	0	100	300	1000	0	100	300	1000
Animals in group	10	10	10	10	10	10	10	10
Liver # examined	10	10	10	10	10	10	10	10
- hypertrophy, centrilob. (Zone 3)	0 -	0 -	0 -	4 *[1.0]*	0 -	0 -	0 -	0 -
Spleen # examined	10	10	10	10	10	10	10	10
- Extramedullary hematopoiesis	2	2	3	8	1	0	3	6
	[1.0]	*[1.0]*	*[1.0]*	*[1.9]*	*[1.0]*	-	*[1.0]*	*[1.5]*

[ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

Overall, only the increased extramedullary hematopoiesis in the spleen was considered to represent an adverse treatment-related pathology finding. The minimal hepatocellular hypertrophy and slightly increased liver weights in high dose males as well as the slightly increased duodenum weights without histopathological correlate were considered treatment-related, but not adverse.

III. CONCLUSIONS

The administration of Reg.No. 399379 (500M106, metabolite of pyraclostrobin) as suspension in 0.5% aqueous CMC to male and female Wistar rats for 28-days caused treatment-related effects on red blood cell parameters (regenerative anemia in both sexes), the duodenum (increased weight in males), the liver (increased weight and hepatocellular hypertrophy in males) and the spleen (extramedullary hematopoiesis).

Therefore, under the conditions of the present study the no observed adverse effect level (NOAEL) was 300 mg/kg bw/d in male and female Wistar rats, respectively.

Genotoxicity studies of 500M106 (Reg.No. 399379)

Report:	CA 5.8.1/43 Woitkowiak C., Landsiedel R., 2016 a Reg.No. 399379 (Metabolite of BAS 500 F, Pyraclostrobin) - Salmonella Typhimurium / Escherichia Coli reverse mutation assay 2016/1119837
Guidelines:	OECD 471 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to Reg.No. 399379 (metabolite of pyraclostrobin; batch: L87-58, purity: 97.6%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in a standard plate and preincubation test. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. In the standard plate and preincubation test Reg.No. 399379 was tested at concentrations of 3.3 to 5200 µg/plate. In the plate incorporation assay a weak bacteriotoxic effect was observed in the tester strain TA 98 in the presence of S9 mix at 5200 µg/plate. In the preincubation assay a weak bacteriotoxic effect was observed in TA 1537 with S9 mix at a concentration of 1000 µg/plate. Precipitation of the test substance was found from 1000 µg/mL onward in the presence or absence of S9 mix.

A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in the presence or absence of metabolic activation in any of the experiments. The slight increase in the number of his^+ revertants observed in tester strain TA 100 with S9 mix was under the threshold of factor 2.0 and only at doses where evident test substance precipitations were found. The number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain in the presence or absence of S9 mix. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.

According to the results of the study, test substance Reg.No. 399379 was not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2016/1119837)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg. No. 399379 (metabolite of pyraclostrobin)

Description: Solid, orange

Lot/Batch #: L87-58

Purity: 97.6% (tolerance ± 1.0%)
Impurities: 1.6% Reg.No. 369315 (azo), 0.2% Reg.No. 364380 (azoxy)

Stability of test compound: The stability of the test item under storage conditions over the study period was guaranteed by the sponsor (expiry date June 01, 2017). The stability in DMSO over 4 h was verified analytically.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Solvent/final concentration: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

To demonstrate the efficacy of the S9 mix in this assay, the S9 batch was further characterized with benoz(a)pyrene in the strains TA 100 and TA 98 as required in OECD 471.

3. Activation:

S9 was produced from the livers of induced male Wistar rats. The rats received phenobarbital (80 mg/kg bw; i.p.) and β-naphthoflavone (80 mg/kg bw; orally) each on three consecutive days. 24 h after the last administration the animals were sacrificed and the livers were prepared. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
Salmonella typhimurium:
The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay:	In the first experiment triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2600, 5200 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without S9) for all tester strains indicated above.
Pre-incubation assay:	In the second experiment triplicate plates were prepared for each concentration (neg. control; 3.3, 10, 33, 100, 333, and 1000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains. Due to high precipitation in the first assay the doses were adjusted.

B. TEST PERFORMANCE:

1. Dates of experimental work: 22-Mar-2016 to 01-Apr-2016

2. Plate incorporation assay:

To test tubes containing 2 mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Minimal glucose agar plates. In the experiments with E. coli the warm soft agar contains 0.5 mM tryptophan instead of histidine + biotin. After incubation for 48-72 h at 37°C, his^+ or trp^+ revertants were counted.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.
After incubation in the dark for 48 to 72 hours at 37°C the bacterial colonies were counted.

4. Statistics:

No special statistical tests were performed.

5. Acceptance and assessment criteria:

Generally, the experiment was considered valid if the following criteria were met:

- The number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain.
- The sterility controls revealed no indication of bacterial contamination.
- The positive control substances both with and without S9 mix induced a distinct increase in the number of revertant colonies within the range of the historical positive control data or above.
- Fresh bacterial culture containing approximately 10^9 cells per mL were used.

The test substance was considered positive in this assay if the following criteria were met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. at least doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and E. coli WP2 uvrA) or tripling (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance was generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains were within the historical negative control data range under all experimental conditions in at least two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions. The stability of the test substance at room temperature in the vehicle DMSO was verified analytically.

B. TOXICITY AND SOLUBILITY

A weak bacteriotoxic effect (slight decrease in the number of his+ revertants) was observed in the standard plate test and only in the tester strain TA 98 with S9 mix at 5200 µg/plate.
In the preincubation assay, bacteriotoxicity (slight decrease in the number of his+ revertants) was observed in tester strains TA 1537 with S9 mix at a concentration of 1000 µg/plate only.
Strong test substance precipitation was found from about 1000 µg/plate onward with and without S9 mix. Furthermore, it was very difficult to distinguish between revertant colonies and precipitation at concentrations of 2600 and 5200 µg/plate in the 1st Experiment.

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Table 5.8.1-61: Bacterial gene mutation assay with Reg.No. 399379 - Mean number of revertants

Experiment 1: Plate incorporation assay*										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabolic activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	26.7 ± 3.2	16.7 ± 3.5	127 ± 23	93 ± 19	8.7 ± 1.5	9.3 ± 2.1	11.0 ± 1.0	6.0 ± 2.6	27.0 ± 2.0	22.3 ± 2.3
Test substance										
33 µg/plate	26.3 ± 5.0	20.3 ± 3.8	105 ± 5	96 ± 3	9.7 ± 2.9	10.0 ± 2.6	8.7 ± 0.6	6.7 ± 2.1	25.0 ± 4.6	27.0 ± 6.2
100 µg/plate	21.7 ± 5.7	19.0 ± 4.4	117 ± 25	111 ± 17	10.0 ± 4.4	8.0 ± 2.0	9.0 ± 3.6	6.0 ± 1.0	22.3 ± 4.9	24.0 ± 6.2
333 µg/plate	25.7 ± 5.9	19.7 ± 3.2	162 ± 9	118 ± 6	12.7 ± 1.5	10.3 ± 4.2	10.7 ± 1.5	8.3 ± 2.1	29.3 ± 8.6	21.3 ± 4.5
1000 µg/plate	25.3 ± 10.4 P	19.0 ± 2.0 P	194 ± 4 P	101 ± 5 P	10.7 ± 1.2 P	11.7 ± 1.5 P	9.3 ± 2.1 P	8.3 ± 2.1 P	27.3 ± 6.4 P	15.7 ± 3.5 P
2600 µg/plate	18.7 ± 5.9 P	15.0 ± 3.6 P	219 ± 23 P	96 ± 10 P	9.0 ± 1.0 P	9.7 ± 1.5 P	7.7 ± 2.5 P	5.7 ± 1.2 P	20.3 ± 2.1 P	15.7 ± 3.2 P
5200 µg/plate	16.3 ± 3.2 P	13.3 ± 2.5 P	209 ± 5 P	101 ± 19 P	8.7 ± 2.1 P	9.0 ± 3.0 P	8.0 ± 2.0 P	5.7 ± 2.3 P	21.0 ± 3.0 P	18.7 ± 5.5 P
Pos. control§	1036 ± 255	940 ± 90	1489 ± 310	4367 ± 198	171 ± 21	5174 ± 307	106 ± 13	1389 ± 174	137 ± 4	1221 ± 68
Experiment 2: Preincubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabolic activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	31.0 ± 9.5	18.3 ± 3.5	121 ± 5	100 ± 13	8.7 ± 1.5	9.7 ± 0.6	10.0 ± 2.6	7.0 ± 2.0	20.3 ± 9.5	25.3 ± 4.9
Test substance										
3.3 µg/plate	30.0 ± 7.2	17.3 ± 2.1	111 ± 12	98 ± 8	10.3 ± 2.5	9.0 ± 1.0	10.7 ± 4.0	8.7 ± 1.5	16.3 ± 3.8	22.0 ± 3.0
10.0 µg/plate	31.0 ± 1.7	15.7 ± 3.5	106 ± 3	111 ± 11	9.7 ± 3.1	10.3 ± 3.5	11.0 ± 1.0	8.3 ± 0.6	15.7 ± 4.5	19.3 ± 2.5
33.0 µg/plate	31.3 ± 9.6	24.0 ± 5.3	127 ± 11	113 ± 11	11.3 ± 0.6	11.0 ± 2.6	10.7 ± 3.2	8.7 ± 2.1	23.0 ± 8.2	22.7 ± 7.4
100.0 µg/plate	34.0 ± 4.6	19.0 ± 7.2	129 ± 15	116 ± 14	10.3 ± 2.5	10.0 ± 1.7	12.3 ± 2.1	7.3 ± 3.5	18.3 ± 1.5	20.0 ± 7.5
333.0 µg/plate	28.7 ± 1.2	20.7 ± 5.7	205 ± 19	112 ± 9	15.0 ± 3.6	9.3 ± 2.5	7.0 ± 3.5	7.7 ± 2.5	21.3 ± 4.5	22.7 ± 7.0
1000.0 µg/plate	25.0 ± 1.0 P	15.7 ± 0.6 P	233 ± 28 P	106 ± 16 P	10.3 ± 4.9 P	9.0 ± 4.4 P	6.3 ± 4.5 P	6.3 ± 2.3 P	30.0 ± 7.9 P	22.3 ± 9.9 P
Pos. control§	1135 ± 235	982 ± 49	1707 ± 177	2841 ± 72	196 ± 17	2946 ± 145	120 ± 18	876 ± 200	102 ± 5.0	345 ± 113

*: Numbers may differ from original data due to rounding; P: precipitation; HCD: Historical Control Data
§ = Compound and concentrations see Material and Methods (I.A.2.) above

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiment with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested [see Table 5.8.1-61]. The slight increase in the number of his+ revertants observed in tester strain TA 100 with S9 mix was under the threshold of factor 2.0 and only at doses where evident test substance precipitations were found.
In the 1st Experiment the counting of the revertant colonies of the two highest dose groups was extremely difficult and finally done manually. It cannot be excluded that the precipitates interfered with scoring. So, according to the OECD Guideline the test substance was tested up to one concentration that was insoluble in the final treatment mixture in the 2nd Experiment.
In this study with and without S9 mix, the number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

III. CONCLUSION

According to the results of the present study, the test substance Reg.No. 399379 (metabolite of pyraclostrobin) is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/44 Schulz M., Landsiedel, R., 2016 b Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in L5178Y mouse lymphoma cells ($TK^{+/-}$ locus assay, microwell version) 2016/1171390
Guidelines:	OECD 490 (2015), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17 No. L 142, EPA 870.5300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 399379 (metabolite of pyraclostrobin, batch: L87-58, purity: 97.6%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the TK locus in Mouse Lymphoma L5178Y cells. Two independent experiments were conducted in the presence (4-h incubation) or absence (4-h and 24-h incubation) of metabolic activation with two parallel cultures each. Based on the results of a preliminary cytotoxicity assay, concentrations of up to 100 µg/mL were used in the main experiment. Methylmethanesulfonate (MMS), 7,12-dimethylbenz[a]anthracene (DMBA) and Cyclophosphamide (CPA) served as positive controls in the experiments without and with metabolic activation, respectively. After the incubation period treatment media were replaced by culture medium in both experiments and the cells were incubated for 48 h for expression of mutant cells. This was followed by incubation of cells in selection medium containing TFT for about 9 - 11 days.

In both experiments without S9 mix, no biologically relevant increase in the number of mutant colonies was observed independent of the treatment duration. However, in the presence of S9 mix, Reg.No. 399379 led to a concentration-dependent statistically significant increase in the number of mutant colonies in both experiments. From 50 µg/mL (Exp. 1) and from 18.75 µg/mL (Exp. 2) onward, the MF was clearly above the vehicle control and historical control data and was also exceeding the calculated borderline MF using the GEF of 126. In both experimental parts in the presence of S9 mix, markedly increased mutation rates were obtained, when RTG was reduced to values below 50%. Therefore, it cannot be ruled out that cytotoxicity contributed to the observed genotoxicity. In these experimental parts the ratio of small to large colonies was close to the observation after treatment with the positive control CPP. Thus, it has to be considered that the test substance may have induced clastogenic effects. The positive controls showed a distinct increase in induced mutant colonies, indicating that the tests were sensitive and valid.

Based on the results of the study it is concluded that under the conditions of this test Reg.No. 399379 (metabolite of pyraclostrobin) and/or its metabolites induced forward mutations and/or chromosomal aberrations in vitro in the mouse lymphoma assay with L5178Y TK+/- cells in the presence of metabolic activation.

(BASF DocID 2016/1171390)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg. No. 399379 (metabolite of pyraclostrobin)

Description:	Solid, orange
Lot/Batch #:	L87-58
Purity/content:	97.6% (tolerance ± 1.0%) Impurities: 1.6% Reg.No. 369315 (azo), 0.2% Reg.No. 364380 (azoxy)
Stability of test compound:	The stability of the test item under storage conditions over the study period was guaranteed by the sponsor (expiry date June 01, 2017).
Vehicle:	DMSO (1% final concentration)

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	1% (v/v) DMSO in culture medium
Positive control -S9:	Methyl methanesulfonate (MMS) 15 and 5 µg/mL during the 4-h and 24-h exposure (dissolved in medium)
Positive control +S9:	Cyclophosphamide (CPP) 2.5 µg/mL (dissolved in medium); 7,12-dimethylbenz[a]anthracene (DMBA) 1 µg/mL (dissolved in DMSO)

3. Activation: S9 was produced from the livers of phenobarbital/β-naphthoflavone induced rats. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and an appropriate amount of S9-fraction is mixed with an equal volume of S9-cofactor solution. The co-factors in the S9-mix had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2 \times 6H_2O$	8 mM

4. Test organism:	L5178Y mouse lymphoma cells were used. They have a high proliferation rate (doubling time about 9-10 h), a high plating efficiency (~90%) and a stable karyotype with a near diploid (40 ± 1) chromosome number. Stocks of the L5178Y cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination, karyotype stability, and spontaneous mutant frequency. Prior to treatment, spontaneous TK-deficient mutants were eliminated from the stock cultures by growing the cells for 1 day in pretreatment medium A and 3 days in pretreatment medium B (see below).
5. Culture media:	
Culture medium (basis):	RPMI-0 (RPMI 1640 medium with 1% of 10000 IU/10000 µg/mL Penicillin/Streptomycin, 1% Sodium Pyruvate (10 mM))
Medium (treatment +S9):	RPMI-0 + 5% FCS (RPMI-5)
Medium (treatment -S9):	RPMI-0 + 10% FCS (RPMI-10)
Medium (CE/selection):	RPMI-0 + 20% FCS (RPMI-20)
Pretreatment medium A:	("THMG" medium): RPMI-10 medium containing hypoxanthine (5 µg/mL), thymidine (3 µg/mL), methotrexate (0.1 µg/mL), and glycine (7.5 µg/mL).
Pretreatment medium B:	("THG" medium): RPMI-10 medium containing hypoxanthine (5 µg/mL), thymidine (3 µg/mL), and glycine (7.5 µg/mL).
Selection medium:	Complete culture medium supplemented with 4 µg/mL trifluorothymidine (TFT).
6. Locus examined:	thymidine kinase (TK)
7. Test concentrations:	
a) Preliminary toxicity assay:	Nine concentrations ranging from 8.2 to 2100 µg/mL (±S9)
b) Mutation assay:	
1st experiment:	1.56, 3.13, 6.25, 12.5, 25, 50, 100 µg/mL with and without metabolic activation (4-h exposure)
2nd experiment:	1.56, 3.13, 6.25, 12.5, 25, 50, 100 µg/mL without metabolic activation (24-h exposure)
2nd experiment:	4.69, 9.38, 18.75, 37.5, 50, 75, 100 µg/mL with metabolic activation (4-h exposure)

B. TEST PERFORMANCE:

1. Dates of experimental work: 18-Apr-2016 to 16-June-2016

2. Preliminary cytotoxicity assay:

A pre-test was performed in order to determine the concentration range of the mutagenicity experiments. Both, pH value and osmolarity were determined at the maximal concentration of the test item and in the solvent control without metabolic activation. Cells were treated for 4 h in the presence and absence of S9 mix with the test substance at concentrations from 8.2 to 2100 µg/mL. The relative suspension growth was determined.

3. Mutation Assay:

Pretreatment of cells:

During the week prior to treatment $3x10^5$ cells were seeded into 75 cm^2-flasks and incubated for 1 day with "THMG" medium and for the following 3 days in “THG” medium to eliminate spontaneous TK-deficient mutants.

Cell treatment and expression period:

The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4 hours. The second experiment was performed with a treatment period of 24 hours in the absence of metabolic activation and a treatment period of 4 hours in the presence of metabolic activation.

For each test group, $1.5x10^7$ ($1x10^7$ during 24 h exposure) cells per flask (75 cm^2 flasks) suspended in 20 mL RPMI medium were exposed to various concentrations of the test item either in the presence or absence of metabolic activation. Positive and solvent controls were performed in parallel. After 4 h (24 h in the second experiment), the test item was removed by centrifugation (173 x g, 5 min) and the cells were washed at least once in RPMI-5 medium. From each culture a sample of treated cells (2 x 10^5 cells/mL or 6 x 10^6 cells/flask) was pipetted in 75 cm² flasks and was incubated for a 2-day expression period. To maintain exponential growth during this phase, each culture was counted daily and the cell numbers were adjusted at each day to 2 x 10^5 cells/mL in 30 mL RPMI-10 medium. The cell numbers were determined using a cell counter.

Seeding for selection

For the selection of the mutants, the cells were centrifuged and resuspended in 50 mL selection medium. Per culture 200 µL were dispensed in each well of two 96-well plates (2000 cells/well). After incubation for at least 9 days, both the number of negative wells and the number of wells containing small or large colonies were scored for calculation of the mutant frequency (MF).

Cloning efficiency

For determination of cytotoxicity, at the end of the treatment (Cloning efficiency 1 (survival)) and expression period (Cloning efficiency 2 (viability)) the cells were centrifuged resuspended. Per culture 200 µL were dispensed in each well of two 96-well plates (1.6 cells/well). After incubation for 9 - 11 days the plates were scored for empty wells.
For calculation of the relative suspension growth (RSG) and the relative total growth (RTG) the cell counts determined within the expression period at 2nd and 3rd passage after exposure in the case of 4-hour exposure and 1st, 2nd and 3rd passage after exposure in the case of 24-hour exposure were used.

Colony counting

The number of empty wells and the number of wells containing colonies were scored and reported. The colonies are classified into large colonies (indication of gene mutation) and small colonies (indication of chromosome breakage). Small colonies are defined as less than 1/4 of the diameter of the well. Size is the key factor and morphology (the optical density of the small colonies is considerably higher) should be secondary.

Calculations and processing of the data:

Cloning efficiency 1 (survival) and 2 (viability) was determined for each test group and given as absolute and relative cloning efficiency (CE_x and RCE_x, respectively).

$$CE_x = \frac{-\ln(\text{total number of empty wells} \div \text{total number of seeded wells (96)})}{\text{number of seeded cells per well (1.6)}} \times 100$$

$$RCE_x = \frac{CE_x \text{ of the test group}}{CE_x \text{ of the negative / vehicle control}} \times 100$$

The RTG is the standard measure of cytotoxicity. This measure includes the relative growth in suspension (RSG) during the expression period and the relative cloning efficiency (RCE_2; viability) at the time the mutants are selected.
For taking into account any loss of cells during the 4-hour treatment period a relative growth during treatment factor (RGDT, %) was calculated by comparing the growth of each treated culture relative to the control:

$$\text{RGDT} = \frac{\text{Cell count after 4 h of the test group}}{\text{Cell count after 4 h of the negative/v ehicle control}} \times 100$$

The total suspension growth (SG) and the RSG (in %) were calculated for each test group as follows:

Total suspension growth after 4 hour-exposure

$$SG = \frac{\text{Cell count after 24 h}}{2 \times 10^5 \text{ cells per mL}^{1,2}} \times \frac{\text{Cell count after 48 h}}{2 \times 10^5 \text{ cells per mL}^2} \times \frac{\text{RGDT}}{100}$$

Total suspension growth (SG) after 24 hour-exposure:

$$SG = \frac{\text{Cell count after 24 h}}{2 \times 10^5 \text{ cells per mL}} \times \frac{\text{Cell count after 48 h}}{2 \times 10^5 \text{ cells per mL}^2} \times \frac{\text{Cell count after 72 h}}{2 \times 10^5 \text{ cells per mL}^2}$$

$$\text{RSG} = \frac{\text{SG of the test group}}{\text{SG of the negative/vehicle control}} \times 100$$

$$RTG = \frac{RSG \times RCE_2}{100}$$

[1] Cell number seeded following 4-hour treatment
[2] If cell number was lower than 2 x 10^5 cells per mL all remaining cells were seeded
The uncorrected mutant frequency per 10^6 cells ($MF_{uncorr.}$) was calculated for each test group as follows:

$$MF_{uncorr} = \frac{-\ln(\text{total number of empty well s} \div \text{total number of seeded wells (96)})}{\text{number of seeded cells per well (2000)}} \times 10^6$$

The corrected mutant frequency (MF_{corr}) was calculated regarding the values of CE_2:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_2} \times 100$$

Determination of borderline mutant frequency based on GEF

The GEF (global evaluation factor) evaluation method requires that the MF exceeds a value based on the global distribution of the background MF of the test method. This value is defined as the mean of the negative/vehicle MF distribution plus one standard deviation. Based on a large data base (n = 493 experiments) from six laboratories a GEF of 126 mutant colonies per 10^6 cells (mean MF_{corr} = 99 x10^{-6} colonies; standard deviation = 27 x10^{-6} colonies) was calculated for the microwell method. To be judged positive the mutation frequency has to exceed a threshold of 126 colonies per 10^6 cells (GEF) above the concurrent negative/vehicle control value.

4. Statistics:

An appropriate statistical method to test for linear trend (MS EXCEL function RGP; 10) was performed to assess a possible linear dose-relation in mutant frequencies. The dependent variable was the corrected mutant frequency and the independent variable was the concentration. A trend was judged as statistically significant whenever the one-sided p-value (probability value) was below 0.05 and the slope was greater than 0. However, both, biological and statistical significance have been considered together.

5. Acceptability criteria:

A mutation assay is considered acceptable if it meets the following criteria (the current recommendations of the IWGT are considered):

- The absolute cloning efficiency obtained at the time of mutant selection (CE_2) of the negative/vehicle controls should fall in the range of 65 - 120%.
- The suspension growth (SG) of the negative/vehicle controls referring to the expression period following treatment should fall in the range of 8 - 32 for 4-hour exposure and 32 - 180 for 24-hour exposure.
- The mutant frequency of the negative/vehicle controls should fall within the range of 50 - 170 $x10^{-6}$ colonies.
- The positive controls should yield an absolute increase in total MF that is an increase above the spontaneous background MF (an induced MF [IMF]) of at least 300 $x10^{-6}$ colonies. The small colony MF should account for at least 40% of that IMF, means a small colony IMF of at least 120 x 10^{-6} colonies. Alternatively, the positive controls should induce at least 150 x 10^{-6} small colonies above the spontaneous background MF. The upper limit of cytotoxicity observed in the positive controls should have a RTG that is greater than 10%.
- The highest applied concentration of the test substance should be 2 mg/mL, 2 µL/mL or 10 mM, unless limited by cytotoxicity or solubility of the test substance. If toxicity occurs, the highest concentration should lower the RTG to 10 to 20% of survival. If precipitation occurs, the highest evaluated concentration should be the lowest concentration, where precipitation is observed by the unaided eye.

6. Evaluation criteria:

A test item is classified as mutagenic **if all** of the following criteria are met:

- The induced mutation frequency reproducibly exceeds a threshold of 126 colonies per 10^6 cells (GEF: Global Evaluation Factor) above the corresponding solvent control.
- Evidence of reproducibility of any increase in mutant frequencies, means the mutagenic response occurs at least in both parallel cultures of one experiment.
- A statistically significant dose-related increase in mutant frequencies using an appropriate statistical trend test.

The test substance is considered non-mutagenic **if at least one** of the following criteria are met:

- The mutation frequency is below a threshold of 126 colonies per 10^6 cells (GEF: Global Evaluation Factor) above the concurrent solvent control value.
- No evidence of reproducibility of an increase in mutant frequencies is obtained.
- No statistical significant dose-related increase in mutant frequencies using an appropriate statistical trend test.

However, in the evaluation of the test results the historical variability of the mutation rates in the solvent controls of this study were taken into consideration.
Results of test groups are generally rejected if the relative total growth is less than 10% of the vehicle control.
Whenever a test item is considered mutagenic according to the above mentioned criteria, the ratio of small versus large colonies is used to differentiate point mutations from clastogenic effects. If the increase of the mutation frequency is accompanied by a reproducible and dose dependent shift in the ratio of small versus large colonies clastogenic effects are indicated.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY

In culture medium test substance precipitation occurred at 65.6 µg/mL and above at the end of treatment in the absence and the presence of S9 mix. After 4 hours treatment in the absence of S9 mix cytotoxicity indicated by reduced relative suspension growth of about or below 20% was observed at 131.3 µg/mL and above, while in the presence of S9 mix the cultures were not continued due to strong cytotoxicity at concentrations ≥ 65.6 µg/mL.

B. TREATMENT CONDITIONS

Osmolarity and pH values were not influenced by test substance treatment. In the 1st experiment, test substance precipitation in the culture medium was noted at the end of treatment at the highest concentration (100 µg/mL) in absence and presence of metabolic activation. In the 2nd experiment no precipitation was noted at the end of treatment.

C. CYTOTOXICITY

In the 1st experiment, no clear cytotoxic effects (indicated by reduced cloning efficiencies or reduced relative total growth (RTG) of below 20% of control) were observed irrespective of the presence of S9 mix. However, in both experimental parts at the highest applied concentrations, the RTG was reduced [see Table 5.8.1-62].
In contrary, in the 2nd experiment a decreased relative total growth was observed in the absence of S9 mix at 25 µg/mL (RTG: 17.7%) and above. In the presence of S9 mix a RTG of 14.0% was noted at 100 µg/mL [see Table 5.8.1-63].

D. MUTAGENICITY ASSAYS

No biologically relevant increase in the number of mutant colonies was observed in the 1st and 2nd experiment without metabolic activation after 4 and 24 hour exposure. In line with the recommendations of the current OECD Guideline No. 490, the mutation rate obtained in the 2nd experiment without S9 (24 hour exposure) at 50 µg/mL ($MF_{corr.}$: 166.0 per 10^6 cells) was excluded from assessment due to extremely strong cytotoxicity (RTG: 4.4%).

In contrast, a statistically significant and concentration related increase in the number of mutant colonies was observed after the addition of a metabolizing system in both experiments [see Table 5.8.1-62 and Table 5.8.1-63]. The corrected mutant frequencies were above the calculated threshold for a biologically relevant increase taking the Global Evaluation Factor (GEF) into consideration. The increase in mutation rates in the experiments in the presence of S9 mix displayed a statistically significant concentration dependent trend.

In both experiments at higher concentrations, the ratio of small to large colonies was close to the observation of effects after treatment with the positive control CPP. Thus, it has to be considered that the test substance may have induced clastogenic effects.

Both positive control substances known to induce gene mutations, MMS and DMBA, and the well-known clastogen CPP led to clearly increased mutant frequencies as expected. Currently only a small data set for the positive control substance DMBA is available for comparison. The mutant frequencies of the positive control clearly exceeded the respective calculated thresholds for a mutagenic effect based on the GEF. In addition, the corrected mutant frequencies were within or above our historical positive control data range (222.2 – 1496.6 per 10^6 cells; see report Appendix 7).

Table 5.8.1-62: Gene mutation in mammalian cells - 1st experiment

Test group	$MF_{corr.}$ (per 10^6 cells)	Mean colony counts (%)		Toxicity data			Cloning efficiency (viability)	
		small	large	SG	RSG	RTG	absolute	relative
Without metabolic activation; 4-hour exposure period								
Vehicle (DMSO)	56.6[a]	82	18	25.2	100.0	100.0	110	100
Test item (µg/mL)								
1.56	34.6	86	14	25.8	102.0	93.9	101	92
3.13	54.0	83	17	23.9	94.8	90.2	105	95
6.25	55.2	80	20	23.4	92.7	83.9	100	91
12.5	57.0	90	10	21.7	86.1	75.5	97	88
25	26.3	86	14	17.7	70.2	78.2	123	111
50	30.5	88	13	15.4	61.0	71.3	129	117
100	47.2	80	20	12.7	50.2	49.0	107	98
MMS 15 µg/mL	800	83	17	14.3	56.7	29.4	57	52
With metabolic activation; 4-hour exposure period								
Vehicle (DMSO)	51.0[b]	70	30	14.8	100.0	100.0	102	100
Test item (µg/mL)								
1.56	59.0	79	21	11.0	74.2	85.9	118	116
3.13	55.2	91	9	12.6	85.1	83.0	100	98
6.25	61.2	79	21	12.3	82.8	92.4	114	112
12.5	87.9	90	10	9.1	61.7	71.4	118	116
25	170.0	88	13	6.9	46.7	43.5	95	93
50	**265.9**	84	16	5.8	38.9	41.2	108	106
100	**298.6***	86	14	5.4	36.3	31.1	87	86
CPP 2.5 µg/mL	342.1	90	10	10.5	71.1	58.6	84	82
DMBA 1 µg/mL	524.0	71	29	7.1	48.0	32.3	69	67

#: culture was not continued due to exceedingly severe cytotoxic effects
*: treatment with S9 mix was statistically significant for an increased mutant frequency using the linear trend test ($p<0.05$)
[a] Borderline MF for a positive response: 183 per 10^6 cells
[b] Borderline MF for a positive response: 177 per 10^6 cells

Table 5.8.1-63: Gene mutation in mammalian cells – 2nd experiment

Test group	$MF_{corr.}$ (per 10^6 cells)	Mean colony counts (%)		Toxicity data			Cloning efficiency (viability)	
		small	large	SG	RSG	RTG	absolute	relative
Without metabolic activation; 24-hour exposure period								
Vehicle (DMSO)	77.9[a]	93	7	64.8	100.0	100.0	97	100
Test item (µg/mL)								
1.56	79.1	94	6	66.9	103.3	101.7	96	98
3.13	69.2	92	8	67.0	103.5	93.6	88	90
6.25	72.5	92	8	51.1	78.9	74.7	92	95
12.5	104.3	90	10	29.7	45.9	45.5	97	99
25	97.4	96	4	10.3	15.9	17.7	108	111
50	166.0	93	7	3.5	5.4	4.4	81	83
100	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
MMS 5 µg/mL	744.7	90	10	41.1	63.5	36.2	56	57
With metabolic activation; 4-hour exposure period								
Vehicle (DMSO)	68.2	84	16	15.2	100.0	100.0	138	100
Test item (µg/mL)								
4.69	101.9	88	13	13.4	88.0	71.9	113	82
9.38	142.6	85	15	11.0	72.7	58.9	112	81
18.75	**239.9**	89	11	9.2	60.4	40.5	93	67
37.5	**348.8**	93	7	8.0	52.4	34.9	92	67
50	**427.6**	92	8	6.5	42.7	26.9	87	63
75	**455.8**	91	9	5.1	33.7	22.1	91	66
100	**528.7***	92	8	4.1	26.8	14.0	72	52
CPP 2.5 µg/mL	453.8	93	7	11.3	74.5	45.6	85	61
DMBA 1 µg/mL	1507.4	77	23	6.1	40.0	13.3	46	33

n.c.: culture was not continued due to exceedingly severe cytotoxic effects
*: treatment with S9 mix was statistically significant for an increased mutant frequency using the linear trend test ($p<0.05$)
[a] Borderline MF for a positive response: 204 per 10^6 cells
[b] Borderline MF for a positive response: 194 per 10^6 cells

III. CONCLUSION

Under the experimental conditions chosen in this test, it is concluded that Reg.No. 399379 (metabolite of pyraclostrobin) and/or its metabolites induced forward mutations and/or chromosomal aberrations in vitro in the mouse lymphoma assay with L5178Y $TK^{+/-}$ cells in the presence of metabolic activation.

Report:	CA 5.8.1/45 Chang S., 2016 b Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in human lymphocytes in vitro 2016/1109533
Guidelines:	OECD 487 (2014), Commission Regulation (EU) No 640/2012, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.49
GLP:	yes (certified by Hessisches Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany)

Executive Summary

Reg.No. 399379 (metabolite of pyraclostrobin; batch: L87-58; purity: 97.6%) was tested for its potential to induce micronuclei in human lymphocytes in vitro in the absence and presence of metabolic activation by S9 mix. At least two independent experiments were performed where the cells were incubated for 4 (±S9 mix) or 20 hours (-S9 mix) with the test substance at concentrations in the range of 1.1 up to 2049 µg/mL. The vehicle DMSO served as negative control, mitomycin C (4 h) and demecolcin (20 h) as positive controls in the absence of metabolic activation and cyclophosphamide as positive control in the presence of metabolic activation. Treatments started after a 48 hour stimulation period with phytohemeagglutinine. Twenty hours after commencement of treatment, cytochalasin B was added and the cultures were fixed and stained finally after another 20 hours. Cytokinesis-block proliferation index and cytostasis were determined in 500 binucleated cells/culture as cytotoxicity parameters and number of micronucleated cells were determined in 1000 binucleated cells/culture for evaluation of cytogenetic damage.

In the pulse (4h) experiments IA, IB and II in presence and absence of S9 mix, no cytotoxicity was observed up to the highest evaluated concentration, which is limited by precipitation. However, the continuous exposure experiment (20h) without metabolic activation (experiment II), the lowest precipitating concentration could not be evaluated due to strong cytotoxic effects. No relevant influence on osmolarity or pH value was observed.

In this study, neither a statistically significant nor a biologically relevant increase in the number of micronucleated cells was observed after treatment with the test item. The micronucleus rates of the cells after treatment with the test item were close to the range of the respective solvent controls and within the range of the laboratory historical control data (HCD). The positive control chemicals led to the expected increase in cells containing micronuclei, thus demonstrating the sensitivity of the test system.

In conclusion, Reg.No. 399379 (metabolite of pyraclostrobin) is considered to be non-mutagenic in this in vitro micronucleus test when tested up to precipitating or the highest evaluable concentration.

(BASF DocID 2016/1109533)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg. No. 399379 (metabolite of pyraclostrobin)

Description:	Solid, orange
Density:	NA
Lot/Batch #:	L87-58
Purity/content:	97.6% (tolerance ± 1.0%) Impurities: 1.6% Reg.No. 369315 (azo), 0.2% Reg.No. 364380 (azoxy)
Stability of test compound:	The stability of the test item under storage conditions over the study period was guaranteed by the sponsor (expiry date June 01, 2017).
Vehicle used:	DMSO (0.5% or 1% final concentration)

2. Control Materials:

Negative:	No negative control was employed in this study.
Vehicle control:	Culture medium with 0.5% (Exp. 1) and 1% (Exp. 2) DMSO
Positive control:	Without metabolic activation: Mitomycin C (MMC, 1 µg/mL; pulse treatment) dissolved in deionized water; Demecolcin (150 ng/mL; continuous treatment) dissolved in deionized water With metabolic activation: Cyclophosphamide (CPA, 12.5-17.5 µg/mL) dissolved in saline (0.9% NaCl)

3. Activation: S9 was produced from the livers of rats pretreated with β-naphthoflavone/phenobarbital. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction was thawed at room temperature and mixed with an appropriate volume of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

A final protein concentration of 0.75 mg/mL was used. The S9 preparation had a protein concentration of 27.2 mg/mL.

4. Test organism: Human peripheral blood lymphocytes (drawn from healthy non-smoking donors not receiving medication)

5. Culture media:

Culture medium: Dulbecco´s Modified Eagles medium/Ham´s F12 (1:1) with GlutaMAX™ (200 mM) supplemented with 10% (v/v) fetal bovine serum (FBS), Pen/Strep (100 U/mL/100 µg/mL), HEPES (10 mM), heparin (125 U.S.P.-U/mL), phytohemeagglutinine (PHA, 3 µg/mL)

6. Test concentrations: (**bold**: groups evaluated for cytogenicity)

Experiment IA
(4-h exposure, ±S9): **2.6, 4.6, 8.0, 14**, 24.5, 42.8, 74.9, 131, 328, 820, 2049 µg/mL

Experiment IB
(4-h exposure, ±S9): **1.9, 3.3, 5.8**, 10.2, 17.8, 31.2, 54.6, 95.6, 167, 585, 2049 µg/mL

Experiment II
(4-h exposure, +S9): 1.1, 2.0, **3.5, 6.1, 10.7**, 18.7, 32.7, 57.1, 100, 175 µg/mL
(20-h exposure, -S9): 1.1, 2.0, 3.5, 6.1, **10.7, 18.7, 32.7**, 57.1, 100, 175 µg/mL

B. TEST PERFORMANCE

1. Dates of experimental work: 06-Apr-2016 to 24-May-2016

2. Dose selection:

Dose selection was performed according to the current OECD Guideline for the in vitro micronucleus test. The highest test item concentration should be 2000 µg/mL, 2 µL/mL or 10 mM, whichever is the lowest. At least three test item concentrations should be evaluated for cytogenetic damage.

A preliminary cytotoxicity test was performed to determine the concentrations to be used in the main experiment. Cytotoxicity is characterized by the percentages of reduction in the CBPI (Cytokinesis-block proliferation index) in comparison with the controls (% cytostasis) by counting 500 cells per culture in duplicate. The experimental conditions in this pre-experimental phase were identical to those required and described below for the mutagenicity assay. The pre-test was performed with 11 concentrations of the test item separated by no more than a factor of √10 and a solvent and positive control. All cell cultures were set up in duplicate. Exposure time was 4 hours (with and without S9 mix). The preparation interval was 40 hours after start of the exposure.

With regard to the purity (97.6 %) of the test item, 2049.0 µg/mL of Reg.No. 399379 (metabolite of pyraclostrobin) were applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations ranging from 1.1 to 2049 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.

Precipitation of the test item was observed at the end of treatment at 8.0 µg/mL and above in the absence of S9 mix and at 14.0 µg/mL and above in the presence of S9 mix. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment IA.
On request of the sponsor, Experiment IA was repeated with 1% DMSO as solvent to achieve a solution and to analyze the precipitation limit in more detail by using the same top concentration (Exp. IB). In this experiment, precipitation of the test item was observed at the end of treatment at 5.8 µg/mL and above in the absence and presence of S9 mix.
Considering the precipitation in the experiments IA and IB, 175 µg/mL (with and without S9 mix) were chosen as top concentration in Experiment II.

3. Micronucleus test:

Pulse exposure:
About 48 h after seeding two blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with complete medium (with 10 % FBS) containing the test item. After 20 hours the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in and washed with saline. The washing procedure was repeated once as described. After washing the cells were re-suspended in complete culture medium containing 10 % FBS (v/v). Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Continuous exposure:
About 48 h after seeding two blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with complete medium (with 10 % FBS) containing the test item. After 20 hours the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in and washed with saline. The washing procedure was repeated once as described. After washing the cells were re-suspended in complete culture medium containing 10 % FBS (v/v). Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Preparation of cells:
The cultures were harvested by centrifugation 40 h after beginning of treatment. The cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in approximately 5 mL saline G and spun down once again by centrifugation for 5 minutes. Then the cells were re-suspended in 5 mL KCl solution (0.0375 M) and incubated at 37°C for 20 minutes. 1 mL of ice-cold fixative mixture of methanol and glacial acetic acid (19 parts plus 1 part, respectively) was added to the hypotonic solution and the cells were re-suspended carefully. After removal of the solution by centrifugation the cells were re-suspended for 2 x 20 minutes in fixative and kept cold. The slides were prepared by dropping the cell suspension in fresh fixative onto a clean microscope slide. The cells were stained with Giemsa.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Chi square test.

5. Cytotoxicity evaluation:

Evaluation of cytotoxicity and cytogenetic damage:

Evaluation of the slides was performed using NIKON microscopes with 40 x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The micronuclei have to be stained in the same way as the main nucleus. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 binucleate cells per culture were evaluated for cytogenetic damage on coded slides. The frequency of micronucleated cells was reported as % micronucleated cells. To describe a cytotoxic effect, the CBPI (Cytokinesis-block proliferation index) was determined in 500 cells per culture and cytotoxicity is expressed as % cytostasis. A CBPI of 1 (all cells are mononucleate) is equivalent to 100 % cytostasis.

$$CBPI = \frac{(MONC \times 1) + (BINC \times 2) + (MUNC \times 3)}{n}$$

CBPI	Cytokinesis-block proliferation index
n	Total number of cells
MONC	Mononucleate cells
BINC	Binucleate cells
MUNC	Multinucleate cells

$$\text{Cytostasis \%} = 100 - 100\,[(CBPI_T - 1) / (CBPI_C - 1)]$$

T	Test item
C	Solvent control

6. Evaluation criteria:

Acceptability criteria:

- The rate of micronuclei in the solvent controls falls within the historical laboratory control data range.
- The rate of micronuclei in the positive controls is statistically significant increased.
- The quality of the slides must allow the evaluation of a sufficient number of analyzable cells.

Evaluation criteria:

A test item can be classified as non-clastogenic and non-aneugenic if:

- the number of micronucleated cells in all evaluated dose groups is in the range of the historical laboratory control data and
- no statistically significant or concentration-related increase of the number of micronucleated cells is observed in comparison to the respective solvent control.

A test item can be classified as clastogenic and aneugenic if:

- the number of micronucleated cells is not in the range of the historical laboratory control data and
- either a concentration-related increase in three test groups or a statistically significant increase in the number of micronucleated cells is observed.

If the above-mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

An increase in the number of micronucleated mononucleate cells may indicate that the test item has aneugenic potential.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in DMSO over 4 h was verified analytically (see separate report 01Y0701/14Y117).

B. CYTOTOXICITY

The highest treatment concentration in this study, 2049 µg/mL was chosen with regard to the purity (97.6 %) of the test item and with respect to the OECD Guideline 487 for the in vitro mammalian cell micronucleus test.
In Experiment IA, precipitation of the test item was observed at the end of treatment at 8.0 µg/mL and above in the absence of S9 mix and at 14.0 µg/mL and above in the presence of S9 mix. In Experiment IB, precipitation of the test item was observed at the end of treatment at 5.8 µg/mL and above irrespective of the presence of S9 mix. In addition, precipitation occurred in Experiment II in the absence of S9 mix at 57.1 µg/mL and above and in the presence of S9 mix at 10.7 µg/mL and above at the end of treatment.
No relevant influence on osmolarity or pH was observed.
When using 1% DMSO as solvent, the osmolarity is generally high compared to the physiological level of approximately 300 mOsm. This effect however, is based on a final concentration of 1% DMSO in medium. As the osmolarity is measured by freezing point reduction, 1% of DMSO has a substantial impact on the determination of osmolarity. The outcome of the study is not affected by this impact.
In Experiment IA and IB and in Experiment II in the presence of S9 mix, no cytotoxicity was observed up to the highest evaluated concentration, which is limited by precipitation. However, in Experiment II in the absence of S9 mix (20 hour treatment) strong cytotoxicity was observed at the first precipitating concentration (57.1 µg/mL), preventing the evaluation of the cultures.

B. MICRONUCLEUS ASSAY

In this study, neither a statistically significant nor a biologically relevant increase in the number of micronucleated cells was observed after treatment with the test item [see Table 5.8.1-64 and Table 5.8.1-65].

Table 5.8.1-64: Summary of results of the in vitro micronucleus test in human lymphocytes with Reg.No. 399379 (metabolite of pyraclostrobin) without S9 mix

Exp.	Preparation interval	Test item concentration in µg/mL	Proliferation index CBPI	Cytostasis in %[a]	Micronucleated cells in %[b]
Exposure period 4 hours without S9 mix					
IA	40 hours	Solvent control[1]	1.98		0.30
		Positive control[3]	1.97	0.9	**10.05***
		2.6	2.08	n.c.	0.55
		4.6	1.93	4.8	0.30
		8.0[P]	1.88	10.7	0.20
IB	40 hours	Solvent control[2]	2.09		0.65
		Positive control[3]	1.74	32.2	**7.25***
		1.9	2.08	0.4	0.30
		3.3	2.05	3.9	0.65
		5.8 [P]	1.98	9.9	0.35
Exposure period 20 hours without S9 mix					
II	40 hours	Solvent control[2]	1.85		0.30
		Positive control[4]	1.66	21.9	**5.20***
		10.7	1.72	15.1	0.15
		18.7	1.78	8.3	0.20
		32.7	1.72	14.6	0.15

a: For the positive control groups and the test item treatment groups the values are related to the solvent controls
b: The number of micronucleated cells was determined in a sample of 2000 binucleated cells
*: The number of micronucleated cells is statistically significantly higher than corresponding control values
P: Precipitation occurred at the end of treatment
n.c.: Not calculated as the CBPI is equal or higher than the solvent control value
[1]DMSO 0.5 % (v/v)
[2]DMSO 1.0 % (v/v)
[3]MMC 1 µg/mL
[4]Demecolcin 150 ng/mL

Table 5.8.1-65: Summary of results of the in vitro micronucleus test in human lymphocytes with Reg.No. 399379 (metabolite of pyraclostrobin) with S9 mix

Exp.	Preparation interval	Test item concentration in µg/mL	Proliferation index CBPI	Cytostasis in %[a]	Micronucleated cells in %[b]
Exposure period 4 hours with S9 mix					
IA	40 hours	Solvent control[1]	2.09		1.00
		Positive control[3]	1.77	29.0	**8.25***
		4.6	1.97	10.7	0.75
		8.0	2.01	7.0	0.60
		14.0[P]	1.89	17.7	0.70
IB	40 hours	Solvent control[2]	2.05		0.65
		Positive control[4]	1.75	28.3	**4.00***
		1.9	2.03	2.1	0.45
		3.3	2.04	0.8	0.55
		5.8 [P]	2.11	n.c.	0.45
II	40 hours	Solvent control[2]	2.22		0.75
		Positive control[5]	1.77	36.8	**6.65***
		3.5	2.23	n.c.	0.50
		6.1	2.18	3.8	0.40
		10.7[P]	2.03	16.1	0.35

a: For the positive control groups and the test item treatment groups the values are related to the solvent controls

b: The number of micronucleated cells was determined in a sample of 2000 binucleated cells

*: The number of micronucleated cells is statistically significantly higher than corresponding control values

P: Precipitation occurred at the end of treatment

n.c.: Not calculated as the CBPI is equal or higher than the solvent control value

1 DMSO 0.5 % (v/v)

2 DMSO 1.0 % (v/v)

3 CPA 15 µg/mL

4 CPA 12.5 µg/mL

5 CPA 17.5 µg/mL

The micronucleus rates of the cells after treatment with the test item were close to the range of the respective solvent control values and within the range of the laboratory historical control data (HCD). The HCD in the absence of S9 mix were 0.15-1.25% (95% control limits: 0.02 to 1.15%) for (4h) pulse treatment and 0.05-1.43% (95% limits: 0.05 – 1.05%) for continuous (20h) treatment. The HCD in the presence of S9 mix were 0.15-1.35% (95% limits: 0.08 – 1.20%) for pulse treatment.
All positive controls used, i.e. Demecolcin (150.0 ng/mL), MMC (1.0 µg/mL) or CPA (15.0, 12.5 or 17.5 µg/mL), caused distinct increases in the number of cells with micronuclei (4.0 – 10.05%), indicating the sensitivity of the test system.

III. CONCLUSION

In conclusion, it can be stated that under the experimental conditions reported, Reg.No. 399379 (500M106) did not induce micronuclei as determined by the in vitro micronucleus test in human lymphocytes. Therefore, the test item is considered to be non-mutagenic in this in vitro micronucleus test when tested up to precipitating or the highest evaluable concentrations.

Based on the positive invitro gene mutation assay in mammalian cells (mouse lymphoma $TK^{+/-}$ assay (MLA), higher tier in vivo mutagenicity assays were conducted. As the high number of small mutant colonies in the MLA indicated the possible induction of clastogenic effects, an in vivo micronucleus assay in mice was performed. The possible induction of gene mutations in vivo was investigated in a transgenic rodent assay using the Muta™Mouse model. The exposure of the target organs in these two assays was confirmed in a study investigating the absorption and distribution of ^{14}C-Reg.No. 399379 in mice using the same vehicle as in the in vivo mutagenicity assays.

Report:	CA 5.8.1/46 [redacted] 2016 a Reg.No. 399379 (Metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus assay in bone marrow cells of the mouse 2016/1295116
Guidelines:	OECD 474 (2016), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395
GLP:	yes (certified by Hessisches Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany)

Report:	CA 5.8.1/47 Grauert E., Hidding B., 2017 a Reg.No. 399379 (Metabolite of BAS 500 F, Pyraclostrobin) - Homogeneity and Concentration Control Analyses in 0.5 % carboxymethyl cellulose / steril water (w/v) 2017/1020704
Guidelines:	not applicable
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 399379 (500M106; metabolite of pyraclostrobin; batch: L87-58; purity: 97.6%) was tested for chromosomal damage (clastogenicity) and for the ability to induce spindle poison effects (aneugenic activity) in NMRI mice using the micronucleus test method. For this purpose, the test substance was administered once orally to groups of 7 male mice at dose levels of 500, 1000 and 2000 mg/kg bw. The vehicle served as negative and cyclophosphamide as positive control. The animals were sacrificed 24 or 48 (additional high dose and vehicle group) hours after the administration and the bone marrow was prepared and 4000 polychromatic erythrocytes per animal were scored for micronuclei.

The oral administration of Reg.No. 399379 did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing micronuclei. The rate of micronuclei was close to the concurrent negative control and was within the range of the historical control data. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur. A separate study demonstrated that Reg.No. 379379 and its metabolites reached the bone marrow. Weak signs of systemic toxicity were observed in all dose groups consisting of ruffled fur.

The positive control cyclophosphamide led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system.

In conclusion, under the experimental conditions of this study, the test substance Reg. No. 399379 (500M106) does not induce cytogenetic damage in bone marrow cells of NMRI mice in vivo.

(BASF DocID 2016/1295116)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	Reg.No. 399379 (metabolite of pyraclostrobin)
	Description:	Solid, orange
	Lot/Batch #:	L87-58
	Purity:	97.6% (tolerance ± 1.0%) Impurities: 1.6% Reg.No. 369315 (azo), 0.2% Reg.No. 364380 (azoxy)
	Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed until 01 June 2018 as indicated by the sponsor. Homogeneity of the test substance was guaranteed on account of the high purity and was ensured by mixing before test substance preparation.
	Vehicle used:	0.5% aqueous Carboxy Methyl Cellulose (CMC)

2.	**Control Materials:**	
	Negative:	No negative control was employed in this study.
	Vehicle control:	0.5% aqueous CMC
	Positive control:	Cyclophosphamide (CPA) 40 mg/kg, dissolved in water

3. Test animals:

Species:	Albino mice
Strain:	Crl:NMRI
Sex:	Males
Age:	6 - 7 weeks at commencement of treatment
Mean body weight at dosing:	36.3 ± 1.3 g
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	7
Acclimation period:	At least 5 days
Diet:	Standardized pelleted feed (Maus/Ratte Haltung "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland)
Water:	Drinking water in bottles, ad libitum
Housing:	During the study the mice were housed individually in Makrolon cages, type MII

4. Environmental conditions:

Temperature:	22 ± 2°C
Humidity:	45 - 65%
Air changes:	frequency not indicated (fully air-conditioned rooms)
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)
Husbandry:	Single housing in Makrolon Type II cages with wire mesh top and granulated soft wood bedding
Diet:	Standard pelleted diet (Teklad Certified Global 18% Protein Rodent diet)

5. Test compound doses:

Micronucleus assay:	500, 1000 and 2000 mg/kg The test substance was administered once by oral gavage using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 15-Sep-2016 to 04-Oct-2016

2. Preliminary range finding test:

No preliminary range finding test was performed based on existing data showing that the compound is virtually non-toxic to mice.

3. Micronucleus test:

Treatment and sampling:	For the 24-hour sacrifice interval, groups of 7 male mice were treated once with either vehicle or 500, 1000 or 2000 mg test item/kg bw or the positive control Cyclophosphamide at 40 mg/kg bw by oral gavage. For the 48-hour sacrifice interval, additional groups of 7 male mice were treated with either the vehicle control or the high dose 2000 mg/kg bw. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The animals were observed for evident clinical signs of toxicity at intervals of around 0-1 h, 2-4 h, 5-6 h, 24 h and/or 48 h. Twenty-four or 48 hours after administration, mice were sacrificed and the two femora were prepared. The epiphyses were cut off and the marrow was flushed out with foetal calf serum. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald/Giemsa. At least one slide was made from each bone marrow sample. Additional bone marrow smears (3 additional slides per animal) were made and were air-dried and fixed with ice-cold absolute methanol (fixation time: 10 minutes). These slides were stored frozen at ≤ -18°C until possible further use.
Slide evaluation:	Microscopic evaluation of the slides was at 100x magnification. Per animal 4000 polychromatic erythrocytes (PCE) were analyzed for micronuclei. To describe a cytotoxic effect, the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 500 erythrocytes. The analysis was performed with coded slides. All animals per group were evaluated.

4. Statistics:

The study results were tested for statistical significance using the non-parametric Mann-Whitney test.

5. Acceptance criteria:

The study is considered acceptable if the following criteria are met:

- The concurrent negative control is considered acceptable for addition to the laboratory historical control database (should ideally be within the 95% control limits of the distribution of the historical negative control database).
- At least 5 animals per group can be evaluated.
- The appropriate number of doses and cells have been analyzed.
- PCE to total erythrocyte ratio should not be less than 20% of the negative control.
- The positive control shows a statistically significant increase of micronucleated PCEs compared to the negative control and is comparable to those in the historical positive control database.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- At least one of the treatment groups exhibits a statistically significant increase in the frequency of micronucleated immature erythrocytes compared with the concurrent negative control,
- This increase is dose-related at least at one sampling time when evaluated with an appropriate trend test, and
- Any of these results are outside the distribution of the historical negative control data (e.g. Poisson-based 95% control limits).

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle was verified analytically (see Study 01Y0701/14Y119 included in Volume III of the Reg.No. 399379 28-day study (BASF DocID 2016/1288407)). The homogeneity of the test substance in the vehicle was guaranteed by constant stirring. As indicated by relative standard deviations in the range of 1.8 to 2.5% the dosing suspensions were homogenous and within the expected range (90-110%) [see Table 5.8.1-66].

Table 5.8.1-66: Analysis of Reg.No. 399379 dosing suspension for homogeneity and test-item content

Nominal concentration [mg/mL]	Sampling	Concentration measured [g/100 ml] Mean ± SD[#]	Relative standard deviation [%]	% of nominal concentration
50	20.09.2016	44.7 ± 1.0	2.3	89.5
100	20.09.2016	100.6 ± 1.8	1.8	100.6
200	20.09.2016	189.0 ± 4.7	2.5	94.5

[#] Values may not calculate exactly due to rounding of values

B. PRELIMINARY RANGE FINDING TEST

Existing information indicated that Reg.No. 399379 is of low toxicity to mice. Thus, no range finding study was conducted in the context of this micronucleus study in mice.

C. MICRONUCLEUS ASSAY

1. Clinical signs of toxicity

No mortality was observed up to the highest dose tested (2000 mg/kg bw). A dose dependent increase of the incidence of ruffled fur was noted, which (with the exception of one mid dose mouse) was reversible within 24 hours.

2. Evaluation for micronuclei

Micronucleus frequencies of Reg.No. 399379 treated animals were near to the concurrent vehicle control values irrespective of the applied dose or the sampling time [see Table 5.8.1-67] as well as within the historical control range [see Table 5.8.1-68].
The positive control cyclophosphamide resulted in a statistically significant increase of the number of micronuclei in polychromatic erythrocytes, thus demonstrating the sensitivity of the test system.

3. Proof of bone marrow exposure

The PCE/NCE ratio was not affected by treatment with the test substance [see Table 5.8.1-67], i.e. no adverse effect on the bone marrow was noted. However, administration of ^{14}C-Reg.No. 399379 to male mice at a dose of 500 mg/kg by oral gavage revealed radioactivity equivalent to 109.4 µg Eq/g plasma and 46.1 µg Eq/g bone marrow 4 hours after administration [see below BASF DocID 2016/1225930]. These data clearly demonstrate that the bone marrow as highly blood perfused organ is exposed to Reg.No. 399379 and its metabolites.

Table 5.8.1-67: Micronucleus test in mice administered Reg.No. 399379 by oral gavage

Test Group	Dose [mg/kg bw]	Sampling time	MN/4000 PCE [Mean ± SD]	% MN [Mean ± SD]	Range		Ratio PCE /total Ery	% ratio Vehicle
					min	max		
Vehicle (0.5% CMC)	0	24	4.6 ± 1.9	0.12 ± 0.05	2	7	0.648	100.00
Dose 1	500	24	6.7 ± 3.2	0.17 ± 0.08	2	11	0.625	96.45
Dose 2	1000	24	6.9 ± 2.4	0.17 ± 0.06	4	9	0.655	101.08
Dose 3	2000	24	6.9 ± 2.0	0.17 ± 0.05	4	10	0.636	98.15
Positive (CPA)	40	24	92.4 ± 15.1*	2.31 ± 0.38	74	121	0.606	93.52
Vehicle (0.5% CMC)	0	48	5.0 ± 1.3	0.13 ± 0.03	3	7	0.679	100.00
Reg.No. 399379	2000	48	6.4 ± 3.5	0.16 ± 0.09	2	12	0.673	99.12

MN = Micronuclei; PCE: Polychromatic erythrocytes; CPA: Cyclophosphamide
* $p < 0.005$ non-parametric Mann-Whitney test

Table 5.8.1-68: Historical control data for micronuclei in negative and positive controls
(September 2014 to September 2016; 4000 PCE/animal evaluated)

	Negative Controls Males	Positive Controls (CPA) Males
Mean (%)	0.123	2.713
95% Control limit	-0.030 – 0.276	0.724 – 4.702
Standard Deviation (%)	0.076	0.995
2 x Standard Deviation	0.153	1.989
Range of mean group value (%)	0.000 – 0.425	0.925 – 5.425
Range (individual animal data)	0 – 17	37 – 217
No. of Experiments	37	37

III. CONCLUSION

Based on the result of this study Reg.No. 399379 (500M106) does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study, i.e. is devoid of clastogenic or aneugenic activity in vivo. A kinetic study demonstrated that Reg.No. 399379 reached the target organ (bone marrow) via the blood.

Report:	CA 5.8.1/48 [redacted], 2016 b 14C-Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin) - Study on the kinetics on mice 2016/1225930
Guidelines:	OECD 417, EPA 870.7485, JMAFF, (EC) No 440/2008 of 30 May 2008 - Part B No. L 142
GLP:	yes (certified by Landesanstalt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Rheinland-Pfalz, Mainz)

Executive Summary

A single target dose of 500 mg/kg bw of ^{14}C-Reg.No. 399379 (500M106) was administered to four male mice. Four hours after dosing, mice were sacrificed under isoflurane anesthesia and blood cells, plasma, bone marrow, liver, glandular und vestibule stomach were sampled and worked up for the detection of the radioactive residues in order to demonstrate that the test item and/or its metabolites reached the systemic circulation including organs/tissues. Corresponding samples of untreated mice served as controls.
The mean total radioactive residues (TRR) 4 h post dosing were 109.4 µg Eq/g in plasma and 46.1 µg Eq/g in bone marrow. In liver, blood cells, vestibule stomach and glandular stomach, mean total radioactive residues of 267.0, 33.8, 7798.5 and 397.2 µg Eq/g were found, respectively. Taken together, the data demonstrate that ^{14}C-Reg.No. 399379 and/or its metabolites reach the systemic circulation including bone marrow, vestibule and glandular stomach as well as liver after single oral administration of the test substance at a target dose of 500 mg/kg bw.

(BASF DocID 2016/1225930)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material (labelled):	^{14}C-Reg.No. 399379 (metabolite of pyraclostrobin)
Description:	Solid, orange
Lot/Batch #:	1166-1102
Purity (radiochemical):	>95% (96.8% according to the CoA)
Purity (chemical):	87.7%
Specific activity of AI:	6.44 MBq/mg
Specific activity:	29.6 MBq/g in acetonitrile
Stability of test compound:	The stability was verified within the current study.

2. Test Material (non-labelled):	Reg.No. 399379 (metabolite of pyraclostrobin)
Description:	Solid, orange
Lot/Batch #:	L87-58
Purity:	97.6%
Stability of test compound:	The stability was guaranteed by the sponsor (expiry date: 01 June 2017).
3. Vehicle:	0.5% CMC-solution (sodium carboxymethylcellulose) in tap water
4. Test animals:	
Species:	Mouse
Strain:	Crl:NMRI
Sex:	Male
Age:	4 weeks
Weight at dosing:	31.8 – 37.7 g
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld, Germany
Acclimation period:	At least 5 days
Diet:	Kliba lab diet (mouse / rat "GLP") pellets, Provimi Kliba SA, Kaiseraugst, Basel, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	During acclimatization: Polycarbonate Cages (Type III)
	During the experiment: individual housing in Polycarbonate Cages (1291H; PC, 820 cm^2, Techniplast) with steel wire mesh ground (7 x 7 mm mesh wire).
Environmental conditions:	
Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	15 per h
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 19-July-2016 to 22-Aug-2016

2. Test substance preparation:

^{14}C-Reg.No. 399379 in acetonitrile (51.51 MBq) was taken to dryness under a stream of nitrogen and resuspended with an appropriate volume of a 0.5% aqueous CMC containing 47.49 mg/g of the non-radioactive test item by stirring with a magnetic stirrer and additional ultrasonication.

Before start and at the end of the administration, samples were taken to determine the specific activity of the test substance in the preparation and to demonstrate the correct concentration of the test substance, its homogeneity, stability as well as its radiochemical purity.
Based on the current data, the nominal concentration of the test substance preparation was 50.2 mg/mL and the nominal specific activity was 21.5 MBq/mL. The dosed amount of radioactivity per animal was between 5.2 and 6.5 MBq.

2. Homogeneity/Concentration and stability control:

The amount of radioactivity of ^{14}C-Reg.No. 399379 and the total amount of Reg.No. 399379 (sum of ^{14}C and non-radioactive) in the test substance preparation was determined by LSC, Radio-HPLC as well as HPLC-UV in samples that were taken before and after administration of the test substance preparation to the animals. The samples were prepared for analysis using conventional methods described in standard operating procedures. The analyses of these samples allow to demonstrate the homogeneity, correctness of the concentration and the stability of the test substance in the test substance preparation.

3. Study design and test groups:

The study was designed to obtain data to demonstrate that the test substance reaches the systemic circulation and defined organs / tissues. For this purpose, blood cells, plasma, bone marrow from the two femora, liver, glandular und vestibule stomach were prepared.
Additionally, a number of other organs were collected and stored frozen for potential future investigations (lung, heart, kidney, testes, epididymis and gut).
Treated animals were sacrificed 4 hours after administration, control animals were not treated and were sacrificed independently of any administration.

Two experiments were performed according to the following scheme:

Experiment 0	
Animals:	4 males
Radioactivity per animal:	untreated control
Dosing:	Untreated
Analysis:	total radioactivity (for determination of the detection limit)
Experiment 1	
Animals:	4 males
Radioactivity per animal:	4 – 8 MBq
Dosing:	1 oral dose, 500 mg/kg bw
Analysis:	total radioactivity

4. Experimental procedure and observations:

The animals were dosed at a volume of 10 mL/kg bw by oral gavage.

A check for moribund and dead animals was conducted at least once daily. A cage side examination was conducted at least once daily for any signs of morbidity, pertinent behavioral changes and signs of overt toxicity.

The body weight was determined on the day of administration prior to dosing for the animals of experiment 1; the animals of experiment 0 were weighed subsequently.

5. Sample work up and measurement:

Whole blood samples were inverted several times to ensure homogeneity and then separated into plasma and blood cells by centrifugation.

After weighing, aliquots of plasma were mixed with scintillation cocktail (Hionic Fluor, Perkin Elmer) and analyzed for radioactivity without any additional treatment.

The liver was homogenized. Subsequently 1 mL Soluene®-350 was added to aliquots of the suspensions and incubated over night at about 37°C and isopropanol was added afterwards. Bleaching was performed with 1 mL 30% H_2O_2 followed by incubation at room temperature at least over night. Hionic Fluor was added before measurement of radioactivity by LSC.

Blood cells, vestibule and glandular stomach were dissolved in Soluene®-350 and processed as described above. Bone marrow from the two femora was removed with a cotton ball, which transferred into a scintillation vial. After addition of Soluene the samples were processed as described for the liver.

All values given in the project and in the report are background corrected. The background values of LSC analyses were 10 to 170 cpm. The quantification limit (LOQ) for LSC measurement was set twice the background. For further calculations (e.g. mean values and standard deviations) samples < LOQ were set to 0.

II. RESULTS AND DISCUSSION

A. ANALYTICS

Concentration control analysis revealed that the dosing suspension had a concentration of 96.9% ± 4.9 (Mean ± RSD) of the nominal content (50.2 mg/mL).
The measured specific activity of the test-substance preparation (16.8 MBq/ml) compared to its target value (20 MBq/mL) was assessed to be acceptable within the precision of the method. Radio-HPLC analyses confirmed the stability of the radiolabelled test substance over the administration period by radiochemical purities of ^{14}C-Reg.No. 399379 (metabolite of pyraclostrobin) > 95%.

B. CLINICAL OBSERVATIONS AND MORTALITY

No mortality occurred during the study period. No clinical signs and findings were observed.

C. BODY WEIGHT

As to be expected for a short-term experiment (4 h), no effects on body weight were noted.

D. BIOKINETICS

After a single administration of ^{14}C-Reg.No. 399379 (Metabolite of BAS 500 F, Pyraclostrobin) to male mice at a target dose level 500 mg/kg bw, the mean actual nominal dose level was 497.1 mg/kg bw [see Table 5.8.1-69].

Quantifiable radioactive residues were observed in all organs and tissues examined. As to be expected the highest residues were found in the vestibular stomach in the mg Eq/g range. Lower residues in the µg Eq/g range were determined in glandular stomach, liver, plasma, bone marrow and blood cells [see Table 5.8.1-69]. Variabilities of the single animal data are assessed to reflect inter-individual variability in a normal range for kinetic studies.

Blood cells, plasma, bone marrow, vestibule and glandular stomach as well as liver from four untreated mice were collected and measured for total radioactivity (background levels). Taking the specific activity of administered ^{14}C-Reg.No. 399379 and the measured sample weights into account, double background levels corresponded to < 0.2 µg Eq/g for plasma, < 0.7 and 0.6 µg Eq/g for blood cells and liver, < 10 µg Eq/g for vestibule and glandular stomach and < 15 µg Eq/g for bone marrow, respectively.
These data demonstrate that the detected residue levels in blood cells, plasma, bone marrow, vestibule and glandular stomach as well as liver in mice dosed with ^{14}C-Reg.No. 399379 (metabolite of pyraclostrobin) at a dose level of 500 mg/kg bw were significantly above background.

Table 5.8.1-69: Radioactive residues (µg Eq/g) in selected organs and tissues of male mice treated with ^{14}C-Reg.No. 399379 at a target dose level of 500 mg/kg bw 4 hours after dosing

Parameter	Animal No.	5	6	7	8	Mean	SD
Body weight	[g]	35.4	35.4	37.7	31.8	35.1	2-4
Specific activity	[MBq/g]	-	-	-	-	334.0	-
Dose	[mg/kg bw]	491.1	493.1	514.3	490.0	497.1	11.5
Radioactive Dose	[MBq/animal]	5.81	5.83	6.48	5.20	5.8	0.5
Blood cells	[µg Eq/g]	48.6	18.9	43.2	24.5	33.8	14.3
Plasma	[µg Eq/g]	136.2	91.0	117.1	93.2	109.4	21.4
Bone marrow	[µg Eq/g]	41.2	32.6	58.4	52.1	46.1	11.4
Liver	[µg Eq/g]	302.5	264.9	235.6	264.8	267.0	27.4
Vestibule stomach	[µg Eq/g]	15840.8	3675.0	9323.7	2354.3	7798.5	6154.9
Glandular stomach	[µg Eq/g]	943.7	297.9	182.6	164.6	397.2	369.1

III. CONCLUSION

The current study demonstrated that radioactive residues of ^{14}C-Reg.No. 399379 are present in the systemic circulation and in bone marrow, vestibule and glandular stomach as well as liver of mice, four hours after single oral administration of the test substance at a target dose level of 500 mg/kg bw.

Report:	CA 5.8.1/49 [redacted] 2017 a Reg.No. 399379 (metabolite of BAS 500 F, Pyraclostrobin): Transgenic mice (MUTA mouse) gene mutation assay 2017/1002442
Guidelines:	OECD 488
GLP:	yes (certified by Ministry of Health and Welfare, Tokyo, Japan)

Executive Summary

Administration of Reg.No. 399379 (metabolite of pyraclostrobin; batch L85-218; purity: 97.7%) to groups of 6 Wistar rats at dose levels of 0, 100, 300 and 1000 mg/kg bw/day for 28-days resulted in adverse signs of systemic toxicity at 1000 mg/kg bw/day in both sexes.

Treatment with Reg.No. 399379 did not affect the survival, body weight development or food consumption or organ weights. The only histopathological finding consisted of an infiltration of inflammatory cells in one control and two top dose males (1000 mg/kg), which was considered to represent an incidental finding.

The evaluation of the mutant frequency in the target gene (lacZ), which in the strain of mice used is incorporated in about 80 copies into the mouse genome in a λ phage vector on chromosome 3, did not indicate an increased mutation rate. In contrast, the positive control N-ethyl-N-nitrosourea resulted in a statistically significant increase in mutant frequency, indicating the sensitivity of the test system.

Under the conditions of the present study Reg.No. 399379 (500M106) is not.

(BASF DocID 2017/1002442)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	Reg.No. 399379 (Metabolite of pyraclostrobin)
	Description:	Solid, orange
	Lot/Batch #:	L85-218
	Purity:	97.7% (tolerance ± 1.0%) 1.4% Reg.No. 369315, 0.2% Reg.No. 364380, 0.1% Reg.No. 4108340
	Stability of test compound:	The test substance was stable over the study period until 01 Jun 2017.

2. Vehicle and/or positive control: 0.5% aqueous Carboxymethylcellulose (vehicle)
N-ethyl-*N*-nitrosourea (ENU; positive control)

3. Test animals:

Species:	Mouse
Strain:	CD_2-LacZ80/HazfBR (Muta™Mouse)
Sex:	Male
Age:	8 weeks
Weight at dosing:	24.2 to 28.2 g
Source:	Japan Laboratory Animals, Inc.
Acclimation period:	7 days
Diet:	CRF-1 (lot No. 160607, Oriental Yeast) ad libitum
Water:	Drinking water from water bottles, ad libitum
Housing:	Group housing (2 to 3 animals/cage) in plastic cages (18.2 x 26.0 x 12.8 cm (WxDxH)) with cellulose bedding (ALPHA-dri™, Shepherd Specialty Papers)
Environmental conditions:	
Temperature:	20 - 26°C (central air-conditioning) Actually measured 22.9 to 23.3°C
Humidity:	35 - 70% (central air-conditioning) Actually measured: 47 to 60%
Air changes:	≥ 12 per hour
Photo period:	12 h light / 12 h dark (07:00 - 19:00 / 19:00 - 07:00)

B. STUDY DESIGN

1. Dates of experimental work: 24-Oct-2016 to 28-Dec-2018
Inlife dates:
01-Nov-2016 (start of administration) -
01-Dec-2016 (necropsy for organ harvest)

The study was performed at Biosafety Research Center.

2. Animal assignment and treatment:

Reg.No. 399379 was administered to groups of 6 male mice by gavage at target concentrations of 0, 100, 300 and 1000 mg/kg bw/day for 28 days. Animals were assigned to groups based on their body weights on Day 1 using LATOX-F/V5 software. After finalisation of treatment period the animals were left untreated for another 3 days for manifestation of potential mutagenic effects.
Positive control animals were treated twice with ENU at a dose of 100 mg/kg bw on study days 7 and 8 and left untreated for another 10 days for manifestation of mutagenic effects.

3. Test item preparation and analysis:

The test item was administered as suspension in 0.5% aqueous CMC. Appropriate amounts of the test item were weighed and ground with mortar and pestle. Subsequently, small portions of vehicle (0.5% aqueous CMC) was added and the preparation transferred to a measuring cylinder. The mortar was flushed with vehicle and the cylinder filled up to volume. The suspensions were kept homogenous by stirring with a magnetic stirrer. Suspensions were divided into aliquots for daily use and stored at room temperature until use. Freshly prepared suspensions were used for up to 6 days.

The stability of Reg.No. 399379 in suspension at room temperature for a period of 7 days was demonstrated before the start of the study (BASF project No. 01Y0701/14Y119; copy of the report included in Volume III of the report).

The positive control administration solution was prepared by weighing 50 mg ENU into a graduated test tube, which was filled up to 5 ml with a sodium-phosphate buffer (pH 6.0) to make up a 10.0 mg/mL solution. The positive control formulation was prepared just before use.

The homogenous distribution of Reg.No. 399379 in the diet was verified for all concentrations (see below) using the first suspension preparation.

Analysis of diet preparations for homogeneity and test-item content

Nominal concentration [mg/ mL]	Concentration measured [g/100 ml] Mean ± SD[#]	Relative standard deviation [%]	% of nominal concentration
10.0	9.544 ±0.073	0.8	95.4
30.0	28.85 ± 0.26	0.9	96.2
100.0	102.9 ± 0.8	0.8	102.9

[#] Values may not calculate exactly due to rounding of values

As indicated by relative standard deviations below 1% the prepared dosing suspensions were homogenous.

4. Statistics on in-life parameters

The data of body weight, body weight gain, organ weight, absolute organ weight / body weight ratio and food consumption from the negative control group and each test substance-treated group were tested by Bartlett's test for homogeneity of variance (two-sided, significance level of 0.05) first. If homogeneity was determined (not significant on Bartlett's test), then Dunnett's multiple comparison test was performed to assess the statistical significance of differences between the negative control group and each test substance-treated group (two-sided, familywise significance level of 0.05). If there was no homogeneity (significant on Bartlett's test), Steel's test (two-sided, significance level of 0.05) was performed to analyze the differences.

C. METHODS

1. Observations:

Animals were observed for clinical signs once daily until organ removal. During the administration period they were observed twice daily.

2. Body weight:

The body weight of the animals was determined at Day 1 (day of assignment to groups) and days 8, 15, 22, 29 and 31 (just before sacrifice). The weights of positive control animals were determined on days 1, 7 (before first dosing), and 18 (day of sacrifice).

3. Food consumption

Food consumption was determined on days 3, 7, 14, 22, and 28.

4. Water consumption:

Monitoring of drinking water consumption was not indicated in the report.

5. Ophthalmoscopy:

Not performed.

6. Clinical biochemistry:

Not performed.

7. Sacrifice and pathology:

All animals were sacrificed by exsanguination under isoflurane anesthesia. The external surface and orifices were observed, followed by observation of the organs and tissues in the abdominal, thoracic, pelvic and cranial cavities. All macroscopic abnormalities were recorded as to the location, size, color tone, etc. The following organs were collected, weighed and histopathologically examined:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and top dose).

C	W	H		C	W	H		C	W	H	
			adrenals				larynx				skin
			aorta	✓	✓	✓	liver				spinal cord (3 levels)
			bone marrow				lung				spleen
			brain	✓			lymph nodes				sternum w. marrow
			caecum				mammary gland (♀+&♂)	✓	✓	✓	stomach (fore- & glandular)
			colon				muscle, skeletal	✓			testes
✓			duodenum				nerve, peripheral (sciatic n.)				thymus
✓			epididymides§ &				nose/nasal cavity‡				thyroid glands
			esophagus				ovaries and oviduct				trachea
			eyes (with optic nerve)				pancreas	✓			urinary bladder
			femur (with knee joint)				parathyroid glands				uterus with cervix
			gross lesions				Peyer's patches				vagina
			Harderian gland				pharynx				
✓			heart				pituitary		✓		body (anesthetized animals)
			ileum				prostate				
			jejunum (w. Payer's plaque)				rectum				
✓			kidneys				salivary glands				
			lacrimal glands				seminal vesicles~				

§ vas deferens/cauda epididymis; & The seminiferous tubules and vas deferens/cauda epididymis were cut a little and be placed in a Petri dish containing 1.5 mL of cold Dulbecco's phosphate-buffered saline (PBS). The germ cells suspended in this PBS were filtered using a cell strainer (pore size 40 µm, corning). About 1 mL of cell suspension was put into microtube and frozen in liquid nitrogen.

Organs or pieces of organs not fixed for histopathological evaluation were stored in liquid nitrogen. In this study glandular stomach (site of first contact) and liver (main organ of metabolization of xenobiotics) were examined for potential mutations.

8. Assessment of mutagenicity:

In the following the principle of the mutagenicity assay is described. A detailed, step-by-step description of the procedures performed in this experiment is given in the study report.

The Muta™Mouse contains about 80 copies of the complete bacterial lacZ gene integrated in a bacteriophage λ vector (λgt10) at chromosome 3. Administration of potential mutagens result – along to mutations at other genes – in mutations of the lacZ gene. Genomic DNA of the organs of interest is isolated according to standard procedures. The enzymes in commercial packaging kits excise the λ-vector including the lacZ gene (wild-type or mutated) from the genomic DNA and insert it together with the full-length λ DNA into λ phage heads to produce infectious phage particles. In this study the Agilent Technologies Transpack packaging extract was used to separately generate infective phages from every animal and organ of interest.

The generated λ pages were used to infect E. coli C (lacZ$^-$, galE$^-$). A small aliquot of the infected E. coli C broth is mixed with non-infected E. coli C and plated for tittering.
In this study 30µl of the infected E. coli C broth (total volume about 2700 µl) was diluted 1:10 and 30 µl of this dilution was added to 1 ml non infected E. coli C prior to plating. In infected E. coli C the λ phage multiplies and infects surrounding E. coli C after lysis of the infected bacterium. By this, every single λ phage generates a plaque in the lawn of uninfected E. coli. After incubation over night the number of plaques is counted and multiplied by 900 to determine the total number of assembled phages. Packaging of genomic DNA is repeated until at least 300,000 plaques per organ and animal were generated.

The major part of the infected E. coli C broth is plated on a medium for selection. This plate contains P-gal (phenyl-β-D-galactopyranoside). E. coli cells receiving the wild-type lacZ gene convert P-gal to galactose and are unable to grow and subsequently die, as they cannot convert the toxic UDP-galactose into the normal metabolite UDP-glucose because of the lack of UDP galactose epimerase (galE$^-$). In contrast, E. coli C receiving the mutated lacZ do not form galactose and are able replicate and to produce λ phages. These phages infect surrounding bacteria and thus form plaques in the lawn formed by non-infected E. coli C, which likewise grow on the selection media. The principle of the selection is depicted below.

(Scheme included in WHO Environmental Health Criteria 233 (2006); β-gal = β-galactosidase, galE = uridine diphosphate galactose-4-epimerase)

The mutant frequency is calculated as depicted below and expressed as mutants per 10^6 plaques.

$$Mutant\ frequency = \frac{Number\ of\ mutant\ plaques}{Total\ number\ of\ plaques}$$

9. Statistics on mutant frequencies

The data on the mutant frequency from the negative control group and each test substance-treated group were tested by Bartlett's test for homogeneity of variance (two-sided, significance level of 0.05) first. If homogeneity was determined (not significant on Bartlett's test), then Dunnett's multiple comparison test was performed to assess the statistical significance of differences between the negative control group and each test substance-treated group (two-sided, familywise significance level of 0.05). If there was no homogeneity (significant on Bartlett's test), Steel's test (two-sided, significance level of 0.05) was performed to analyze the differences.
The data on the mutant frequency from the negative control group and the positive control group were tested by F test for homogeneity of variance (two-sided, significance level of 0.05) first. If homogeneity of variance was determined (not significant on F test), Student's t test (two-sided, significance level of 0.05) was performed to assess the statistical significance of differences between the negative control group and the positive control group. If there was no homogeneity (significant on F test), Aspin-Welch's t test (two-sided, significance level of 0.05) was performed to analyze the differences.

10. Validity of the study

The study was considered valid as

- the mutant frequency of the positive control groups for liver and stomach markedly increased and were statistically different from the negative control group
- the mutant frequency in the negative control groups were within the acceptable range (mean ± 3SD) calculated from the historical data base of the laboratory

II. RESULTS AND DISCUSSION

A. Observations

1. Clinical signs of toxicity

No test substance-related effects were observed.

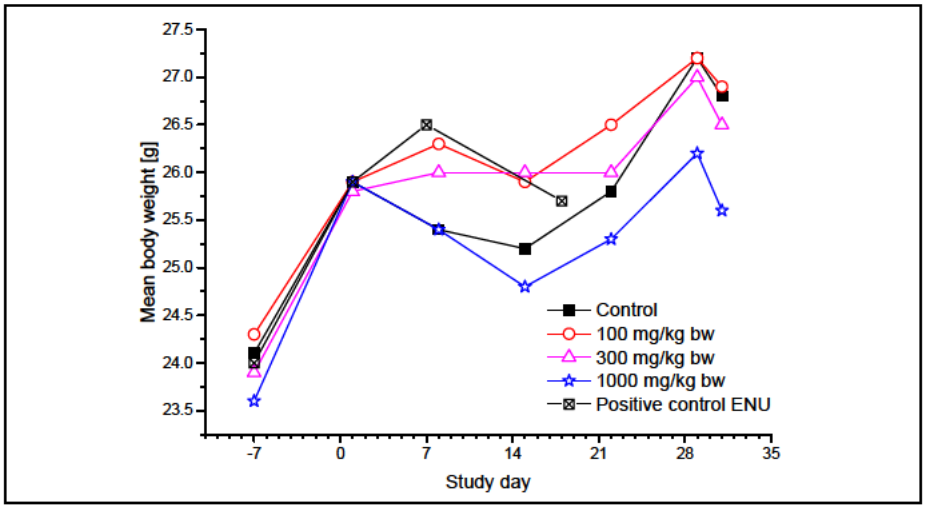
2. Mortality

No animal died prematurely in this study.

B. Body weight and body weight gain

No treatment-related effects on body weight development were noted between treated and control males [Figure 5.8.1-7]. The observed differences in mean body weights were small and due to biological variability.

Figure 5.8.1-7: Body weight development of Muta™Mice administered Reg.No. 399379 for 28 days



C. Food consumption

No substantial differences of food consumption were noted between the control and treated groups.

D. Necropsy

1. Organ weight

No effects on absolute or relative liver or stomach weights were observed [see Table 5.8.1-70].

Table 5.8.1-70: Absolute and relative weights of Muta™Mice administered Reg. No. 399379 for 28 days

Organ weight	Dose [mg/kg]	Absolute weight		Δ%	Relative weight [% of b.w.]		Δ% #
Terminal weight [g]	0	26.8	±1.8				
	100	26.9	±1.7	(0.4)			
	300	26.5	±0.9	(-1.1)			
	1000	25.6	±0.8	(-4.5)			
ENU	100	25.7	±1.6	(-4.1)			
Liver [g]	0	1.23	±0.06		4.61	±0.12	
	100	1.20	±0.12	(-2.4)	4.45	±0.21	(-3.5)
	300	1.17	±0.04	(-4.9)	4.43	±0.22	(-3.9)
	1000	1.14	±0.06	(-7.3)	4.44	±0.06	(-3.7)
ENU	100	1.21	±0.06	(-1.6)	4.70	±0.13	(2.0)
Stomach [g]	0	0.17	±0.02		0.65	±0.09	
	100	0.17	±0.02	(0.0)	0.63	±0.09	(-3.1)
	300	0.17	±0.02	(0.0)	0.64	±0.08	(-1.5)
	1000	0.16	±0.02	(-5.9)	0.64	±0.08	(-1.5)
ENU	100	0.18	±0.01	(5.9)	0.69	±0.08	(6.2)

Values may not calculate exactly due to rounding of figures

2. Gross and histopathology

There were no treatment-related gross necropsy findings. The only finding consisted of a single white nodule at the right epididymis of one high dose (1000 mg/kg bw) animal.
Histopathological examination revealed inflammatory infiltrations in the glandular stomach of one control and two high dose animals. This was considered to represent an incidental finding.

E. Assessment of mutagenicity

Up to 3 times packaging of genomic DNA from liver and stomach was performed to obtain the minimum required 300,000 plaque forming units (i.e. functionally reconstituted λ phages). Neither in liver [see Table 5.8.1-71] nor in the stomach [Table 5.8.1-72] of Reg.No. 399379 treated Muta™Mice a statistically significant increase of the mutant frequency was observed when compared with the vehicle.

Table 5.8.1-71: Mutant frequency in the liver of Muta™Mice administered Reg. No. 399379 for 28 days

Substance and dose	Animal ID	Number of plaque forming units	Number of packagings	Number of mutants	Mutant frequency [x10^6]	Group Mean ± SD
0.5% CMC	1001	662,400	1	61	92.1	41.9 ± 26.6
	1002	398,700	1	18	45.1	
	1003	591,300	1	21	35.5	
	1004	306,900	1	12	39.1	
	1005	315,000	1	6	19.0	
	1006	480,600	1	10	20.8	
Reg.No. 399379 100 mg/kg	1101	320,400	1	10	31.2	36.8 ± 9.2
	1102	432,000	1	14	32.4	
	1103	632,700	1	16	25.3	
	1104	439,200	1	16	36.4	
	1105	358,200	1	17	47.5	
	1106	479,700	2	23	47.9	
Reg.No. 399379 300 mg/kg	1201	410,400	1	17	41.4	38.2 ± 6.2
	1202	550,800	1	15	27.2	
	1203	486,900	1	21	43.1	
	1204	395,100	1	17	43.0	
	1205	517,500	1	18	34.8	
	1206	451,800	3	18	39.8	
Reg.No. 399379 1000 mg/kg	1301	319,500	1	10	31.3	28.9 ± 4.9
	1302	456,300	1	12	26.3	
	1303	419,400	2	10	23.8	
	1304	416,700	1	15	36.0	
	1305	502,200	1	16	31.9	
	1306	375,300	1	9	24.0	
ENU 100 mg/kg	1401	347,400	1	41	118.0	108.8* ± 16.9
	1402	523,800	1	60	114.5	
	1403	487,800	1	50	102.5	
	1404	692,100	2	65	93.9	
	1405	594,000	3	80	134.7	
	1406	369,000	1	33	89.4	

*: Significant difference from negative control ($p<0.05$), Student's t test

Table 5.8.1-72: Mutant frequency in the glandular stomach of Muta™Mice administered Reg. No. 399379 for 28 days

Substance and dose	Animal ID	Number of plaque forming units	Number of packagings	Number of mutants	Mutant frequency [x10^6]	Group Mean ± SD
0.5% CMC	1001	454,500	2	25	55.0	38.8 ± 10.4
	1002	302,400	1	13	43.0	
	1003	332,100	1	12	36.1	
	1004	416,700	3	10	24.0	
	1005	391,500	1	13	33.2	
	1006	437,400	1	18	41.2	
Reg.No. 399379 100 mg/kg	1101	376,200	2	16	42.5	35.4 ± 13.1
	1102	308,700	1	7	22.7	
	1103	375,300	1	7	18.7	
	1104	358,200	1	12	33.5	
	1105	505,800	1	21	41.5	
	1106	506,700	1	27	53.3	
Reg.No. 399379 300 mg/kg	1201	398,700	1	11	27.6	35.6 ± 9.3
	1202	522,900	1	24	45.9	
	1203	380,700	1	15	39.4	
	1204	517,500	1	23	44.4	
	1205	350,100	1	8	22.9	
	1206	302,400	3	10	33.1	
Reg.No. 399379 1000 mg/kg	1301	544,500	2	38	69.8	51.8 ± 11.5
	1302	371,700	2	21	56.5	
	1303	662,400	3	27	40.8	
	1304	314,100	1	12	38.2	
	1305	625,500	2	34	54.4	
	1306	393,300	1	20	50.9	
ENU 100 mg/kg	1401	449,100	1	104	231.6	320.3* ± 55.5
	1402	643,500	3	245	380.7	
	1403	300,600	1	100	332.7	
	1404	525,600	2	149	283.5	
	1405	320,400	1	104	324.6	
	1406	398,700	1	147	368.7	

*: Significant difference from negative control ($p<0.05$), Aspin-Welch's t test

The results of this study do not meet the criteria for a positive response as lined out in paragraph 41 of the OECD Guideline 488 (2013): "There are several criteria for determining a positive result, such as a dose-related increase in the mutant frequency, or a clear increase in the mutant frequency in a single dose group compared to the solvent/vehicle control group. At least three treated dose groups should be analysed in order to provide sufficient data for dose-response analysis. While biological relevance of the results should be the primary consideration, appropriate statistical methods may be used as an aid in evaluating the test results. Statistical tests used should consider the animal as the experimental unit." The proof of exposure as required in paragraph 42 for a negative result was provided by the above discussed AD study in mice using radioactive Reg.No. 399379 (see BASF DocID 2016/1225930).

III. CONCLUSION

After treatment of Muta™Mice with Reg.No. 399379 for 28 days followed by a 3 days expression period no statistically or biologically significant increase of the mutant frequency was observed at dose levels up to the limit dose of 1000 mg/kg. Therefore, under the conditions of this test, Reg.No. 399379 (500M106, metabolite of pyraclostrobin) is not genotoxic in vivo.

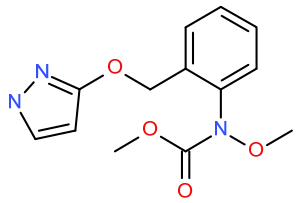
Overall toxicological evaluation of 500M106

As a conclusion based on the weight of evidence provided above, 500M106 (Reg.No. 399379) is not considered to be genotoxic in vivo and thus **not considered to be toxicologically relevant**. Reg.No. 399379 is of low toxicity when administered to rats for 28 days. Identified target organs were duodenum, liver, spleen and the hematological system. The NOAEL in the 28-day study was 300 mg/kg bw/day. Based on this NOAEL and a safety factor of 600 (extra SF of 3 for extrapolation of 28-day to 90-day and SF 2 for extrapolation of subchronic to chronic) an ADI of 0.5 mg/kg bw/day can be established.

Based on the results of the in vitro comparison study, it cannot be excluded that metabolite 500M106 might be also formed in humans under in vivo conditions. Therefore, dietary exposure assessments have been performed (see M-CA 6.9).

(Surface) Water metabolites

500M60 (other denominators: Reg.No. 411847, BF 500-11)



500M60 (Reg.No. 411847, BF 500-11) is a photolysis metabolite found in surface water.

A) QSAR Predicitions for 500M60 (Reg.No. 411847, BF 500-11)

OECD Toolbox (Version: 3.2) [see DocID 2014/1172955]

For 500M60 (Reg.No. 411847) the OECD toolbox does not indicate any alerts.

OASIS TIMES (MIX V.2.27.13 TB; Mutagenicity S-9 activated v08.08) [see molecule 2 of report BASF DocID 2014/1172952 and 2014/1172953]

There were no Ames mutagenicity alerts for 500M60 (Reg.No. 411847) or in-silico generated metabolites. In all cases the structures were out of domain. The prediction in vitro chromosome aberration for 500M60 (Reg.No. 411847) was negative (out of domain), whereas it was positive for 4 out of 11 in-silico generated metabolites. The respective alert was ‘phenols’ (in domain).

Discussion: The alert ‘phenols’ identified for the in vitro chromosome aberration was also present for pyraclostrobin (phenols) and metabolite 500M76 (Reg.No. 413038). Whereas the in vitro study with pyraclostrobin was negative, the in vitro study with 500M76 (Reg.No. 413038) - like that with 500M60 (Reg.No. 411847) - was positive. However, the in vitro results were not confirmed in the higher tier in vivo mouse micronucleus studies. Therefore, the weight of evidence indicates that 500M60 (Reg.No. 411847) is not genotoxic.

VEGA: Mutagenicity model (CAESAR, version 2.1.9) [see molecule 2 of report DocID 2014/1172954 for both VEGA models]

500M60 (Reg.No. 411847) is out of model applicability domain. The prediction is ‘non mutagen’.

VEGA: Mutagenicity model (SarPy; version 1.0.4-BETA)

500M60 (Reg.No. 411847) is out of model applicability domain. The prediction is ‘mutagen’ with the structural alerts SA 96 and SA98. As discussed above, this alert was also obtained for pyraclostrobin and a number of pyraclostrobin metabolites, which like 500M60 (Reg.No. 411847) were actually tested negative. The predicition is therefore rejected.

DEREK (Version 2.0.0.201011301322; Knowledge base version: 11_20_05_2010)

The metabolite 500M60 (Reg.No. 411847) was not evaluated in this system.

Genotoxicity studies on 500M60 (Reg.No. 411847, BF 500-11)

Study already submitted:

In the original submission an Ames test on Reg.No. 411847 was submitted and assessed (BASF DocID 1999/12017). A standard plate test and a pre-incubation test with and without metabolic activation was performed at concentrations of 20 - 5000 µg/plate in S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 as well as in E. coli WP2 uvrA. A slight bacteriotoxicity was occasionally noted at concentrations ≥ 2500 µg/plate while no precipitations were noted at any concentration. No increase of revertant colonies was observed in any of the tests performed.

New studies submitted in this dossier:

Report:	CA 5.8.1/50 Schulz M., Landsiedel R., 2012c Reg.No. 411847 (metabolite of BAS 500 F, Pyraclostrobin) - In vitro gene mutation test in CHO cells (HPRT locus assay) 2012/1148607
Guidelines:	OECD 476, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17, EPA 870.5300
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg. No. 411847 (metabolite of pyraclostrobin; batch: L83-76; purity: 94.5%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells. Two independent experiments were conducted in the presence and absence of metabolic activation at concentrations of up to 2000 µg/mL. The treatment intervals in the absence of metabolic activation were 4 and 24 h in the first and second experiment, respectively. With metabolic activation treatment was 4 h for the orginal and confirmatory experiment. Ethylmethanesulfonate (EMS) and methylcholanthrene (MCA) served as positive controls in the experiments without and with metabolic activation, respectively.

Cytotoxic effects indicated by reduced cloning efficiencies of below 20% of the respective vehicle control were observed at 1000 µg/mL in the experiments without metabolic activation and at ≥ 1500 µg/mL with metabolic activation.

Neither in the original nor in the confirmatory experiments a relevant increase in the mutant frequency was observed. The positive control substances, however, induced a marked increase in mutant frequency.

Based on the results of the study it is concluded that under the conditions of the test Reg. No. 411847 does not induce forward mutations in mammalian cells in vitro.

(BASF DocID 2012/1148607)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Description:	Reg. No. 411847 (metabolite of pyraclostrobin)
Lot/Batch #:	Solid, beige
Purity:	L83-76
Stability of test compound:	94.5% (tolerance ± 1.0%)
	The stability of the test substance under storage conditions was guaranteed until 01-Jan-2014. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance solutions. The stability of a comparable batch (01183-172) at room temperature in the vehicle DMSO and in water over a period of 4 hours was verified analytically.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study
Solvent control:	1% (v/v) DMSO in culture medium
Positive control -S9:	Ethyl methanesulfonate (EMS) 300 µg/mL
Positive control +S9:	Methylcholantrene (MCA) 20 µg/mL

3. Activation:

S9 was produced from the livers of at least 5 induced male Wistar [Crl:WI(Han)] rats. The rats were administered injections of phenobarbital (80 mg/kg; i.p.) and ß-napthtoflavone (80 mg/kg; per gavage) on three consecutive days. 24 h after the last administration the animals were sacrificed.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organism: Chinese hamster CHO cells. Stocks of the CHO cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).

5. Culture media:

Culture medium: Ham's F12 medium with L-glutamine and hypoxanthine supplemented with 10% (v/v) fetal calf serum (FCS).

Pretreatment medium: ("HAT" medium): FCS-supplemented Ham's F12 medium with L-glutamine containing per mL 13.6 µg hypoxanthine, 0.18 µg aminopterin and 3.88 µg thymidine.

Selection medium: ("TG" medium): L-Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL
All media were supplemented with
- 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL)
- 1% (v/v) amphotericine B (250 µg/mL)
During pulse exposure (4 hours) to the test substance, Ham‘s F12 medium was used without FCS supplementation. In the case of continuous treatment (24 hours) Ham‘s F12 medium with FCS supplementation was used.

6. Locus examined: hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

7. Test concentrations:

a) Preliminary toxicity assay: Nine concentrations ranging from 11.7 to 3000 µg/mL

b) Mutation assay:
1st experiment: 62.5, 125, 250, 500, 1000 and 2000 µg/mL with and without metabolic activation
2nd experiment: 93.8, 187.5, 375, 750, 1500 and 2000 µg/mL with and 31.3, 62.5, 125, 250, 500, 1000 µg/mL without metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 05-Mar-2012 to 18-Jun-2012

2. Preliminary cytotoxicity assay:

Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25 cm² flasks with 9 test substance concentrations in serum-free Ham's F12 medium for about 4 hours (with and without metabolic activation) or 24 hours (only without metabolic activation; serum-supplemented medium) after an attachment period of 20-24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS) or PBS, covered with Ham's F12 and incubated for a further 5 - 8 days. After this incubation period, colonies were fixed, stained and counted. In addition to the cloning efficiency the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

3. Mutation Assay:

Pretreatment of Cells: Cells with a passage number ≥ 2 after thawing from the frozen cells stock were seeded into 75 cm^2-flasks and incubated for 3-4 days with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants. Afterwards, a passage into culture medium followed and the cells were incubated for further 3-4 days.

Cell treatment: For each test group, about $1x10^6$ cells per flask were seeded into 175 cm^2 flasks containing about 20 mL Ham's F12 medium supplemented with 10% FCS and incubated for about 20 - 24 hours with 5% CO_2 at 37°C and ≥ 90% humidity for cell attachment. 2 flasks were used for each test group.

After the cell attachment period the medium was replaced by the treatment medium. In case of experiments without metabolic activation the treatment medium consisted of 18 mL Ham's F12 medium without FCS plus 2 mL positive control substance. For the vehicle control and the test groups, 20 mL of the treatment medium was supplemented with 0.2 mL of the vehicle or test substance preparation. In case of metabolic activation, the treatment medium consisted of 14 mL Ham's F12 medium without FCS, 2 mL positive control and 4 mL S9-mix.

Analogously, for the vehicle control and test groups, 16 mL of medium was supplemented with 0.2 mL vehicle or test substance preparation and 4 mL S9-mix.

Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix (or for 24 hours without S9-mix in the second experiment) at 5% CO_2, 37°C and ≥ 90% humidity.

Expression:

After incubation for 4 or 24 hours, respectively, the treatment medium was replaced by at least 20 mL Ham's F12 medium with 10% FCS after having been rinsed several times with Hanks' balanced salt solution (HBSS). The following 1st passage was carried out after an incubation period of about 3 days following the 4 hour exposure or 2 days following the 24 hour exposure period. After an entire expression period of 7 - 9 days the cells were transferred into the selection medium (2nd passage).

Selection:

For the mutant selection, six 75-cm^2 flasks each were seeded with $3x10^5$ cells from each treatment group in selection medium (TG medium) and incubated for about 6 to 7 days. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.

Determination of Cytotoxicity:

Cloning efficiency 1 (survival):

The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25 cm^2 flasks using 5 mL Ham`s F12 medium with 10% FCS. After a 20-24 hour attachment period the cells were incubated with vehicle, test substance or the positive control for 4 or 24 hours as described above. At the end of the treatment period the cells were washed with HBSS and the treatment medium was replaced by Ham's F12 medium with 10% FCS. After a further incubation for about 5 to 8 days the colonies were fixed, stained and counted.

Cloning efficiency 2 (viability):

The viability (cloning efficiency 2; CE_2) was determined after the expression period. About 200 cells were separated during the transfer into selection medium and seeded in two flasks (25 cm^2) containing 5 mL Ham's F12 medium incl. 10% (v/v) FCS each.
After seeding of the cells, the flasks were incubated for 5 - 8 days to form colonies. These colonies were fixed, stained and counted.

Calculations:

Mutant frequency:

Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{total\ number\ of\ mutant\ colonies}{number\ of\ seeded\ cells} x\,10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\ absolute}} x\,100$$

Cloning efficiency (CE, %)

absolute:

$$CE_{absolute} = \frac{total\ number\ of\ colonies\ in\ the\ test\ group}{total\ number\ of\ seeded\ cells\ in\ the\ test\ group} x100$$

relative, in comparison to control:

$$CE_{relative} = \frac{CE\ of\ the\ dose\ group}{CE\ of\ the\ vehicle\ control} x100$$

4. Statistics:

An appropriate statistical trend test was performed to assess a dose-related increase of mutant frequencies. The number of mutant colonies obtained for the test substance treated groups was compared with that of the respective vehicle control groups. A trend is judged as statistically significant whenever the p-value (probability value) is below 0.10 and the slope is greater than 0. Both, biological and statistical significance will be considered together. However, due to the negative findings, a statistical evaluation was not carried out.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies ($MF_{corr.}$) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose-response relationship. Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose-response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

A test substance is generally considered negative in this test system if:

- The corrected mutation frequency ($MF_{corr.}$) in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance under storage conditions was guaranteed until 01 Jan 2014 as indicated by the Sponsor. The stability of a comparable batch (01183-172) at room temperature in the vehicle DMSO and in water over a period of 4 hours was verified analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY

After 4 hours treatment in the absence and presence of S9 mix cytotoxicity indicated by reduced relative cloning efficiency of about or below 20% relative survival was observed at ≥ 1500 µg/mL. Treatment for 24 hours in the absence of S9 mix reduced relative cloning efficiency of below 20% relative survival at concentrations ≥ 750 µg/mL.

Precipitation of the test substance was observed at ≥ 1500 µg/mL at the end of treatment in the absence and presence of metabolic activation. No marked effect on osmolarity and pH value was observed.

Based on these data the highest concentration tested in the mutagenicity experiments was 2000 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

No relevant increase in the number of mutant colonies was observed in the original and confirmatory experiments with and without metabolic activation [see Table 5.8.1-73 and Table 5.8.1-74]. The mutant frequencies obtained displayed no dose-dependency and were within the range of the historical negative control data.

Treatment with the positive controls EMS and MCA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

Cytotoxic effects indicated by reduced cloning efficiencies of below 20% of control value were observed in both experiments in the absence and presence of S9 mix at concentrations ≥ 1000 µg/mL and > 1000/750 µg/mL, respectively [see Table 5.8.1-73 and Table 5.8.1-74].

The pH and osmolarity of the tested concentrations were not altered at the concentrations tested. Test substance precipitation was observed from 1000 µg/mL onwards in the presence and absence of metabolic activation.

Table 5.8.1-73: Gene mutation in mammalian cells - 1st experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	15	4.17	4.23	71.1	100.0	98.9	100.0
Reg. No. 411847							
62.5 µg/mL	18	5.00	5.58	80.8	113.5	89.3	90.3
125.0 µg/mL	7	1.94	2.12	76.5	107.6	92.0	93.0
250.0 µg/mL	2	0.56	0.55	69.1	97.2	100.4	101.5
500.0 µg/mL	15	4.17	4.96	67.6	95.1	90.4	91.4
1000.0 µg/mL	15	4.17	4.42	11.3	15.8	90.6	91.7
2000.0 µg/mL	n.c.[2]	n.c.[2]	n.c.[2]	0.0	0.0	n.c.[2]	n.c.[2]
Positive control EMS							
300.0 µg/mL	333	92.50	117.38	86.9	122.1	81.3	82.2
With metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	5	1.39	1.26	86.1	100.0	102.8	100.0
Reg. No. 411847							
62.5 µg/mL	n.c.[1]	n.c.[1]	n.c.[1]	94.4	109.6	n.c.[1]	n.c.[1]
125.0 µg/mL	3	0.83	0.83	88.1	102.3	103.3	100.5
250.0 µg/mL	14	3.89	3.89	91.1	105.8	99.9	97.2
500.0 µg/mL	9	2.50	2.54	86.8	100.7	96.0	93.4
1000.0 µg/mL	3	0.83	0.82	39.0	45.3	99.1	96.5
2000.0 µg/mL	n.c.[2]	n.c.[2]	n.c.[2]	0.0	0.0	n.c.[2]	n.c.[2]
Positive control MCA							
20.0 µg/mL	244	67.78	77.16	68.1	79.1	87.9	85.5

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
n.c.[1] culture was not continued since a minimum of only four analyzable concentrations is required
n.c.[2] culture was not continued due to strong cytotoxicity

Table 5.8.1-74: Gene mutation in mammalian cells - 2nd experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	Relative
Without metabolic activation; 24-hour exposure period							
Vehicle (DMSO)	32	8.89	9.77	74.6	100.0	90.0	100.0
Reg. No. 411847							
31.3 µg/mL	n.c.[1]	n.c.[1]	n.c.[1]	84.6	113.4	n.c.[1]	n.c.[1]
62.5 µg/mL	37	10.28	11.70	72.6	97.3	88.0	97.8
125.0 µg/mL	11	3.06	3.71	71.9	96.3	82.4	91.5
250.0 µg/mL	28	7.78	8.01	68.5	91.8	92.1	102.4
500.0 µg/mL	17	4.72	5.25	43.0	57.6	90.8	100.8
1000.0 µg/mL	n.c.[2]	n.c.[2]	n.c.[2]	0.0	0.0	n.c.[2]	n.c.[2]
Positive control EMS							
300 µg/mL	1160	322.22	520.42	69.3	92.8	62.0	68.9
With metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	15	4.17	5.24	56.8	100.0	80.1	100.0
Reg. No. 411847							
93.8 µg/mL	22	6.11	7.74	85.0	149.8	88.0	109.8
187.5 µg/mL	16	4.44	4.57	73.4	129.3	94.6	118.1
375.0 µg/mL	42	11.67	13.94	77.4	136.3	83.4	104.1
750.0 µg/mL	10	2.78	3.21	37.3	65.6	86.5	108.0
1500.0 µg/mL	n.c.[2]	n.c.[2]	n.c.[2]	0.0	0.0	n.c.[2]	n.c.[2]
2000.0 µg/mL	n.c.[2]	n.c.[2]	n.c.[2]	0.0	0.0	n.c.[2]	n.c.[2]
Positive control MCA							
20 µg/mL	436	121.11	140.33	68.1	120.0	86.4	107.8

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
n.c.[1] culture was not continued since a minimum of only four analyzable concentrations is required
n.c.[2] culture was not continued due to strong cytotoxicity

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test Reg. No. 411847 does not induce forward mutations in the HPRT locus in CHO cells in vitro.

Report: CA 5.8.1/51
Engelhardt G., Leibold E., 2003a
In vitro chromosome aberration assay with Reg.No. 411847 in V79 cells
2003/1004383
Guidelines: OECD 473, EEC 2000/32 B.10, EPA 870.5375
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

Reg.No. 411847 (metabolite of pyraclostrobin; batch: 01183-172; purity: 98.9%) was tested in vitro for the ability to induce chromosome and numerical aberrations in Chinese Hamster V79 cells in two independent experiments in the presence and absence of metabolic activation at concentrations of 62.5 µg/mL to 1000 µg/mL with a pulse treatment of 4 hours. The cells were prepared 18 hours after the start of treatment. Vehicle (DMSO) and positive controls (cyclophosphamide (CPP) and ethylmethanesulfonate (EMS) for the experiment with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system.

In the test without metabolic activation Reg.No. 411847 induced a statistically significant and dose-dependent increase in the number of structurally aberrant metaphases including and excluding gaps in two experiments performed independently of each other. An increase in the number of polyploid/endopolyploid metaphases was observed in the experimental part without metabolic activation. In the experiment with metabolic activation, a dose-related increase in the number of structurally aberrant metaphases including and excluding gaps and an increase in the number of endopolyploid metaphases starting from the mid dose onwards was also observed.

Based on the results of this study, Reg.No. 411847 is considered to have both a clastogenic and aneugenic potential in vitro in Chinese hamster V79 cells.

(BASF DocID 2003/1004383)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 411847 (Metabolite of pyraclostrobin)

Description:	Crystalline, white
Lot/Batch #:	01183-172
Purity:	98.9%
Stability of test compound:	The stability of the test substance was guaranteed by the sponsor, and the sponsor holds this responsibility. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance formulations and on account of the high purity. The stability of the test substance dissolved in the vehicle DMSO and in aqueous solution over a period of 4 hours was verified analytically.
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO
Positive control, -S9:	Ethylmethanesulfonate 350 µg/mL
Positive control, +S9:	Cyclophosphamide 0.5 µg/mL

3. Activation: S9 was produced from the livers of 5 induced male Wistar Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg Aroclor 1254 (as solution in corn oil with a concentration of 20 g/100 mL) per kg body weight. 5 days after administration the animals were sacrificed. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction was thawed at room temperature and 1 volume of S9-fraction was mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10%

4. Test organisms: Chinese hamster V79 cells

5. Culture medium: MEM medium with glutamine supplemented with
- 10% (v/v) fetal calf serum (FCS)
- 1% (v/v) penicillin/streptomycin (10000 IU/10000 µg/mL)
- 1% (v/v) amphotericine B (250 µg/mL)

During exposure to the test substance (4-hour treatment), MEM medium was used without FCS supplementation.

6. Test concentrations:

a) Preliminary toxicity assay: Nine to ten concentrations ranging from 1 to 2800 µg/mL were used in experiments with or without metabolic activation for exposure duration of 4 and 18 hours.

b) Mutation assay:
1st experiment: 62.5, **125**, **250**, **500** and 750 µg/mL without metabolic activation; **125**, **250**, **500**, 750 and 1000 µg/mL with metabolic activation (18 h preparation interval)
[bold concentrations were evaluated]

2nd experiment: 100, 200, **300**, **400**, **500**, 600 and 700 µg/mL without metabolic activation (18 h preparation interval)

[bold concentrations were evaluated]

B. TEST PERFORMANCE:

1. Dates of experimental work: 30-Sep-2002 to 14-Jan-2003

2. Preliminary cytotoxicity assay:

A range-finding cytotoxicity test was conducted with V79 cultures exposed for 4 and 18 hours to test substance concentrations of 1 - 2800 µg/mL both with and without metabolic activation. At the end of the exposure period, cell count, cell attachment, mitotic index and the quality of metaphases were determined in order to derive appropriate test substance concentrations for the main test.

3. Cytogenicity Assay:

Two independent experiments were performed. In the first experiment cells were exposed for 4 hours with the test substance either without or with metabolic activation and harvested after 18 hours. For clarification/conformation of the results of the first experiment, the second experiment was done with the same exposure and harvest time but only treatment without metabolic activation. Duplicate curltures were used for all experimental groups.

Cell treatment:	Cells were exposed to the test substance, solvent or positive control in pulse treatment experiments for 4 hours with or without metabolic activation. The cells were incubated in Quadriperm® dishes at 37°C, 5% CO_2 and ≥ 90% humidity. Two chambers of a Quadriperm dish were used for each concentration (=duplicate cultures). The preparation interval was 18 h post treatment-begin. For determination of cytotoxicity, additional cell cultures (using 25 cm² plastic flasks) were treated in the same way as in the main experiment. Growth inhibition was estimated by comparing the cell number in the treated groups with the concurrent control.
Spindle inhibition:	0.2 µg/mL of Colcemid was added to the cultures 2 - 3 hours prior to harvesting.
Cell harvest:	At the end of the incubation time the culture medium was completely removed. For hypotonic treatment, 5 mL of a 0.4% KCl solution (37°C) was added for about 20 minutes. The cells were fixed by addition of 5 mL methanol/glacial acetic acid (3:1 v/v). The fixative was changed twice.

Slide preparation:	The slides were removed from the Quadriperm chambers, briefly allowed to drip off and passed through a Bunsen burner flame. After drying, the cells were stained with Giemsa and Titrisol. After rinsing and clarifying in Xylene the cover slips were mounted in Corbit-Balsam.
Metaphase analysis:	Slides were coded prior to analysis. As a rule, the first 100 consecutive well-spread metaphases of each culture were counted for all test groups, and if cells had 20 - 22 chromosomes, they were analyzed for structural chromosome aberrations. Numerical chromosome aberrations were also recorded. If there is a clear increase in chromosomally damaged cells, the number of metaphases to be analyzed is reduced from the planned 200 mitoses/test group. A mitotic index based on 1000 cells per culture was determined for all evaluated test groups in both experiments.

4. Statistics:

B)

The proportion of metaphases with aberrations was calculated for each group.

A comparison of each dose group with the vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test was Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible significant increase in the number of structural chromosomal aberrations.
- The proportion of aberrations exceeded both the concurrent negative control range and the historical negative control range.

A test substance is generally considered non-clastogenic in this test system if:

- There was no significant increase in the number of chromosomally damaged cells at any dose above concurrent control frequencies.
- The aberration frequencies were within the historical negative control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance at room temperature in the vehicle DMSO and in water over a period of 4 hours was verified analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY:

A preliminary cytotoxicity assay was performed to determine appropriate test substance concentrations for the main test. Dose selection was based on the cell count, cell attachment, mitotic index and the quality of metaphases. Based on the preliminary cytotoxicity assay, 750 µg/mL without metabolic activation and 1000 µg/mL with metabolic activation were selected as the top doses for the main test.

C. CYTOGENICITY ASSAYS:

RegNo. 411847 led to a dose-dependent and statistically significant increase in the number of structural chromosomal aberrations, including and excluding gaps without metabolic activation in two independently experiments [see Table 5.8.1-75 to Table 5.8.1-77]. In the experiment with metabolic activation, a dose-related, not statistically significant increase in the number of structurally aberrant metaphases was observed [see Table 5.8.1-76]. However, the incidences were within the historical control range and thus not considered to be indicative of a clastogenic effect.

In addition to the clastogenic effects aneugenic effects were observed with and without metabolic activation [see Table 5.8.1-75 to Table 5.8.1-77]. Here the increase in the incidence of endopolyploid metaphases was higher than that of polyploid metaphases. The aneugenic effects were less pronounced with metabolic activation.

Vehicle and positive controls were all in the expected range demonstrating the validity of the test.

The osmolarity and pH values were not influenced by the test substance treatment. Precipitation of the test substance did not occur up to the highest tested concentration. No suppression of the mitotic activity was observed up tho the highest concentration tested. Regarding cell countst, a growth inhibition was observed from at concentration ≥ 500 - 600 µg/mL without metabolic activation and at ≥ 750 µg/mL with metabolic activation.

III. CONCLUSION

Based on the results of the study it is concluded that Reg.No. 411847 has both, a clastogenic and an aneugenic potential under the in vitro conditions of this study. The relevance of this in vitro result was evaluated in a higher tier in vivo study (see below) according to Commission Regulation (EU) No 283/2013, Section 5.4.2.

Table 5.8.1-75: Chromosome aberration test with Reg.No. 411847 without metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	11.7	1	1.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	B	100	13.1	3	3.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	1	0.0	1	1.0
	A + B	**200**	**12.4**	**4**	**2.0**	**4**	**2.0**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**1**	**0.5**
Reg.No. 411847																			
125 µg/mL	A	100	10.2	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	1	0.9	1	0.9	4	3.8
	B	100	11.7	6	6.0	6	6.0	4	4.0	0	0.0	0	0.0	0	0.0	1	0.9	7	6.5
	A + B	**200**	**11.0**	**9**	**4.5**	**8**	**4.0**	**4**	**2.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**2**	**0.9**	**11**	**5.1**
250 µg/mL	A	100	9.1	7	7.0	6	6.0	3	3.0	0	0.0	0	0.0	0	0.0	2	1.7	17	14.3
	B	100	11.3	6	6.0	6	6.0	4	4.0	1	1.0	0	0.0	0	0.0	0	0.0	9	8.3
	A + B	**200**	**10.2**	**13**	**6.5***	**12**	**6.0**	**7**	**3.5**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**2**	**0.9**	**26**	**11.4**
500 µg/mL	A	50	10.3	12	24.0	12	24.0	10	20.0	1	2.0	0	0.0	0	0.0	0	0.0	1	2.0
	B	50	9.8	13	26.0	13	26.0	9	18.0	2	4.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100#**	**10.1**	**25**	**25.0****	**25**	**25.0****	**19**	**19.0****	**3**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**1.0**
Positive control EMS																			
350 µg/mL	A	50	10.7	8	16.0	8	16.0	3	6.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	9.9	8	16.0	8	16.0	4	8.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100**	**10.3**	**16**	**16.0****	**16**	**16.0****	**7**	**7.0***	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Table 5.8.1-76: Chromosome aberration test with Reg.No. 411847 with metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	13.2	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	14.1	1	1.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	A + B	**200**	**13.7**	**3**	**1.5**	**2**	**1.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**
Reg.No. 411847																			
125 µg/mL	A	100	12.9	2	2.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	10.9	5	5.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**11.9**	**7**	**3.5**	**4**	**2.0**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
250 µg/mL	A	100	16.3	5	5.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	1	1.0	1	1.0
	B	100	14.1	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	1	1.0
	A + B	**200**	**15.2**	**8**	**4.0**	**5**	**2.5**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**1**	**0.5**	**2**	**1.0**
500 µg/mL	A	100	11.9	7	7.0	6	6.0	4	4.0	0	0.0	0	0.0	0	0.0	1	1.0	3	2.9
	B	100	12.2	4	4.0	4	4.0	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	2	2.0
	A + B	**200**	**12.1**	**11**	**5.5**	**10**	**5.0**	**7**	**3.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**5**	**2.4**
Positive control CPP																			
0.5 µg/mL	A	50	13.5	9	18.0	9	18.0	3	6.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	12.7	8	16.0	8	16.0	5	10.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100**	**13.1**	**17**	**17.0****	**17**	**17.0****	**8**	**8.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)

Table 5.8.1-77: Chromosome aberration test with Reg.No. 411847 without metabolic activation (18 hours treatment, harvest after 18 hours) - Experiment 2

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	11.8	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	2	2.0	0	0.0
	B	100	9.5	4	4.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	A + B	**200**	**10.7**	**7**	**3.5**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**3**	**1.5**	**0**	**0.0**
Reg.No. 411847																			
300 µg/mL	A	100	12.2	10	10.0	7	7.0	4	4.0	0	0.0	0	0.0	0	0.0	3	2.6	12	10.4
	B	100	9.5	7	7.0	5	5.0	2	2.0	0	0.0	0	0.0	0	0.0	3	2.7	7	6.4
	A + B	**200**	**10.9**	**17**	**8.5***	**12**	**6.0****	**6**	**3.0***	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**6**	**2.7**	**19**	**8.4**
400 µg/mL	A	100	10.1	17	17.0	13	13.0	12	12.0	2	2.0	0	0.0	0	0.0	5	4.3	11	9.5
	B	100	12.3	11	11.0	10	10.0	9	9.0	0	0.0	0	0.0	0	0.0	5	4.5	7	6.3
	A + B	**200**	**11.2**	**28**	**14.0****	**23**	**11.5****	**21**	**10.5****	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**10**	**4.4**	**18**	**7.9**
500 µg/mL	A	50	9.9	11	22.0	11	22.0	8	16.0	2	4.0	0	0.0	1	1.9	0	0.0	3	5.6
	B	50	10.3	14	28.0	13	26.0	11	22.0	2	4.0	0	0.0	0	0.0	0	0.0	1	2.0
	A + B	**100#**	**10.1**	**25**	**25.0****	**24**	**24.0****	**19**	**19.0****	**4**	**4.0***	**0**	**0.0**	**1**	**1.0**	**0**	**0.0**	**4**	**3.8**
Positive control EMS																			
350.0 µg/mL	A	50	8.5	11	22.0	11	22.0	9	18.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	8.1	14	28.0	14	28.0	11	22.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100**	**8.3**	**25**	**25.0****	**25**	**25.0****	**20**	**20.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged vells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Report:	CA 5.8.1/52 [redacted] 2012d Reg.No. 411847 (metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in bone marrow cells of the mouse 2012/1218557
Guidelines:	OECD 474 (1997), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12, EPA 870.5395
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 411847 (metabolite of pyraclostrobin; batch: L83-76; purity: 94.5%) was tested for chromosomal damage (clastogenicity) and for the ability to induce spindle poison effects (aneugenic activity) in NMRI mice using the micronucleus test method. For this purpose, the test substance was administered once orally to groups of 5 male mice at dose levels of 125, 250 and 500 mg/kg body weight. The vehicle served as negative and cyclophosphamide and vincristine sulfate as positive controls. The animals were sacrificed 24 or 48 (additional high dose and vehicle group) hours after the administration and the bone marrow of the two femora was prepared and investigated for micronuclei. The results of plasma analysis demonstrated the bioavailability of the test item.

The oral administration of Reg.No. 411847 did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing either small or large micronuclei. The rate of micronuclei was below the concurrent negative control and was within the range of the historical control data. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur. Signs of systemic toxicity were not observed up to the highest dose of 500 mg/kg bw.

Both of the positive control chemicals, i.e. cyclophosphamide for clastogenic effects and vincristine for induction of spindle poison effects, led to the expected increase in the rate of polychromatic erythrocytes containing small (cyclophosphamide) or small and large (vincristine sulphate) micronuclei, thus demonstrating the sensitivity of the test system.

In conclusion, under the experimental conditions of this study, the test substance Reg.No. 411847 (metabolite of pyraclostrobin) does not induce cytogenetic damage in bone marrow cells of NMRI mice in vivo.

(BASF DocID 2012/1218557)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

	Reg.No. 411847 (metabolite of pyraclostrobin)
Description:	Solid, beige
Lot/Batch #:	L83-76
Purity:	94.5% (tolerance ± 1.0%)
Stability of test compound:	The stability of the test substance under storage conditions was guaranteed until 01 Jan 2014 as indicated by the sponsor. Homogeneity of the test substance was guaranteed by mixing before preparation of the test substance emulsions.
Vehicle used:	DMSO and subsequent emulsion in corn oil

2. Control Materials:

Negative:	No negative control was employed in this study.
Vehicle control:	DMSO and subsequent emulsion in corn oil
Positive control:	Cyclophosphamide (CCP) 20 mg/kg for the determination of clastogenic effects Vincristine sulphate (VCR) 0.15 mg/kg for the determination aneugenic effects

3. Test animals:

Species:	Albino mice
Strain:	Crl:NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	5 - 8 weeks
Mean weight at dosing:	31.3 g
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	Not indicated in the report
Micronucleus assay:	5 males/dose (exception for the additional 1000 mg/kg bw dose group: 10 animals)
Acclimation period:	At least 5 days
Diet:	Standardized pelleted feed (Maus/Ratte Haltung "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland)
Water:	Drinking water in bottles, ad libitum
Housing:	During the study the mice were housed individually in Makrolon cages, type MII

4. Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	frequency not indicated (fully air-conditioned rooms)
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	500 and 1000 mg/kg
Micronucleus assay:	125, 250 and 500 mg/kg (an additional test group treated with 1000 mg/kg was treated in parallel to verify the bioavailability of the test substance after oral administration) The test substance was administered once by oral gavage using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 24-Apr-2012 to 28-Aug-2012

2. Preliminary range finding test:

Male and female mice were treated once by oral gavage of test item doses of 500 and 1000 mg/kg bw.

3. Micronucleus test:

Treatment and sampling:	Groups of 5 male mice were treated once by oral gavage with either vehicle, 125, 250, or 500 mg test substance/kg bw. Additional test groups treated with the vehicle control and the high dose were used for the second sampling period. A further test group of 10 animals was treated at 1000 mg/kg bw for determination of plasma levels of the test item. The application volume was 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substances CCP and VCR were administered once by oral gavage (CCP) or i.p. injection (VCR). The animals were observed for evident clinical signs of toxicity throughout the study.

	Twenty-four hours after the administration the mice were sacrificed and the two femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 300xg for 5 minutes. The supernatant was removed and the pellet resuspended in about 50 µl fresh FCS. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide preparation:	One drop of the suspension was applied on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May Gruenwald solution, rinsed, and finally stained with Giemsa solution (7.5%). After rinsing and clarifying in xylene, the preparations were mounted. The slides were coded prior to microscopic evaluation.
Slide evaluation:	In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored. The increase in the number of micronuclei in polychromatic erythrocytes of treated animals as compared to the vehicle control group provides an index of a chromosome-breaking (clastogenic) effect or of a spindle activity (aneugenic) of the substance tested. In addition, the number of small micronuclei ($d < D/4$) and of large micronuclei ($d \geq D/4$) (d = diameter of micronucleus, D = cell diameter) was determined: The size of micronuclei may indicate the possible mode of action of the test substance, i.e. a clastogenic or a spindle poison effect. The ratio of polychromatic to normochromatic erythrocytes was calculated. An alteration of this ratio indicates a toxic effect on erythropoieses and thus, that the test substance actually reached the target organ.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test (modified rank test according to Wilcoxon). Here, the relative frequencies of cells with micronuclei of each animal were used.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and significant increase in the number of micronucleated polychromatic erythrocytes was observed.
- The proportion of cells containing micronuclei exceeded both the values of the concurrent vehicle control range and the vehicle historical control range.

A test substance is generally considered negative in this test system if:

- There was no significant increase in the number of micronucleated polychromatic erythrocytes at any dose above concurrent control frequencies.
- The frequencies of cells containing micronuclei were within the historical control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle DMSO/corn oil was verified analytically. The homogeneity of the test substance in the vehicle was guaranteed by constant stirring during the removal and administration of the test substance formulation and by analytical determination of 3 individual samples of each concentration. The mean test substance concentrations were determined as 12.193, 23.631, 50.447 and 99.792 mg/mL at nominal concentrations of 12.5, 25, 50 and 100 mg/mL, respectively. Thus, the actual concentrations were in the range of 98 to 101% of the nominal concentrations. The homogeneity of the preparations was indicated by relative standard deviations in the range of 4.3 to 5.4%.

In order to demonstrate the bioavailability of the test item blood plasma analyses from blood sampled at necropsy were performed. In blood plasma samples obtained in the preliminary range finding test, Reg.No. 411847 was detectable in the samples from the 1000 mg/kg bw treated animals, but not 500 mg/kg bw. A plausible explanation for this is the assumption that the severely intoxicated mice at 1000 mg/kg bw were not able to completely metabolize the test-item whereas this was the case at the lower dose of 500 mg/kg bw. The aforementioned analysis of the Reg.No. 411847 plasma concentration was performed with a method, which was retrospectively validated. The validation report can be found in M-CA 4 (see BASF DocID 2016/1321627).

In the main study blood was sampled for plasma analysis at necropsy from all animals including the additional 10 animals dosed at 1000 mg/kg bw, which were solely dosed for this purpose.

No test-item was detected in the plasma samples from mice at 125 mg/kg bw after 24 hours and at 500 mg/kg bw after 48 hours. However, in the plasma sampled after 24 hours of 1/5, 3/5 and 2/2 mice Reg.No. 411847 was quantifiable at 250, 500 and 1000 mg/kg bw, respectively. This demonstrates that the test-item is bioavailable and reaches the highly blood perfused bone marrow. This is also demonstrated by the signs of systemic toxicity observed in treated mice.

B. PRELIMINARY RANGE FINDING TEST

In a pretest, deaths were observed at 1000 mg/kg. At 500 mg/kg, all animals survived but distinct signs of toxicity were observed consisting of hunched posture, abdominal position, reduced general condition, irregular respiration and respiration shallow. However, there were no distinct differences in clinical observations between male and female animals. Thus, only male animals were used in the main experiment.

C. MICRONUCLEUS ASSAY

Treatment of mice with Reg.No. 411847 did not lead to a biologically relevant increase in the rate of micronuclei (see Table 5.8.1-78). Micronucleus frequencies in PCE of the animals treated with the indicated doses of the test substance were below the concurrent vehicle control values and within the historical control range.

The PCE/NCE ratio was not affected by treatment with the test substance which indicates that erythropoiesis was not inhibited.

The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE containing exclusively small micronuclei. The administration of the spindle poison vincristine resulted in an incidence of small and large micronuclei in polychromatic erythrocytes.

Dose dependent signs of systemic toxicity were observed in test substance treated mice at all dose levels. These consisted of piloerection, hunched posture (at ≥ 250 mg/kg bw), abdominal position, reduced general condition, and shallow respiration (at ≥ 500 mg/kg bw) and a narcotic like state (1000 mg/kg bw only). At 1000 mg/kg body weight eight out of ten animals died as early as 1 hour after administration. No signs of systemic toxicity were observed in any of the animals treated with the positive control substances or the vehicle.

Table 5.8.1-78: Micronucleus test in mice administered Reg.No. 411847 by oral gavage

Treatment	PCE				NCE		PCE/NCE ratio
	scored	With MN			Number	With MN [‰]	
		total [‰]	small [‰]	large [‰]			
DMSO emulsified in corn oil corn oil	10000	0.6	0.6	0.0	5176	0.0	1.9
Reg.No. 411847							
125 mg/kg	10000	0.2	0.2	0.0	5051	0.8	2.0
250 mg/kg	10000	0.3	0.3	0.0	6023	1.0	1.7
500 mg/kg	10000	0.5	0.5	0.0	5791	0.7	1.7
Positive controls							
Cyclophosphamide	10000	**12.4****	**12.4****	0.0	4635	0.4	2.2
Vincristine	10000	**39.1****	**27.3****	**11.8****	5117	1.6	2.0
48 h preparation interval							
DMSO and emulsified in corn oil	10000	0.6	0.6	0.0	4119	0.0	2.4
Reg.No. 411847							
500 mg/kg	10000	0.5	0.5	0.0	5025	1.4	2.0

** $p \leq 0.01$ (Wilcoxon Test; one sided)
PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes; MN: micronuclei

III. CONCLUSION

Based on the result of this study 500M60 (Reg.No. 411847, BF 500-11) does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study, i.e. is devoid of clastogenic or aneugenic activity in vivo. The analysis of Reg.No. 411847 in rat plasma demonstrated that the test item reached the target organ (bone marrow). Thus, the clastogenicity observed in vitro is not relevant under in vivo conditions.

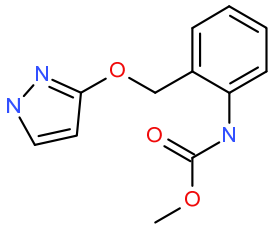
Overall toxicological evaluation of 500M60 (Reg.No. 411847, BF 500-11)

The QSAR evaluation of 500M60 is of low reliability and by weight of evidence there was no conclusive alert for genotoxity.

By weight of evidence 500M60 is considered to be not genotoxic based on the in vitro and in vivo studies conducted. Therfore, 500M60 is considered to be **not toxicologically relevant**.

The highest calculated concentration (PEC_{sw}) is 0.266 µg/L. No relevant human exposure is to be expected.

500M62 (other denominators: Reg.No. 412785, BF 500-13)



500M62 (Reg.No. 412785, BF 500-13) is a photolysis metabolite found in surface water.

A) QSAR Predicitions for 500M62 (Reg.No. 412785, BF 500-13)

OECD Toolbox (Version: 3.2) [see DocID 2014/1172955]

For 500M62 (Reg.No. 412785)the OECD toolbox reveald no alert for protein binding but two complex alerts for DNA binding and DNA alerts for Ames, MN and CA, i.e. '*Radical|Radical >> Radical mechanism via ROS formation (indirect)|Radical >> Radical mechanism via ROS formation (indirect) >> Single-Ring Substituted Primary Aromatic Amines|SN1|SN1 >> Nucleophilic attack after metabolic nitrenium ion formation|SN1 >> Nucleophilic attack after metabolic nitrenium ion formation >> Single-Ring Substituted Primary Aromatic Amines*' and '*AN2|AN2 >> Carbamoylation after isocyanate formation|AN2 >> Carbamoylation after isocyanate formation >> N-Hydroxylamines|Radical|Radical >> Radical mechanism via ROS formation (indirect)|Radical >> Radical mechanism via ROS formation (indirect) >> N-Hydroxylamines|SN1|SN1 >> Nucleophilic attack after metabolic nitrenium ion formation|SN1 >> Nucleophilic attack after metabolic nitrenium ion formation >> N-Hydroxylamines*'

Discussion: The above alerts were also identified for 500M49 (Reg.No. 5916420). This metabolite - like 500M62 (Reg.No. 412785) - was negative in the Ames test. Metabolite 500M49 (Reg.No. 5916420) was also negative for in vitro chromosome aberration in the in vitro micronucleus test and in the mouse lymphoma assay investigating mutagenicity in mammalian cells. Therefore, the predicted alerts do not reflect the experimental data and are thus rejected. Therefore, the weight of evidence indicates that 500M60 (Reg.No. 411847) is not genotoxic.

OASIS TIMES (MIX V.2.27.13 TB; Mutagenicity S-9 activated v08.08) [see molecule 3 of report DocID 2014/1172952 and 2014/1172953]

There was no Ames mutagenicity alert for 500M62 (Reg.No. 412785). However, 4 of 7 or in-silico generated metabolites were predicted positive (in domain) with the alerts 'Single-ring substituted primary aromatic amines' and 'N-Hydroxylamines'. The prediction in vitro chromosome aberration for 500M62 (Reg.No. 412785) was poitive (out of domain), as it was positive for 4 out of 7 in-silico generated metabolites. The respective alerts were 'Single-ring substituted primaryaromatic amines', 'substituted anilines' and 'N-Hydroxylamines'. All positive in vitro CA alerts were 'in domain'.

Discussion: As discussed above for the alerts found in the OECD Toolbox, the OASIS TIMES alerts were also identified for 500M49 (Reg.No. 5916420). This metabolite - like 500M62 (Reg.No. 412785) - was negative in the Ames test. Metabolite 500M49 (Reg.No. 5916420) was also negative for in vitro chromosome aberration in the in vitro micronucleus test and in the mouse lymphoma assay investigating mutagenicity in mammalian cells. Therefore, the predicted alerts do not reflect the experimental data and are thus rejected. Therefore, the weight of evidence indicates that 500M60 (Reg.No. 411847) is not genotoxic.

VEGA: Mutagenicity model (CAESAR, version 2.1.9) [see molecule 3 of report BASF DocID 2014/1172954 for both VEGA models]

500M62 (Reg.No. 412785) is out of model applicability domain. The prediction is 'non mutagen'.

VEGA: Mutagenicity model (SarPy; version 1.0.4-BETA)

500M62 (Reg.No. 412785) is out of model applicability domain. The prediction is 'mutagen' with the structural alerts SA 96 and SA98. As discussed above, this alert was also obtained for pyraclostrobin and a number of pyraclostrobin metabolites, which like 500M62 (Reg.No. 412785) were actually tested negative. The predicition is therefore rejected.

DEREK (Version 2.0.0.201011301322; Knowledge base version: 11_20_05_2010)

Metabolite 500M62 (Reg.No. 412785) was not evaluated in this system.

Studies on 500M62 (Reg.No. 412785, BF 500-13)

Study already submitted:

In the original submission, an Ames test on 500M62 (Reg.No. 412785; BF 500-13) was submitted and assessed (BASF DocID 1999/12020). A standard plate test (SPT) and a pre-incubation test (PIT) with and without metabolic activation was performed at concentrations of 22 - 5000 µg/plate (SPT) and 4 - 2500 µg/plate (PIT) in S. typhimurium strains TA 98, TA 100, TA 1535 and TA 1537 as well as in E. coli WP2 uvrA. A slight bacteriotoxicity was noted at ≥ 2500 µg/plate in the SPT and (depending on the strain) at concentrations ≥ 500 µg/plate in the PIT while no precipitations were noted at any concentration. No increase of revertant colonies was observed in any of the tests performed.

New studies submitted in this dossier:

Report:	CA 5.8.1/53 Nauman S., 2017 Reg.No. 412785 (Metabolite of BAS 500 F, Pyraclostrobin) - Micronucleus test in human lymphocytes in vitro 2017/1024661
Guidelines:	OECD 487 (2016), Commission Regulation (EU) No 640/2012
GLP:	yes (Laboratory certified by Hess. Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz, Wiesbaden, Germany)

Executive Summary

Reg.No. 412785 (metabolite of pyraclostrobin; batch: 01586-60; purity: 99.5%) was tested for its potential to induce micronuclei in human lymphocytes *in vitro* in the absence and presence of metabolic activation by S9 mix. Two independent experiments were performed. In duplicate cultures the cells were incubated for 4 (±S9 mix) or 20 hours (-S9 mix) with the test substance at concentrations in the range of 13.1 to 2010 µg/mL. At least three concentrations were evaluated, which were either limited by cytotoxicity or the precipitation of the test item in the media. The vehicle DMSO (0.5% in culture medium) served as negative control, Mitomycin C (4 h) and Demecolcin (20 h) as positive controls in the absence of metabolic activation and Cyclophosphamide as positive control in the presence of metabolic activation. Treatments started after a 48-hour stimulation period with phytohemeagglutinine. Thereafter, Cytochalasin B was added and the cultures were fixed and stained finally after another 20 hours. Cytokinesis-block proliferation index (CBPI) and cytostasis were determined in 1000 binucleated cells as cytotoxicity parameters. The number of micronucleated cells were determined in 2000 binucleated cells for evaluation of mutagenicity.

In both experiments without metabolic activation and in one experiment with metabolic activation, no cytotoxicity was observed up to the highest evaluated concentration, which showed precipitation without addition of S9 mix from 1149 µg/mL onwards and with addition of S9 mix at the top dose of 2010 µg/mL. In the other experiment with metabolic activation, precipitation at the top dose was accompanied by cytotoxicity.
Independent of metabolic activation, no increase in the number of cells carrying micronuclei was observed in both experiments. The solvent control revealed values that were within the range of the laboratory historical negative control data. The positive control chemicals led to the expected increase in cells containing micronuclei that was within the range of the historical positive control data, thus demonstrating the sensitivity of the test system and the validity of the study.

In conclusion, under the experimental conditions Reg.No. 412785 (= 500M62, metabolite of pyraclostrobin) did not induce the formation of micronuclei in human lymphocytes under in vitro conditions in presence and absence of metabolic activation.

(BASF DocID 2017/1024661)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 412785 (metabolite of pyraclostrobin)

Description:	Solid (crystals) / white
Lot/Batch #:	01586-60
Purity:	99.5%
Stability of test compound:	The stability of the test substance is guaranteed (expiry date 01-May-2020)
Solvent used:	DMSO

2. Control Materials:

Negative control:	A negative control was not employed in this study.
Solvent control:	DMSO (0.5% final concentration in culture medium)
Positive control:	Without metabolic activation: Mitomycin C (MMC, 1.5 µg/mL; pulse treatment) dissolved in deionized water; Demecolcin (125 ng/mL; continuous treatment) dissolved in deionized water With metabolic activation: Cyclophosphamide (CPA, 15 µg/mL (Exp. I) or 17.5 µg/mL (Exp. II) dissolved in saline (0.9% NaCl))

3. Activation: S9 was produced from the livers of rats pretreated with β-naphthoflavone/phenobarbital. For testing, S9-fraction (protein content: 30.1 mg/mL (Exp. I) and 34.9 mg/mL (Exp. II)) was thawed at room temperature and mixed with S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following final composition:

Component	Concentration
Phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

50 µL S9 mix per mL culture medium were used, yielding a 2.5% S9 fraction or final protein concentration of 0.75 mg/mL in each culture.

4. Test organism: **Human** peripheral blood lymphocytes

Donor(s): Experiment I: 26 years old male
Experiment II: 23 years old male

5. Culture media:

Culture medium: Dulbecco´s Modified Eagles medium/Ham´s F12 (1:1) with GlutaMAX™ (200 mM) supplemented with 10% (v/v) fetal bovine serum (FBS), Pen/Strep (100 U/mL / 100 µg/mL), HEPES (10 mM), heparin (125 U.S.P.-U/mL), and phytohemeagglutinine (PHA, 3 µg/mL).

6. Test concentrations:

Micronucleus assay

Experiment I

(4-h exposure, -S9): 13.1, 22.9, 40.0, 70.0, 122, 214, **375, 656, 1149** and 2010 µg/mL; precipitation at ≥ 1149 µg/mL (evaluated concentrations are indicated in bold)

(4-h exposure, +S9): 13.1, 22.9, 40.0, 70.0, 122, 214, 375, **656, 1149** and **2010** µg/mL; precipitation at 2010 µg/mL (evaluated concentrations are indicated in bold)

Experiment II

(4-h exposure, +S9): 176, 265, 397, 596, **893, 1340** and **2010** µg/mL; precipitation at 2010 µg/mL (evaluated concentrations are indicated in bold)

(20-h exposure, -S9): 176, 265, **397, 596, 893,** 1340 and **2010** µg/mL; precipitation at ≥ 1340 µg/ml (evaluated concentrations are indicated in bold)

B. TEST PERFORMANCE

1. Dates of experimental work: 22-Feb-2017 to 28-Mar-2017

2. Dose selection:

Dose selection was performed according to the current OECD Guideline for the in vitro micronucleus test. The highest test item concentration should be 2000 µg/mL, 2 µL/mL or 10 mM, whichever is the lowest, or be limited by cytotoxicity (up to 55 ± 5% cytostasis) and/or limited solubility visible by test item precipitation. At least three test item concentrations should be evaluated for cytogenetic damage.

A preliminary cytotoxicity test was performed to determine the concentrations to be used in the main experiment. Cytotoxicity is characterized by the percentages of reduction in the Cytokinesis-block proliferation index (CBPI) in comparison with the controls (% cytostasis) by counting 500 cells per culture in duplicate. The experimental conditions in this pre-experimental phase were identical to those required and described below for the mutagenicity assay. The pre-test was performed with 10 concentrations of the test item separated by no more than a factor of √10 and a solvent and positive control. All cell cultures were set up in duplicate. Exposure time was 4 hours (± S9 mix). The preparation interval was 40 hours after start of the exposure.

With regard to the purity (99.5%) of the test item, 2010 µg/mL were applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations ranging from 13.1 to 2010 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity.
In the pre-test for toxicity, precipitation of the test item was observed at the end of treatment at 1149 µg/mL and above in the absence of S9 mix and at 2010 µg/mL in the presence of S9 mix. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.
Using a CBPI as an indicator for toxicity, no cytotoxic effects were observed in Experiment I after 4 hours treatment in the absence and presence of S9 mix. Therefore, 2010 µg/mL were chosen as top treatment concentration for Experiment II.

3. Micronucleus test:

Pulse exposure (±S9)

About 48 h after seeding two blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with complete medium (with 10 % FBS) containing the test item. After 20 hours, the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in and washed with saline. The washing procedure was repeated once as described. After washing, the cells were re-suspended in complete culture medium containing 10 % FBS (v/v). Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Continuous exposure (-S9)

About 48 h after seeding 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with complete medium (with 10 % FBS) containing the test item. After 20 hours, the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in and washed with saline. The washing procedure was repeated once as described. After washing the cells were re-suspended in complete culture medium containing 10 % FBS (v/v). Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Preparation of cells

The cultures were harvested by centrifugation 40 h after beginning of treatment. The cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in approximately 5 mL saline and spun down once again by centrifugation for 5 minutes. Then the cells were resuspended in 5 mL KCl solution (0.0375 M) and incubated at 37°C for 20 minutes. 1 mL of ice-cold fixative mixture of methanol and glacial acetic acid (19 parts plus 1 part, respectively) was added to the hypotonic solution and the cells were resuspended carefully. After removal of the solution by centrifugation the cells were resuspended for 2 x 20 minutes in fixative and kept cold. The slides were prepared by dropping the cell suspension in fresh fixative onto a clean microscope slide. The cells were stained with Giemsa.

4. Statistics:

Statistical significance was confirmed by the Chi square test ($\alpha < 0.05$), using a validated test script of "R", a language and environment for statistical computing and graphics. Within this test script a statistical analysis was conducted for those values that indicated an increase in the number of cells with micronuclei compared to the concurrent solvent control.

5. Cytotoxicity evaluation:

Evaluation of cytotoxicity and cytogenetic damage

Evaluation of the slides was performed using microscopes with 40 x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The micronuclei had to be stained in the same way as the main nucleus. The area of the micronucleus should not extend the third part of the area of the main nucleus. At least 1000 binucleated cells per culture were evaluated for cytogenetic damage on coded slides. The frequency of micronucleated cells was reported as % micronucleated cells. To describe a cytotoxic effect, the CBPI (Cytokinesis-block proliferation index) was determined in 500 cells per culture and cytotoxicity is expressed as % cytostasis. A CBPI of 1 (all cells are mononucleate) is equivalent to 100 % cytostasis.

The CBPI was calculated as follows:

$$CBPI = \frac{(MONC \times 1) + (BINC \times 2) + (MUNC \times 3)}{n}$$

CBPI	Cytokinesis-block proliferation index
n	Total number of cells
MONC	Mononucleate cells
BINC	Binucleate cells
MUNC	Multinucleate cells

$$\text{Cytostasis \%} = 100 - 100\,[(CBPI_T - 1) / (CBPI_C - 1)]$$

T	Test item
C	Solvent control

6. Evaluation criteria:

Acceptability criteria

- The rate of micronuclei in the solvent controls falls within the historical laboratory control data range.
- The rate of micronuclei in the positive controls is statistically significantly increased.
- The quality of the slides must allow the evaluation of a sufficient number of evaluable cells.

Evaluation criteria

A test item can be classified as non-clastogenic and non-aneugenic if:

- the number of micronucleated cells in all evaluated dose groups is in the range of the historical laboratory control data and
- no statistically significant or concentration-related increase of the number of micronucleated cells is observed in comparison to the respective solvent control.

If the above-mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

An increase in the number of micronucleated mononucleate cells may indicate that the test item has aneugenic potential.

II. RESULTS AND DISCUSSION

A. ANALYTICS

Stability of the test item in DMSO for a period of 4 hours at room temperature was verified analytically (see separate report 01Y0253/12Y146; BASF DocID 2017/1078583 - available upon request).

B. CYTOTOXICITY, PRECIPITATION AND OSMOLARITY

In Experiment I, precipitation of the test item in the culture medium was observed at 1149 µg/mL and above in the absence of S9 mix and at 2010 µg/mL in the presence of S9 mix at the end of treatment. In addition, precipitation occurred in Experiment II in the absence of S9 mix at 1340 µg/mL and above and in the presence of S9 mix at 2010 µg/mL at the end of treatment [see Table 5.8.1-80].
No relevant influence on osmolarity or pH was observed.
In Experiment I in the absence of S9 mix and in Experiment II in the presence of S9 mix, no cytotoxicity was observed up to the highest evaluated concentration, which showed precipitation. In Experiment I in the presence of S9 mix, the highest applied and evaluated concentration showed cytotoxicity and precipitation. In Experiment II in the absence of S9 mix, no cytotoxicity was observed up to the highest applied concentration and precipitation occurred in the two highest applied concentrations (1340 and 2010 µg/mL). Since the cultures treated with 1340 µg/mL did not fulfill the criteria for genotoxic evaluation (at least 1000 evaluable cells per duplicate culture), the highest applied concentration was evaluated for genotoxicity.

C. MICRONUCLEUS ASSAY

In this study, neither a statistically significant nor a biologically relevant increase in the number of micronucleated cells was observed after treatment with the test item [see Table **5.8.1-52**5.8.1-80].
The micronucleus rates of the cells after treatment with the test item were close to the range of the respective solvent control values and within the range of the laboratory historical control data (HCD). The HCD in the absence of S9 mix were 0.15-1.65% (95% control limits 0.08-1.12%) for (4h) pulse treatment and 0.05-1.35% (95% limits: 0.12-1.03%) for continuous (20h) treatment. The HCD in the presence of S9 mix were 0.15-1.30% (95% limits: 0.16-1.08%) for pulse treatment.

All positive controls used, i.e. Demecolcin (150 ng/mL), MMC (1.5 µg/mL) or CPA (17.5 or 15.0 µg/mL), resulted in distinct increases in cells with micronuclei (3.15-14.85%). These responses were within the range of the laboratory historical positive control data thus demonstrating the sensitivity and validity of the test system.

Table 5.8.1-80: Summary of results of the in vitro micronucleus test in human lymphocytes with Reg.No. 412785

Exp.	Exposure period [h]	Test item concentration [µg/mL]	Proliferation index CBPI	Cytostasis [%[a]]	Micronucleated cells [%[b]]
Without S9 mix					
I	4	Solvent control[1]	1.83		0.30
		Positive control[2]	1.34	58.4	**14.85***
		375	1.81	2.2	0.70
		656	1.75	9.4	0.45
		1149[P]	1.78	5.3	0.45
II	20	Solvent control[1]	2.02		0.55
		Positive control[3]	1.90	11.3	**3.15***
		397	1.91	10.9	0.15
		596	1.79	22.9	0.10
		893	1.74	27.1	0.35
		1340[P]	1.63	37.9	n. e.
		2010[P]	1.71	30.8	0.45
With S9 mix					
I	4	Solvent control[1]	2.02		1.10
		Positive control[4]	1.53	48.2	**4.55***
		656	1.84	17.6	0.35
		1149	1.73	28.0	0.40
		2010[P]	1.53	48.1	0.65
II	4	Solvent control[1]	2.20		0.40
		Positive control[5]	1.78	35.3	**5.75***
		893	2.01	15.5	0.30
		1340	1.88	26.5	0.65
		2010[P]	1.90	24.6	0.50

a: For the positive control groups and the test item treatment groups the values are related to the solvent controls.
b: The number of micronucleated cells was determined in a sample of 2000 binucleated cells.
P: Precipitation occurred at the end of treatment by the unaided eye.
CBPI: cytokinesis-block proliferation index
n. e. Not evaluable; the cultures did not fulfill the criteria for genotoxic evaluation (≤ 1000 appropriate binucleate cells per culture).
*: The number of micronucleated cells is statistically significantly higher than corresponding control values ($p \leq 0.05$, Chi-squared test).
[1] DMSO 0.5% (v/v)
[2] MMC 1.5 µg/mL
[3] Demecolcin 125 ng/mL
[4] CPA 15.0 µg/mL
[5] CPA 17.5 µg/mL

III. CONCLUSION

Under the experimental conditions of this study Reg.No. 412785 (metabolite of pyraclostrobin) did not induce micronuclei as determined by the in vitro micronucleus test in human lymphocytes when tested up to precipitating concentrations with and without metabolic activation.

Toxicological evaluation of 500M62 (Reg.No. 412785, BF 500-13)

The QSAR evaluation of 500M62 is of low reliability and by weight of evidence there was no conclusive alert for genotoxity. Metabolite 500M62 was neither genotoxic in the Ames test nor in an in-vitro micronucleus assay in human lymphocytes. In conclusion, 500M62 is considered to be **not toxicologically relevant**.

The highest calculated concentration in surface water (PEC_{sw}) is 0.413 µg/L. No relevant human exposure is to be expected as untreated surface water is not used as drinking water.

CA 5.8.2 Supplementary studies on the active substance

Studies evaluated in the draft monograph of Rapporteur Member State Germany of August 01, 2001:

No supplemental studies on the active substance were evaluated in the previous Annex I listing process besides those indicated in M-CA 5.8.1.

Submission of not yet per-reviewed studies in this dossier:

A study investigating the effects of pyraclostrobin administration on blood plasma and urine iron as well as plasma transferrin levels was performed. Lower serum iron levels were noted, which were however not accompanied by lower serum transferrin levels or higher urinary iron excretion.

Upon request of US EPA, studies were conducted investigating the T-cell dependent antigen response and the natural killer cell mediated immune response. The respective studies are listed in Table 5.8.2-1. The immunotoxicity studies did not reveal a specific effect on the T-cell dependent or NK-cell mediated immune response.

Additionally, a 28-day range finding study was performed to set the dose levels for the subsequent immunotoxicity studies. The T-cell dependent antigen response had to be repeated because of an abnormal, 2-fold higher humoral immune response in control animals when compared to the historical control. These two studies are not listed in Table 5.8.2-1 as the results are not end-point relevant.

In addition, literature data were taken into account (see CA 5.8.2/6 to CA 5.8.2/9). However, these data are judged to represent supplemental information only and do not provide relevant endpoints.

Table 5.8.2-1: Summary of not yet peer-reviewed supplemental studies with pyraclostrobin

Study	Dosages (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
Immunotoxicity, mouse, T-cell dependent immune response 0, 50, 200, 750 ppm	F: 14, 55, 191	55 (systemic) 55 (immunotox NOEL)	Body weight effects, lower thymus and spleen weights, lower number of spleen cells, but no adverse effect on functional T-cell dependent immune response	2012/1084176
Immunotoxicity, mouse, NK-cell mediated immune response 0, 50, 200, 750 ppm	F: 13, 50, 165	50 (systemic) 165 (immunotox)	Body weight effects, lower thymus and spleen weights, lower number of spleen cells, but no effect on NK cell activity	2011/1035857
Effects on iron blood serum and urine levels, 14-day study in rats 0, 50, 500, 1500 ppm	M: 3.8, 33.9, 73.9 F: 4.1, 37.4, 78.3	3.8	Lower serum iron level in blood serum	2003/1009534

Thus, the conclusion for relevant endpoints for the current renewal is as follows:

Other toxicological studies (SANCO/11802 data point 5.8)

Supplementary studies on the active substance	**Immunotoxicity of pyraclostrobin** No evidence for specific immunotoxicity No classification required
	Effect of pyraclostrobin on iron levels in rats Decreased blood plasma iron levels (NOAEL = 3.8 mg/kg bw/day)

Effect of pyraclostrobin on iron levels in rats (CA 5.8.2/1)

Report: CA 5.8.2/1
[redacted], 2003a
BAS 500 F - Determination of iron in urine and serum of Wistar rats - Administration in the diet over 14 days
2003/1009534
Guidelines: none
GLP: no

Executive Summary

The aim of this study was to determine the level of iron in serum and urin after oral administration of pyraclostrobin (BAS 500 F). Pyraclostrobin was administered to groups of 10 female Wistar rats at dietary concentrations of 0, 50, 500 and 1500 ppm over a period of 14 days.

Food consumption and body weight development was impaired in both sexes at ≥ 500 ppm. At the high dose level an overall body weight loss was observed.

Serum iron concentrations were dose- and time dependently decreased in mid and high dose animals up to 50% when compared to the control. Serum transferrin levels or urinary iron excretion were not affected by treatment. No treatment related effects were noted at 50 ppm. Thus, regarding the decrease of serum iron concentration, the no observed effect level was 50 ppm (3.8 mg/kg bw/day in males, 4.1 mg/kg bw/day in females).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin

Description:	solid / crystalline / yellowish
Lot/Batch #:	CP 029053
Purity:	99.0%
Stability of test compound:	Stable - Expiry date July 2005

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Wistar (CrlGlxBrlHan:WI)
Sex:	Male and female
Age:	about 8 weeks
Weight at dosing:	mean ♂: 237 g, ♀ 167 g
Source:	Charles River, Sulzfeld, Germany
Acclimation period:	6 days
Diet:	basic maintenance diet for rat/mouse/hamster, meal from Provimi KLIBA SA, Kaiseraugust, Switzerland, ad libitum
Water:	water, ad libitum
Housing:	type DK III stainless steel wire mesh cages, floor area about 800 cm²

Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	not reported
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 18-Mar-2003 - 10-Apr-2003
(In life dates: 19-Mar-2003 (start of administration) to 03-Apr-2003 (necropsy)

2. Animal assignment and treatment:

Pyraclostrobin was administered to groups of 10 male and 10 female rats at dietary concentrations of 0, 50 (low dose), 500 (mid dose) and 1500 ppm (high dose) for up to 14 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

3. Test substance preparation and analysis:

An acetonic solution of the test substance was prepared. This solution was sprayed on about three kilograms of food under partial vacuum in a laboratory evaporator. Subsequently, acetone was removed at 900 mbar at a temperature of about 40°C. This premix was then adjusted to the desired concentration with the addition afappropriate amounts of food and mixed in a laboratory mixer for about 10 minutes.

The stability of the test substance in the diet over a period of up to 43 days at room temperature was proven earlier. No further analyses of the test substance preparations were carried out for this study.

4. Statistics:

Means and standard deviations (S.D.) of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Statistics of clinical examinations, clinical pathology and pathology

Parameter	Statistical test
food consumption, body weight	A comparison of the dose group with the control group was performed using Welch t-test (two-sided) for the hypothesis of equal means.
Clinical phathology	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians.

C. METHODS

1. Observations:

The animals were examined for evident signs of toxicity or mortality twice daily on working days and once daily on weekends and public holidays. Observations for general clinical signs of toxicity were performed once daily.

2. Body weight:

The body weight of the animals was determined on the day of randomization, at the start of the treatment (day 0), and weekly thereafter.

3. Food consumption and compound intake:

Individual food consumption was determined weekly (according to body weight) and calculated as mean food consumption in grams per animal and day.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$Substance\ intake\ for\ day\ x = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm, and BW_x as body weight on day x of the study (in g).

4. Water consumption:

Not performed in this study.

5. Ophthalmoscopy:

Not performed in this study.

6. Hematology and clinical chemistry:

Blood samples were taken from the retroorbital venous plexus in the morning (day 14) from non-fasted animals. Iron and transferrin levels were determined for all animals.

7. Urinalysis:

For determination of iron in urine spechimens individual animals were transferred to metabolism cages (withdrawal of food and water) and urine was collected overnight.

8. Sacrifice and pathology:

The animals were sacrificed by decapitation under carbon dioxide anesthesia. Five centimeter of duodenum were removed for a possible PCNA examination and fixed for 24 hours in 4% formaldehyde. The caudal part was cut for identification.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above

B. OBSERVATIONS

1. Clinical signs of toxicity

There was nothing abnormal detected in all animals.

2. Mortality

No animal died during the study.

3. Ophthalmoscopy

Not performed in this study.

C. BODY WEIGHT AND BODY WEIGHT GAIN

An overall body weight loss was observed in both male and female rats at the high dose [see Figure 5.8.2-1 and Table 5.8.2-2]. In mid dose males, effects on body weight and body weight gain were observed, too.

Figure 5.8.2-1: Body weight development of rats administered pyraclostrobin for 14 days

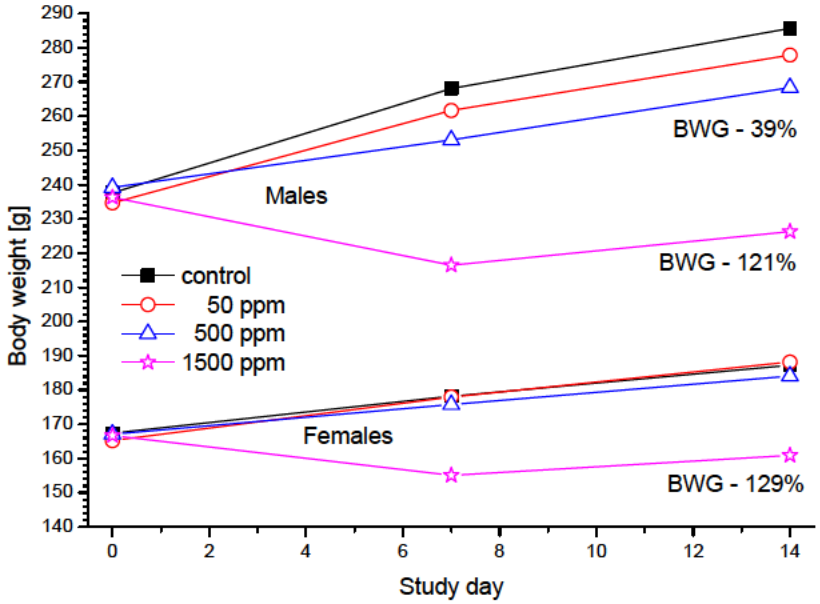


Table 5.8.2-2: Mean body weight of rats administered pyraclostrobin for 14 days

	Males			Females		
day	0	7	14	0	7	14
Dose level [ppm]						
0	237.6 ± 9.3	268.1 ± 13.9	285.7 ± 18.0	167.4 ± 5.4	178.2 ± 8.7	187.2 ± 8.4
50	234.7 ± 7.2	261.7 ± 10.5	277.9 ± 15.1	165.2 ± 5.6	178.0 ± 10.1	188.2 ± 11.1
Δ% (compared to control)#	-1.2	-2.4	-2.8	-1.3	-0.1	0.5
500	239.2 ± 7.5	**253.1 ± 9.7***	**268.4 ± 13.4***	167.1 ± 7.7	175.8 ± 8.1	184.1 ± 8.2
Δ% (compared to control)#	6.7	-5.6	-6.0	-0.2	-1.3	-1.7
1500	236.3 ± 7.5	**216.5 ± 9.5****	**226.3 ± 10.8****	166.7 ± 6.2	**155.1 ± 8.9****	**160.9 ± 6.2****
Δ% (compared to control)#	-0.5	-19.2	-20.8	-0.4	-13.0	-14.1

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)
*: p = 0.05, **: p = 0.01 (Dunnett's test, two-sided)

D. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption was significantly decreased in high and mid dose males and females during the study [see Table 5.8.2-3].

The approximate mean daily test substance intake was calculated to be 3.8, 33.9 and 73.9 mg/kg bw/day in males and 4.1, 37.4 and 78.3 mg/kg bw/day in females at dietary dose levels of 50, 500, and 1500 ppm, respectively.

Table 5.8.2-3: Mean food consumption of rats administered pyraclostrobin for 14 days

	Males		Females	
day	7	14	7	14
Dose level [ppm]				
0	21.8±1.2	20.1±1.4	15.6±1.0	14.9±0.7
50	21.0±0.9	19.4±1.4	15.4±0.9	14.7±1.1
Δ% (compared to control) #	-3.7	-3.5	-0.9	-1.0
500	**13.4±1.6****	**18.0±1.4****	**13.1±1.1****	**13.8±0.9***
Δ% (compared to control) #	-20.3	-10.5	**-16.0**	**-7.1**
1500	**9.0±1.6****	**12.9±1.2****	**6.9±1.2****	**9.7±0.8****
Δ% (compared to control) #	-58.6	-35.7	-55.9	-34.8

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)
*: p = 0.05, **: p = 0.01 (Dunnett's test, two-sided)

E. BLOOD ANALYSIS

1. Serotological findings

In males a dose and time dependent decrease of serum iron levels was observed [see Table 5.8.2-4]. Even though not statistically significant, the decrease in mid dose males after 7 days was considered to be treatment-related. In females no consistent (dose-dependent) effect on iron levels was observed after 7 days. However, the 14 day data indicated a dose-dependent decrease of iron serum levels, which was however less pronounced when compared to males.

Table 5.8.2-4: Iron concentration (µmol/L) in the serum of male and female rats after 7 and 14 days of pyraclostrobin administration

Sex	Blood sampling		0 ppm	50 ppm	500 ppm	1500 ppm
Males	Day 7	Mean % Dev.	47.01 ± 13.51	44.84 ± 6.66 -5	**36.61 ± 5.27** **-22**	**34.85 ± 3.10*** **-26**
	Day 14	Mean % Dev.	54.54 ± 9.83	46.71 ± 6.57 -14	**37.72 ± 5.21**** **-31**	**27.41 ± 4.36**** **-50**
Females	Day 7	Mean % Dev.	59.07 ± 8.47	60.09 ± 14.11 2	46.48 ± 6.38** -21	57.49 ± 15.62 -3
	Day 14	Mean % Dev.	53.61 ± 4.16	58.31 ± 12.22 9	**45.55 ± 9.03*** **-15**	**41.97 ± 9.94**** **-22**

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal Wallis + Wilcoxon-test)

There were no treatment-related changes in the serum transferrin levels of either sex at both time intervals.The slight decrease in transferrin concentration in the serum of the high-dose group males on day 7 was not consistent over time and was regarded to be incidental and not toxicologically relevant [see Table 5.8.2-5].

Table 5.8.2-5: Transferrin concentration (g/L) in the serum of male and female rats after administration of pyraclostrobin for 7 and 14 days

Sex	Blood sampling		0 ppm	50 ppm	500 ppm	1500 ppm
Males	Day 7	Mean % Dev.	5.50 ± 0.34	5.34 ± 0.38 -3	5.45 ± 0.42 -1	4.74 ± 0.37** -14
	Day 14	Mean % Dev.	6.38 ± 0.77	5.80 ± 0.60 -9-	6.07 ± 0.79 -5	5.85 ± 0.47 -8
Females	Day 7	Mean % Dev.	5.40 ± 0.67	5.23 ± 0.27 -3	5.62 ± 0.52 4	4.92 ± 0.39 -9
	Day 14	Mean % Dev.	5.86 ± 0.72	5.68 ± 0.65 -3	6.13 ± 0.56 5	5.63 ± 0.67 -4

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal Wallis + Wilcoxon-test)

2. Clinical chemistry findings

Not performed in this study.

3. Urinalysis

Iron levels in urine were not affected by treatment. The observed numerical differences displayed no statistical significance and were not dose related [see Table 5.8.2-6].

Table 5.8.2-6: Iron concentration (nmol) in the serum of male and female rats after administration of pyraclostrobin for 14 days

Sex	Urine sampling		0 ppm	50 ppm	500 ppm	1500 ppm
Males	Day 15	Mean % Dev.	3.97 ± 1.40	3.27 ± 1.49 -17.6	2.69 ± 1.18 -32.1	3.60 ± 4.39 -9.1
Females	Day 15	Mean % Dev.	2.61 ± 0.90	2.44 ± 1.14 -6.5	2.78 ± 1.48 6.3	2.89 ± 1.17 10.7

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal Wallis + Wilcoxon-test)

B) F. NECROPSY

No gross necropsy observations were made at sacrifice.

III. CONCLUSION

Dietary administration of pyraclostrobin to rats at dose levels of 0, 50, 500 and 1500 ppm over a period of 14 days resulted in a decrease of serum iron concentrations in mid and high dose animals of either sex 14 days after start of treatment. Serum transferrin und urinary iron excretions were not affected by treatment. No treatment-related effects were seen in the low-dose animals.

Thus, regarding the decrease of serum iron concentration, the no observed effect level was 50 ppm (3.8 mg/kg bw/day in males, 4.1 mg/kg bw/day in females).

Immunotoxicity studies (CA 5.8.2/2 – CA 5.8.2/5)

Report: CA 5.8.2/2
[redacted] 2011a
A 28 day oral (dietary) range finding study of BAS 500 F in B6C3F1 mice
2011/1194286
Guidelines: none
GLP: no

Executive Summary

As a range finding study for a subsequent short term studies to determine the immunotoxicological potential of pyraclostrobin, pyraclostrobin (batch: COD-001236; purity: 99.02%) was administered to $B_6C_3F_1$ mice at dietary dose levels of 0, 500, and 1500 ppm for 28 days. The high dose treatment resulted in poor clinical condition and body weight loss within 3 days. As a consequence, the high dose level was terminated after 3 days. Treatment was tolerated at 500 ppm with moderate effects on food consumption and body weight development. Minimal hyperplasia of the duodenal mucosa was observed in both sexes at 500 ppm whereas a minimal decrease of cytoplasmic vaculation in the liver was only observed in 500 ppm males. Lower hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin values were observed in the 500 ppm group males and females. These changes were of minimal degree and thus not considered to be of adverse nature.

The LOEL was identified as 500 ppm, which is equivalent to mean daily doses of about 121 mg/kg bw/day in male and 139 mg/kg bw/day in female mice.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin (96/0494-11)

Description:	semi-solid / orange
Lot/Batch #:	COD-001236
Purity:	99.02%
Stability of test compound:	Stable: Expiry date 01.10.2015

2. Vehicle and/or positive control: Diet was used as vehicle for admistration

3. Test animals:

Species:	Mouse
Strain:	B6C3F1
Sex:	Male and female
Age:	approx. 52 days at start of administration
Weight at dosing:	♂: 22.1 - 25.7 g, ♀ 19.6 - 22.8 g
Source:	Charles River Laboratories, Inc., Raleigh, NC
Acclimation period:	10 days
Diet:	PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002 (meal), ad libitum
Water:	Reverse osmosis-treated (on-site) drinking water, delivered by an automatic watering system, ad libitum
Housing:	Clean, stainless steel, wire-mesh cages suspended above cage-board

Environmental conditions:

Temperature:	22±3°C
Humidity:	50±20%
Air changes:	10 / hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 22-Feb-2011 to 27-May-2011
In life dates: 04-Mar-2011 (start of administration) to 01-Apr-2011 (necropsy), 27-May 2011 (last histopathological examination)

2. Animal assignment and treatment:

Pyraclostrobin was administered to groups of 8 male and 8 female mice at dietary concentrations of 0, 500 (low dose), and 1500 (high dose) ppm for up to 28 days, including the day of euthanasia, except for the 1500 ppm group animals which were killed after 4 days of treatment. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

3. Test substance preparation and analysis:

The diets were prepared by mixing weighed amounts of the pulverized test substance with 100 mL of acetone, which was then completely dissolved in an ultrasonic bath. The resulting solution was sprayed onto a portion of food and mixed. For the control group 100 mL acetone were mixed with the food. Acetone was completely removed at 40°C at 900 mbar. To obtain the appropriate dietary concentration, the premix was mixed with the remaining amount of feed in a laboratory mixer. The test diet formulations were prepared approximately weekly for each of the designated concentrations.

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 43 days at room temperature.

Samples for homogeneity determination were collected each day of diet preparation. According to the SOP three randomly sampled specimen of the low and high dose level were analyzed. The samples were also used for determination of the test-article concentration. For the control diets a single sample was analyzed.

Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sampling	Analysis	Concentration [ppm] Mean ± SD[#]	Relative standard deviation [%]	Mean % of nominal concentration
500 ppm	03.03.11	21.-22.03.11	498.1 ± 2.3	0.5	99.6
500 ppm	10.03.11	21.-22.03.11	488.9 ± 1.7	0.3	97.8
500 ppm	14.03.11	05.05.11	507.0 ± 9.4	1.9	101.4
500 ppm	24.03.11	05.-06.05.11	505.2 ± 10.4	2.1	101.0
1500 ppm	03.03.06	21-22.03.11	1498.7 ± 4.0	0.3.	99.9

[#] based on mean values of the three individual samples

Relative standard deviations of the homogeneity samples in the range of 0.3 to 2.1% indicate the homogenous distribution of pyraclostrobin in the diet preparations. The actual average test-substance concentrations were in the range of 97.5 to 103.5% of the nominal concentrations.

4. Statistics:

Means, standard deviations (S.D.), and standard errors (S.E.) of each test group were calculated for several parameters. All statistical tests were performed using WTDMS^{TM} unless otherwise noted. Further statistical analyses were performed according to following table:

Statistics of clinical examinations, clinical pathology and pathology

Parameter	Statistical test
food consumption, body weight, body weight change, clinical pathology parameters, weight parameters	A parametric one-way ANOVA to determine intergroup differences. If the ANOVA revealed statistically significant intergroup variance, Dunnett's test (two-sided) was used to compare the test-substance-treated group with the control group.

C. METHODS

1. Observations:

The animals were examined for moribundity or mortality twice daily. Observations for overt clinical signs of toxicity were performed at least once daily.

Detailed physical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this the animals were transferred to a standard arena. The findings were ranked according to the degree of severity, if applicable.

2. Body weight:

The body weight of the animals was determined approximately one week prior to test substance administration, on the day of randomization, at the start of the treatment (day 0), and twice weekly thereafter. Furthermore, body weight was determined prior to euthanasia *in extremis*, and at the scheduled necropsy.

3. Food consumption and compound intake:

Individual food consumption was determined twice weekly (according to body weight) and calculated as mean food consumption in grams per animal and day.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

4. Water consumption:

Not performed in this study.

5. Ophthalmoscopy:

Not performed in this study.

C) 6. Hematology and clinical chemistry:

Blood samples were withdrawn from all animals at the scheduled necropsy (study day 28). Blood was collected at the time of euthanasia via the vena cava of animals euthanized by inhalation of carbon dioxide. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.

The following hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)		Prothrombin time
✓	Hemoglobin (Hb)	✓	Neutrophils (percent and absolute)	✓	Platelet count
✓	Hematocrit (Hct)	✓	Eosinophils (% and abs.)		Activated partial thromboplastin time (APPT)
✓	Mean corp. volume (MCV)	✓	Basophils (% and abs.)	✓	Platelet estimate
✓	Mean corp. hemoglobin (MCH)	✓	Lymphocytes (% and abs.)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (% and abs.)		
✓	Reticulocyte count	✓	Large unstained cells (% and abs.)		
✓	Red cell morphology (RBC Morphology)				

Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Albumin/globulin ratio (by calculation)	✓	Aspartate aminotransferase (AST)
	Magnesium	✓	Bilirubin (total)	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Cholesterol (total)	✓	γ-Glutamyl transpeptidase (γ-GT)
✓	Potassium	✓	Creatinine		
✓	Sodium	✓	Globulin (by calculation)		
		✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea nitrogen		

7. Urinalysis:

Not performed in this study.

8. Sacrifice and pathology:

The animals were sacrificed by decapitation under carbon dioxide anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. The following organs were sampled, weighed and examined histopathologically:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups).

C	W	H		C	W	H		C	W	H	
✓	✓		adrenals	✓	✓		kidneys	✓			Skin (with mammary gland)
✓			aorta				lachrymal glands[%]	✓			spinal cord (3 levels)[@]
✓	✓		brain (3 levels)				larynx	✓	✓	✓	spleen
✓			bone	✓	✓	✓	liver	✓			sternum w. marrow
✓			bone marrow[§]	✓			lung	✓			stomach (fore- & glandular)
✓			caecum	✓		✓	lymph nodes[#]	✓	✓		testes
✓	✓		cervix	✓			mammary gland (♀)	✓	✓	✓	thymus
✓			colon	✓			muscle, skeletal	✓			thyroid (with parathyroid)
✓		✓	duodenum	✓			nerve, peripheral (sciatic n.)	✓			trachea
✓	✓		epididymides				nose/nasal cavity	✓		✓	urinary bladder
✓			esophagus	✓	✓		ovaries and oviduct**	✓	✓		uterus
✓			eyes (with optic nerve)	✓			pancreas	✓			vagina
✓			femur (with joint)	✓			Peyer's patches				
✓	✓		gall bladder	✓			pituitary	✓			gross lesions
✓			gross lesions				pharynx		✓		body (anesthetized animals)
✓			Harderian glands	✓	✓		prostate				
✓	✓		heart	✓			rectum				
✓			ileum	✓			salivary glands*				
✓			jejunum (w. Payer's plaque)	✓	✓		seminal vesicles				

[§] from femur; [#] axillary, mandibular and mesenteric; [@] cervical, thoracic, lumbar; * mandibular, ** oviduct not weighed; [%] extraorbital

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above

B. OBSERVATIONS

1. Clinical signs of toxicity

There were test substance-related clinical observations in the 1500 ppm group males and females and 500 ppm group males.

Clinical observations detected in the 1500 ppm group males and/or females included intermittent tremors, dermal atonia, body cool to touch, decreased respiration rate, labored respiration, and yellow material on the urogenital area, anogenital area, ventral trunk, and/or hindlimb(s).

The 500 ppm group males were noted with yellow material on the urogenital area sporadically during the detailed clinical observation. The toxicological relevance of this finding remains unclear. There were no test substance-related clinical observations in the 500 ppm group females.

2. Mortality

All 1500 ppm group males and females were euthanized in extremis on study day 3 due to substantial body weight loss [see Figure 5.8.2-2] and poor physical state. All control and 500 ppm animals survived to the scheduled necropsy.

3. Ophthalmoscopy

Not performed in this study.

C. BODY WEIGHT AND BODY WEIGHT GAIN

High dose (1500 ppm) mice experienced a marked body weight loss of up to 19.5% of the initial weight within 3 days after commencement of treatment [see Table 5.8.2-7 and Figure 5.8.2-2]. Together with the poor clinical condition this was the reason to terminate this dose level pre-term.

Low dose (500 ppm) mice likewise suffered from an initial body weight loss, which was however less pronounced (up to 6.2% of the initial weight). From day 3 onwards, body weight development was roughly comparable to controls, however the initial body weight loss could not be recovered.

Figure 5.8.2-2: Body weight development of mice administered pyraclostrobin for up to 28 days

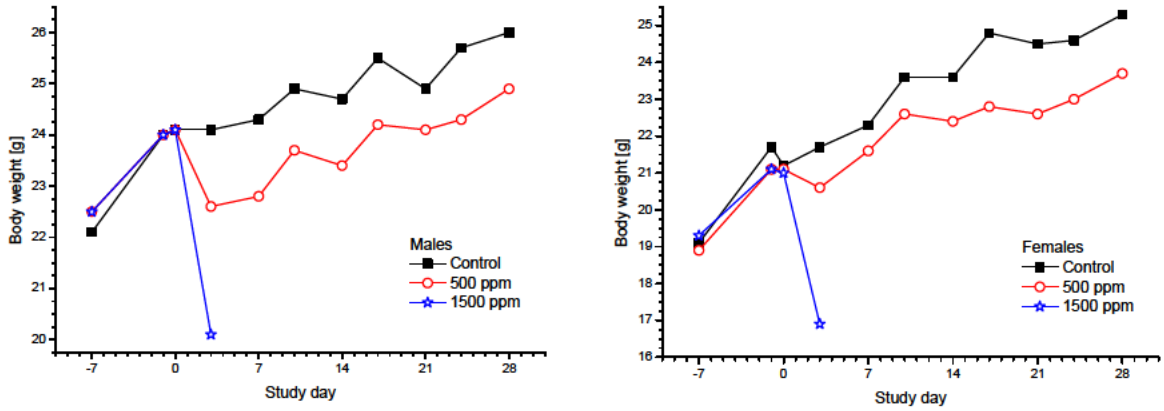


Table 5.8.2-7: Mean body weight of mice administered pyraclostrobin for up to 28 days

	Males			Females		
Dose level [ppm]	0	500	1500	0	500	1500
Body weight [g]						
- Day -7	22.1	22.5	22.5	19.1	18.9	19.3
- Day -1	24.0	24.0	24.0	21.7	21.1	21.1
- Day 0	24.1	24.1	24.1	21.2	21.1	21.0
- Day 3	24.1	22.6**	20.1**	21.7	20.6	16.9**
- Day 28	26.0	24.9	NA	25.3	23.7**	NA
Δ% (compared to control)#		-4.2	-		-6.3	-
Overall body weight gain [g]	3.9	2.4	-2.4	6.2	4.8	-2.4
Δ% (compared to control)#		-38.5	-		-22.6	-

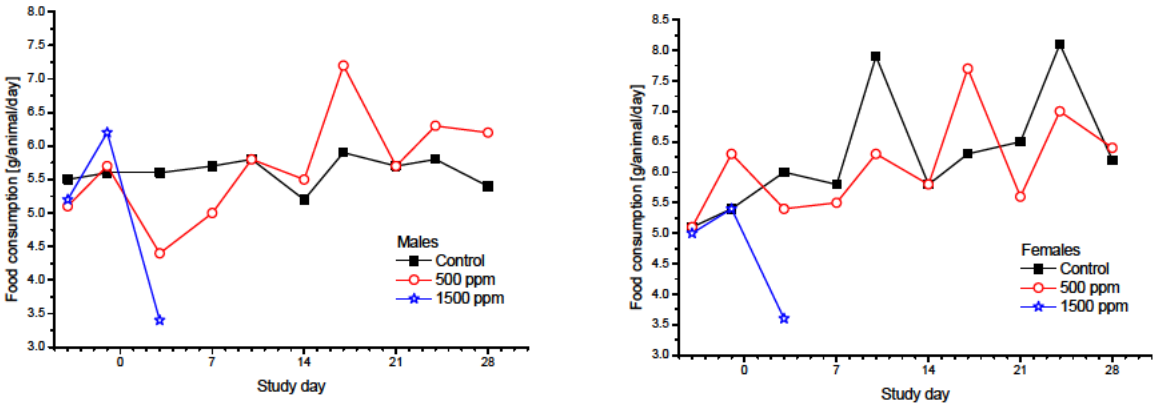
Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

NA not applicable

D. FOOD CONSUMPTION AND COMPOUND INTAKE

In the high dose group a treatment-related statistically significant lower food consumption was noted from study day 0 to 3 [see Figure 5.8.2-3]. Slightly lower food consumption in the 500 ppm groups were noted up through study day 7 in males. Even though the study director considered the food consumption in mid dose females to be slightly affected, there is considerable variability in the food consumption of control and high dose females and a clear treatment-related effect is difficult to determine.

Figure 5.8.2-3: Mean daily food consumption in mice administered pyraclostrobin for up to 28 days



The approximate mean daily test substance intake was calculated to be 121, and 231 mg/kg bw/day in males and 139, and 287 mg/kg bw/day in females at dietary dose levels of 500, and 1500 ppm, respectively.

E. BLOOD ANALYSIS

1. Hematological findings

Statistically significant differences of red blood cell parameters were noted at 500 ppm [see Table 5.8.2-8]. These treatment-related changes were generally of minor degree (max 5.8% difference) and thus not considered to be of adverse nature. Red and white blood cell counts were not affected by treatment. Likewise, the differential blood count did not reveal treatment-related effects.

Table 5.8.2-8: Selected hematology findings in mice administered pyraclostrobin for 28 days

		Males		Females	
Dose	[ppm]	0	500	0	500
Hemoglobin	[mmol/L]	17.2 ± 0.25	**16.2 ± 0.61****	16.5	**15.9****
Hematocrit	[L/L]	54.7	**52.2***	51.9	**50.1****
WBC	[10^9/L]	4.62	5.35	5.74	6.82
RBC	[10^{12}/L]	10.83 ± 0.45	10.57	10.19	10.03
MCV	[fl]	50.5	**49.4****	51.0	**50.0***
MCH	[pg]	15.9	**15.3****	16.2	**15.8****

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's test, two sided)

2. Clinical chemistry findings

Statistically significant decreased of mean alkaline phosphatase (ALP) and alanine aminotransferase (ALT) values was observed in females or males, respectively [see Table 5.8.2-9]. Both changes were not considered to be of toxicological relevance.

Table 5.8.2-9: Selected clinical chemistry findings in mice administered pyraclostrobin for 28 days

		Males		Females	
Dose	[ppm]	0	500	0	500
ALP	[U/l]	103	104	129	108**
ALT	[U/l]	36	30*	35	27

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's test, two sided)

3. Urinalysis

Not performed in this study.

F. NECROPSY

1. Gross and histopathology

There were no treatment-related macropathological findings at 500 ppm. In fact, the only finding was observed in a control female (dark red area(s) in the spleen).

In 1500 ppm animals killed pre-term, macroscopic findings were restricted to one female with a dark red discoloration of the adrenal gland(s) and two females with pale livers. As no histopathological evaluation of high dose animals was performed, the toxicological significance of these macroscopic findings remains open.

2. Organ weight

Terminal body weights in 500 ppm female mice were significantly reduced [see Table 5.8.2-10]. A number of statistically significant differences of absolute and/or relative organ weights were observed in both sexes. The study director considered the changes of kidney and seminal vesicle weights in males to be treatment-related. However, no histopathology was performed on these organs and accordingly the presence of corroborative histopathological changes is unknown. Furthermore, the kidney weight changes in females, being of similar degree, were not considered to be related to treatment. Thus the basis for the former conclusion is unknown. The fact that other statistically significant changes of mainly absolute organ weights were attributed to the lower terminal body weights sheds additional doubt regarding the interpretation of the kidney and seminal vesicle weight changes.

Table 5.8.2-10: Selected mean absolute and relative organ weights of mice administered pyraclostrobin for 28 days (500 ppm, control) and 3 days (1500 ppm) (group means)

Sex		Males				Females			
Organ weight [mg]	Dose [ppm]	Absolute weight [mg]	Δ%	Relative weight [% of b.w.]	Δ%	Absolute weight [mg]	Δ%	Relative weight [% of b.w.]	Δ%
Terminal weight [g]	0	26.0				25.3			
	500	24.9	(-4.2)			23.7**	(-6.3)		
	1500	NA				NA			
Kidney	0	0.501		1.929		0.402		1.585	
	500	0.437**	(-12.7)	1.754**	(-9.1)	0.358**	(-11.0)	1.509	(-4.8)
	1500	NA				NA			
Seminal vesicles	0	0.253		0.974					
	500	0.189**	(-25.1)	0.759**	(-22.1)				
	1500	NA							
Thymus	0	0.037		0.143		0.053		0.209	
	500	0.035	(-6.2)	0.139	(-2.8)	0.041**	(-22.5)	0.174*	(-16.7)
	1500					NA			
Brain	0	0.462		1.780		0.475		1.879	
	500	0.450	(-2.5)	1.807	(1.5)	0.460*	(-3.1)	1.946	(3.6)
	1500	NA				NA			
Heart	0	0.149		0.573		0.146		0.557	
	500	0.138	(-7.5)	0.553	(-3.5)	0.131*	(-10.6)	0.552	(-4.3)
	1500	NA				NA			
Ovaries	0					0.022		0.088	
	500					0.019*	(-16.2)	0.079	(-10.2)
	1500					NA			
Spleen	0	0.057		0.219		0.085		0.337	
	500	0.056	(-1.4)	0.225	(2.7)	0.074*	(-12.9)	0.314	(-6.8)
	1500	NA				NA			

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's test, two sided)
Values may not calculate exactly due to rounding of figures

3. Histopathology

Test substance-related mucosal hyperplasia in the duodenum was noted in the 500 ppm group males and females characterized by bigger crypts lined by large, cuboidal epithelial cells with increased numbers of mitotic figures and larger, wider villi lined by large, columnar epithelium. In the liver, there was minimal decreased cytoplasmic vacuolation of the centrilobular hepatocytes in the 500 ppm group males [see Table 5.8.2-11].

Table 5.8.2-11: Incidence of histopathological lesions in mice administered pyraclostrobin for 28 days

	Male		Female	
Dose [ppm]	0	500	0	500
Animals in group	8	8	8	8
Duodenum # examined	8	8	8	8
- Hyperplasia, mucosal minimal		8		8
Liver # examined	8	8	8	8
- Infiltrate, mononuclear, minimal	2	2	6	5
- Vacuolation, cytoplasmic, decreased , minimal		8		
Spleen # examined	8	8	8	8
- Hyperplasia, white pulp minimal		2	1	
Urinary Bladder # examined	8	8	8	8
- Infiltrate, mononuclear minimal	4	1	2	5

All other histopathological findings were considered incidental as they were evenly distributed or observed in single incidences only.

III. CONCLUSION

Dietary administration of pyraclostrobin to mice at dose levels of 0, 500, and 1500 ppm for 28 days resulted in poor clinical condition and body weight loss within 3 days at 1500 ppm. As a consequence, the high dose level was terminated after 3 days. Treatment was tolerated at 500 ppm with moderate effects on food consumption and body weight development. Minimal hyperplasia of the duodenal mucosa was observed in both sexes at 500 ppm, whereas a minimal decrease of cytoplasmic vaculation in the liver was only observed in 500 ppm males. Lower hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin values were observed in the 500 ppm group males and females. These changes were of minimal degree and thus not considered to be of adverse nature.

The LOEL was determined as 500 ppm, which is equivalent to mean daily doses of about 121 mg/kg bw/day in male and 139 mg/kg bw/day in female mice.

Supplemental information: BASF performed another 28-day subchronic toxicity study in C57BL/6 J Rj at a similar dose range as the study above. However, as the entire pyraclostrobin toxicological data base in mice was developed in B6C3F1 mice, and the laboratories, which finally conducted the immune tox studies described below, use B6C3F1 mice, it was decided not to further pursue testing in C58BL/6 J Rj mice. The findings observed in this 28-day study in C57BL/6 J Rj mice were similar to those observed in the 90-day study in B6C3F1 mice [BASF DocID 1998/11345] and in the above described 28-day study in B6C3F1 mice [BASF DocID 2011/1194286]. Following, this study is not submitted within this dossier.

Report:	CA 5.8.2/3 [redacted] 2012f A 28-day oral (dietary) natural killer cell immunotoxicity study of BAS 500 F in female B6C3F1 mice 2011/1035857
Guidelines:	EPA 870.7800
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The ability of pyraclostrobin (BAS 500 F; batch: COD-001236; purity: 99.02%) to affect the natural killer cell mediated immune response was investigated in female B6C3F1 mice at dietary dose levels of 0, 50, 200 and 750 ppm (corresponding to mean intake levels of 13, 50 and 165 mg/kg body weight/day, respectively) in a 28 day study.

Treatment with pyraclostrobin resulted in systemic toxicity as assessed by clinical signs, food consumption and body weight development at the high dose (750 ppm). Furthermore, a statistically significant reduction of absolute and relative spleen and thymus weights by 40 to 50% was observed. No effect on natural killer cell activity was observed at any dose level. Concurrent treatment with positive control substance, anti asialo GM1 induced clear signs of immunotoxicity, demonstrating the reliability of the test system under the study conditions employed.

Therefore, the no observed adverse effect level (NOAEL) for systemic toxicity was 200 ppm, whereas the NOAEL for cellular mediated immunity as assessed by the functional NKC assay was 750 ppm.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin

Description:	Semi-solid / orange
Lot/Batch #:	COD-001236
Purity:	99.02%
Stability of test compound:	Stable: Expiry date Oct. 1, 2015

2. Vehicle control/Carrier: Rodent diet

3. Positive control: Anti Asialo GM1

Description:	Not available due to container type
Lot/Batch #:	AGH5324
Purity:	100.5% (according to supplier)
Stability of test compound:	According to the supplier the positive control substance was stable over the study period (Expiry date July 30, 2011).
Vehicle for Anti Asialo GM1:	Sterile water for injection and 0.9% sodium chloride for injection, pharmaceutical grade (USP)

4. Test animals:

Species:	Mouse
Strain:	B6C3F1
Sex:	Female
Age:	Approx. 38 days at receipt; approx. 51 days at start of administration
Weight at dosing:	17.0 - 20.2 g
Source:	Charles River Laboratories, Inc., Raleigh, NC, USA
Acclimation period:	13 days
Diet:	PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002 (meal), ad libitum
Water:	Reverse osmosis-treated (on-site) drinking water, ad libitum
Housing:	Single housing in clean, stainless steel, wire-mesh cages suspended above cageboard
Environmental conditions:	
Temperature:	71 ± 5°F (22 ± 3°C)
Humidity:	50 ± 20%
Air changes:	10/hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: 07-Jun-2011 to 19-Jul-2011
(In life dates: 20-Jun-2011 (start of administration) to 18-Jul-2011 (necropsy))

The in-life part of the study was performed at WIL Research, Ashland, OH, USA. The spleens sampled at necropsy were shipped packed in ice to ImmunoTox® Inc, Richmond, VA, USA for the conduct of the NK-cell Assay.

2. Animal assignment and treatment:

Pyraclostrobin was administered to groups of 10 female mice at dietary concentrations of 0, 200 and 750 ppm ad libitum for 28 consecutive days, including the day of the scheduled euthanasia (The first day of diet administration was study day 0.).

Additionally, 10 female mice were administered anti asialo GM1 (positive control substance) via single intravenous injection (0.2 mL/animal) on study day 27, the day prior to scheduled necropsy.

The animals were assigned to the treatment groups by means of computer generated randomization list based on body weights.

Following 28 consecutive days of dose administration, all animals were euthanized.

3. Test substance preparation and analysis:

For the vehicle and positive control groups (groups 1 and 5), the same volume of acetone/kg of diet used for preparation of the test diets was added to a predetermined amount of basal diet and mixed for 5 minutes using a Hobart mixer.

For the test diets (groups 2-4), a sufficient amount of test substance was placed in a metal container and covered, frozen (approximately -20ºC), and then pulverized into a powder and placed in jars. An appropriate amount of acetone was then added to the pulverized test substance and the mixture was sonicated until the test substance was dissolved. The dissolved test substance was then added to a predetermined amount of basal diet and mixed for approximately 10 minutes using a Hobart mixer. The remaining basal diet was added to the pre-mixture to bring the batch size to the final weight and was mixed in a V-blender for approximately 15 minutes using an intensifier bar during the first and last 5 minutes. Diet preparations were performed approx. weekly and stored at room temperature.

The control and test diets were placed in storage containers which remained open for approximately 18-24 hours with the exhaust system on to allow venting off of the acetone in the diet.

Analyses performed using a comparable batch prior to the start of the administration period revealed that the test-substance was stable in the diet over a period of 10 days at ambient temperature (see Appendix C of the study report).

Homogeneity and concentration analyses of the diet preparations were performed twice by sampling diet from the top, middle and bottom of the diet containers prepared for use during study weeks 0 and 3 (i.e. the first and last week of treatment).

Nominal Dose level [ppm]	Sampling	Concentration [ppm] Mean ± SD[#]	Relative standard deviation [%]	Mean % of nominal concentration
50	Jun. 15, 2011	46.6 ± 1.4	3.1	93.1
200	"	187.5 ± 3.5	1.8	93.7
750	"	758.2 ± 18.1	2.4	101.1
50	Jul. 06, 2011	47.6 ± 1.4	2.9	95.2
200	"	191.1 ± 2.1	1.1	95.5
750	"	746.2 ± 4.5	0.6	101.9

Values may not calculate exactly due to rounding of figures

Relative standard deviations of the homogeneity of the pyraclostrobin samples were in the range of 0.6 to 3.1% indicating the homogenous distribution of pyraclostrobin in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 93.1 to 101.9% of the nominal concentrations.

One vial of the positive control substance, anti asialo GM1, administered to group 5 was reconstituted in 1 mL of sterile water for injection and diluted 1:10 with 0.9% sodium chloride for injection. The solution was prepared once and stored refrigerated at approximately 2ºC to 8ºC until use.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change (Analyses performed by WIL Research)	For test substance and the vehicle control groups: Body weight, body weight change, and food consumption were subjected to a parametric one-way ANOVA (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test substance-treated groups to the control group. For the positive control groups: The positive control data were evaluated using the Student's t-Test (Sokal and Rohlf, 1981) and were compared to the vehicle control group

Statistics of clinical pathology

Parameter	Statistical test
NKC assay parameters Weight parameters (terminal body weight, spleen and thymus organ weight) (Analyses performed at ImmunoTox® Inc)	Data were first tested for homogeneity of variances using the Bartlett's Chi Square test (Bartlett, 1937). Homogeneous data were evaluated using a parametric one-way ANOVA (Kruskal and Wallis, 1952). When significant differences occurred, the treatment groups were compared to the vehicle control group using Dunnett's test (Dunnett, 1955; Dunnett, 1964). Non-homogenous data was evaluated using a nonparametric ANOVA (Wilson, 1956). When significant differences occurred, the treatment groups were compared to the vehicle control group using the Gehan-Wilcoxon test, when appropriate (Gross and Clark, 1975). The Jonckheere's test (Hollander and Wolfe, 1973) was used to test for dose-related trends across the vehicle control and treatment groups. The positive control data was evaluated using the Student's t-Test (Sokal and Rohlf, 1981) and compared to the vehicle control group

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality twice daily. Observation for overt clinical signs of toxicity was performed at least once daily.

Detailed clinical observations were performed in all animals approximately weekly, beginning at least 1 week prior to randomization and on the day of the scheduled necropsy.

2. Body weight:

The body weight of the animals was determined before the start of the administration period (approx. 1 week before in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter, ending just prior to the scheduled necropsy.

3. Food consumption and compound intake:

Individual food consumption was recorded approximately weekly, beginning approximately 1 week prior to randomization and ending on the day of the scheduled necropsy. Food intake was calculated as g/animal/day for the corresponding body weight intervals.

The mean daily intake of test substance (mg/kg/day, group means) was calculated based upon individual values for body weight and food consumption (g/kg/day) and the appropriate target concentration of test substance in the food (ppm).

4. Water consumption:

Not performed in the study.

5. Sacrifice and pathology:

The animals were sacrificed by decapitation under carbon dioxide anesthesia. The exsanguinated animals were necropsied. The following tissues and organs were collected and –except for spleen - placed in 10% neutral-buffered formalin for potential further histopathology):

- Spleen
- Thymus
- Lymph nodes (mandibular, mesenteric)
- Peyer's Patches

Individual spleens were placed into individual tubes containing EBSS with 15 mM HEPES and supplemented with gentamicin as a bacteriostat. After weighing the spleen samples were placed on crushed ice and shipped to ImmunoTox®, Inc. for NKC analysis.

6. Natural Killer Cell (NKC) assay:

The ability of NK cells (effector cell) to detect and destroy tumor cells was performed ex-vivo. Briefly, splenocytes of all animals from the study were isolated and adjusted to six concentrations ($2x10^7$ cells/mL, $1x10^7$ cells/mL, $5x10^6$ cells/mL, $2.5x10^6$ cells/mL, $1.25x10^6$ cells/mL, $0.625x10^6$ cells/mL) to obtain effector-to-target ratios of 200:1, 100:1, 50:1, 25:1, 12.5:1 and 6.25:1, respectively. YAC-1 tumor cells (target cell) were adjusted to a concentration of $1.5x10^7$ cells/30 mL, labeled by incubation with 500 µCi of ^{51}Cr and then adjusted to $5x10^4$ cells/mL nucleated cells/mL. To determine the maximum release of ^{51}Cr 0.1 mL were incubated with 0.1 ml Triton X-100. For determination of NK cell activity 0.1 ml of the $5x10^4$ YAC-1 cells/mL were incubated for 4 hours with the spleen cells (0.1 mL; effector cells) in 96-well microtiter plates at each effector concentration. Quadruplicate wells were used per effector concentration. Following incubation, the plates were centrifuged and 0.1 mL of the supernatant was removed from each well and counted. The percent cytotoxicity at each effector concentration was determined for each treatment group and compared to the respective values for the vehicle control.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

Treatment-related clinical signs were observed at the high dose (750 ppm) and consisted of decreased defecation and feces smaller than normal. These observations were related to the reduced food consumption. Yellow material on various body surfaces were found in all groups including positive and negative control starting on study day 3 and continuing sporadically until the end of the study. The incidence was slightly increased incidence at 750 ppm. At 50 and 200 ppm no dose-response was noted. Furthermore, the incidence of this finding at 50 and 200 ppm was well comparable to the incidence observed in control animals of the parallel conducted study investigating the humoral immune response [see below 2012/1020986].

A single 750 ppm group animal (#6403) had clinical observations of hypoactivity and dermal atonia on study day 2; however, the relationship of these findings to the test substance was uncertain given the isolated occurrence.

2. Mortality

No mortality was observed in this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Treatment-related effects on body weight development were restricted to the high dose. During the first week of treatment a body weight loss was observed. During the following 3 weeks, high dose animals gained weight again, however at a lower rate than in control and treated groups at ≤ 200 ppm [see Table 5.8.2-12, Figure 5.8.2-4].

Statistically significant effects on body weight change in the positive control group were considered to be of incidental nature due to biologically variability.

Figure 5.8.2-4: NK cell assay: Body weight development of female mice administered pyraclostrobin for 28 days

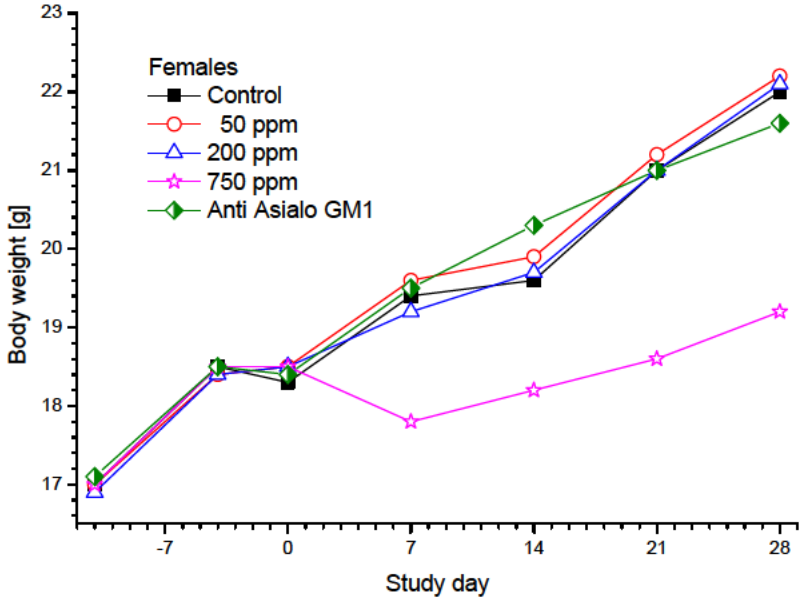


Table 5.8.2-12: Mean body weight of mice administered BAS 500 F or Anti Asialo GM1 for 28 days

Treatment	BAS 500 F				Anti Asialo GM1
Dose level	0 ppm	50 ppm	200 ppm	750 ppm	
Body weight [g]					
- Day -11	17.0	17.0	16.9	17.0	17.1
- Day -4	18.5	18.4	18.4	18.5	18.5
- Day 0	18.3	18.5	18.5	18.5	18.4
- Day 7	19.4	19.6	19.2	**17.8****	19.5
- Day 14	19.6	19.9	19.7	**18.2***	20.3
- Day 21	21.0	21.2	21.0	**18.6****	21.0
- Day 28	22.0	22.2	22.1	**19.2****	21.6
Δ% (compared to control) #		0.9	0.5	-12.7	-1.8
Overall body weight gain [g]	3.8	3.7	3.6	**0.8****	3.2
Δ% (compared to control) #		-2.6	-5.3	-78.4	-15.8

Values may not calculate exactly due to rounding of figures
* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett's test)

C. FOOD CONSUMPTION AND COMPOUND INTAKE

Test substance-related lower food consumption was observed in the 750 ppm group animals throughout the study except for study day 21 to 28 compared to the vehicle control [see also Table 5.8.2-13]. There were no test substance-related effects on food consumption in the 50 and 200 ppm groups.

The approximate mean daily test substance intake in the groups with a diet containing 50, 200 and 750 ppm test substance corresponded to 13, 50 and 165 mg/kg bw/day, respectively.

Table 5.8.2-13: Mean cumulative food consumption of mice administered BAS 500 F or Anti Asialo GM1 for 28 days

Treatment	BAS 500 F				Anti Asialo GM1
Dose level	0 ppm	50 ppm	200 ppm	750 ppm	
Daily mean food consumption [g/animal]					
- Day 0 to 7	4.8	4.8	4.9	**3.0****	5.0
- Day 7 to 14	4.9	4.8	4.8	**4.0****	5.1
- Day 14 to 21	6.0	5.0	5.6	**4.3****	5.9
- Day 21 to 28	6.0	5.5	5.4	5.0	5.5

** $p \leq 0.01$ (Dunnett's test)

D. NECROPSY

1. Terminal body and organ weight

A treatment-related decrease of absolute and relative spleen and thumus weights was noted at the high dose level [see Table 5.8.2-14]. The lower spleen weights correlated with the lower number of spleen cells. No histopathological evaluation of the thymus was performed. However, based on the substantial effect on body weight, a stress related reduction of thymus weights can be assumed. In a subchronic toxicity a similar decrease of thymus weights was associated with thymus atrophy.

Table 5.8.2-14: Spleen and thymus weights in mice treated with pyraclostrobin for 28 days

Organ weight [mg]	Dose [ppm]	Absolute weight [mg]	Δ%	Relative weight [% of bw]	Δ%	H/NH
Terminal weight. [g]	0	22.0	-			H
	50	22.2	(0.9)			
BAS 500 F	200	22.1	(0.5)			
	750	**19.2****	(-12.7)			
Spleen	0	60.6	-	0.27	-	NH
	50	70.4	(16.2)	0.32	(18.5)	
BAS 500 F	200	57.4	(-5.3)	0.26	(-3.7)	
	750	**33.1****	(-45.4)	**0.17****	(-37.0)	
Thymus	0	49.1	-	0.22	-	H
	50	50.9	(3.7)	0.23	(4.5)	
BAS 500 F	200	46.7	(-4.9)	0.21	(-4.5)	
	750	**25.0****	(-49.1)	**0.13****	(-40.9)	

** $p \leq 0.01$ (Dunnett's Test for homogenous data (H), Wilcoxon Rank Test for non-homogenous data (NH)
Values may not calculate exactly due to rounding of figures

2. Gross pathology

The only treatment-related necropsy finding was a smaller thymus in two 750 ppm females (#6412 and #6443).

All other gross necropsy findings occurred in single cases and without relation to dose and thus were considered to be of incidental nature.

E. IMMUNOLOGICAL ANALYSES

There was no test substance-related effect on Natural Killer Cell activity at any dose level [see Table 5.8.2-15]. However, in line with the lower spleen weights a lower number of spleen cells was noted at the high dose.

The significantly higher (25%) spleen cell numbers at the low dose level were not test substance-related due to lack of an effect in the 200 ppm group.

For the positive control (anti asialo GM1) a statistically significant decrease in the NKC activity was observed at effector target ratios of ≥ 50:1.

Table 5.8.2-15: Natural Killer Cell Activity in female mice treated for 28 days with pyraclostrobin at the indicated doses or anti asialo GM1 as positive control

Treatment	Pyraclostrobin				AAGM1
Dose	0 ppm	50 ppm	200 ppm	750 ppm	1:10
Effector:Target Ratio					
200:1	6.2 ± 0.5	5.3 ± 0.4	6.0 ± 0.5	6.3 ± 1.1	**1.1± 0.6****
100:1	2.0 ± 0.2	1.9 ± 0.3	1.8 ± 0.3	1.4 ± 0.5	**0.1 ± 0.4****
50:1	1.2 ± 0.3	0.9 ± 0.3	1.2 ± 0.3	0.6 ± 0.3	**0.1 ± 0.3***
25:1	1.1 ± 0.4	0.8 ± 0.4	0.9 ± 0.3	0.6 ± 0.3	0.4 ± 0.3
12.5:1	1.1 ± 0.3	1.1 ± 0.3	0.9 ± 0.3	0.8 ± 0.3	0.5 ± 0.3
6.25:1	0.8 ± 0.3	0.8 ± 0.1	0.3 ± 0.3	0.7 ± 0.3	0.5 ± 0.2
Spleen Cells (x 10^7)	9.5 ± 0.6	11.9 ± 0.9*	9.1 ± 0.5	**5.3 ± 0.4****	10.0 ± 0.4

* $p \leq 0.05$,** $p \leq 0.01$ (Dunnett's Test for homogenous data, Wilcoxon Rank Test for non-homogenous data, the positive control was compared to the vehicle control using Student's t Test)

III. CONCLUSION

Dietary administration of pyraclostrobin to female B6C3F1 mice for 28 consecutive days resulted in impaired body weight development, lower food consumption and reduced absolute and relative spleen and thymus weights at the high dose of 750 ppm. No adverse effects were noted at 50 and 200 ppm. There was no effect on the NKC assay at any dose level.

Therefore, the no observed adverse effect level (NOAEL) for systemic toxicity was 200 ppm (approx. 50 mg/kg body weight/day), and the no-observed-effect level (NOEL) for the functional NKC assay for cellular mediated immunity was 750 ppm (approx. 165 mg/kg body weight/day).

Report: CA 5.8.2/4
[redacted] 2012e
A 28-day oral (dietary) antibody forming cell immunotoxicity study of BAS 500 F in female B6C3F1 mice
2012/1020986
Guidelines: EPA 870.7800
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The immunotoxic potential of pyraclostrobin (BAS 500 F; batch: COD-001236; purity: 99.02%) in female B6C3F1 mice was analyzed using dietary dose levels of 0, 50, 200 and 750 ppm (corresponding to mean intake levels of 13, 50 and 202 mg/kg body weight/day, respectively) for 28 days.

Dietary administration of pyraclostrobin to female B6C3F1 mice for 28 consecutive days resulted in decreased body weight gain and 30 to 40% reduced absolute and relative spleen and thymus weights at the highest dose of 750 ppm. No adverse effects were noted at 50 and 200 ppm.

Test substance-related lower spleen cell numbers were noted in the 750 ppm group (-45%). An apparent suppression of the humoral component of the immune system was noted in all test substance-treated groups when evaluated using the concurrent control of this AFC assay. However, the functional response of the vehicle control was substantially above the historical control mean and the pyraclostrobin-exposed groups were within or close to the historical control range of the same sex and strain of mice.

Treatment with the positive control substance cyclophosphamide (CPS), administered once daily at 50 mg/kg/day on study day 24-27 by intraperitoneal injection resulted in decreased thymus and spleen weights, reduced number of spleen cells and a complete suppression of the humoral immune response.

Based on the increased humoral immune response in control animals, the results of this study are not conclusively interpretable. Thus, the test needed to be repeated [see BASF DocID 2012/1084176].

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin
Description: Semi-solid / orange
Lot/Batch #: COD-001236
Purity: 99.02%
Stability of test compound: Stable: Expiry date Oct. 1, 2015

2. Vehicle control: Rodent diet

3. Positive control: Cyclophosphamide monohydrate (CPS)
Description: Solid / white
Lot/Batch #: 079K1569
Purity: 100.5% (according to supplier)
Stability of test compound: Stable: Expiry date July 01, 2012
Vehicle for CPS: Phosphate buffered saline (PBS); sterile water for injection

4. Antigen used for immunization: Sheep red blood cells (sRBC) -Prepared by ImmunoTox® and shipped to WIL Research

5. Test animals:
Species: Mouse
Strain: B6C3F1
Sex: Female
Age: Approx. 37 days at receipt; approx. 51 days at start of administration
Weight at dosing: 16.8 - 20.5 g
Source: Charles River Laboratories, Inc., Raleigh, NC, USA
Acclimation period: 14 days
Diet: PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002 (meal), ad libitum
Water: Reverse osmosis-treated (on-site) drinking water, ad libitum
Housing: Single housing in clean, stainless steel, wire-mesh cages suspended above cageboard.
Environmental conditions:
Temperature: 71 ± 5°F (22 ± 3°C)
Humidity: 50 ± 20%
Air changes: 10/hour
Photo period: 12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: 07-Jun-2011 to 05-Aug-2011
In life dates: 21-Jun-2011 (start of administration) to 19-Jul-2011 (necropsy)

The in-life part of the study was performed at WIL Research, Ashland, OH, USA. The spleens sampled at necropsy were shipped packed in ice to ImmunoTox® Inc, Richmond, VA, USA for the conduct of the SBRC or t-cell dependent antibody response (TDAR) assay.

2. Animal assignment and treatment:

Pyraclostrobin was administered to groups of 10 female mice at dietary concentrations of 0, 200 and 750 ppm for 28 days (The first day of diet administration was study day 0.). Additionally, 10 female mice were administered Cyclophosphamide monohydrate (CPS; positive control substance) via intraperitoneal injection once daily during study days 24-27 at a dose level of 50 mg/kg/day and a dose volume of 10 mL/kg/day.

The animals were assigned to the treatment groups by means of computer generated randomization list based on body weights.

On study day 24 all animals were immunized via an intravenous tail vein injection with 0.2 mL of 7.5 x 10^7 sRBC in EBSS with HEPES (except for a single animal in the 750 ppm group which was immunized via intraperitoneal injection with 0.4 mL of 7.5 x 10^7 sRBC in EBSS with HEPES).

3. Test substance preparation and analysis:

For the test diets (groups 2-4), a sufficient amount of test substance was placed in a metal container and covered, frozen (approximately -20ºC), and then pulverized into a powder and placed in jars. An appropriate amount of acetone was then added to the pulverized test substance and the mixture was sonicated until the test substance was dissolved. The dissolved test substance was then added to a predetermined amount of basal diet and mixed for approximately 10 minutes using a Hobart mixer. The remaining basal diet was added to the pre-mixture to bring the batch size to the final weight and was mixed in a V-blender for approximately 15 minutes using an intensifier bar during the first and last 5 minutes. Diet preparations were performed approx. weekly and stored at room temperature.

For the vehicle and positive control groups (Groups 1 and 5), the same volume of acetone/kg of diet used for preparation of the test diets was added to a predetermined amount of basal diet and mixed for 5 min using a Hobart mixer.

The control and test diets were placed in storage containers which remained open for approximately 18-24 hours with the exhaust system on to allow venting off of the acetone in the diet.

Homogeneity and concentration analyses of the diet preparations were performed twice by sampling diet from the top, middle and bottom of the diet containers prepared for use during study weeks 0 and 3 (i.e. the first and last week of treatment).

Nominal Dose level [ppm]	Sampling	Concentration [ppm] Mean ± SD[#]	Relative standard deviation [%]	Mean % of nominal concentration
50	Jun. 15, 2011	46.6 ± 1.4	3.1	93.1
200	"	187.5 ± 3.5	1.8	93.7
750	"	758.2 ±18.1	2.4	101.1
50	Jul. 06, 2011	47.6 ± 1.4	2.9	95.2
200	"	191.1 ± 2.1	1.1	95.5
750	"	746.2 ± 4.5	0.6	101.9

Values may not calculate exactly due to rounding of figures

Relative standard deviations of the homogeneity of the pyraclostrobin samples were in the range of 0.6 to 3.1% indicating the homogenous distribution of pyraclostrobin in the diet preparations. The actual (mean) average test-substance concentrations were in the range of 93.1 to 101.9% of the nominal concentrations.

The positive control Substance, CPS, was prepared in a PBS solution at a concentration of 5 mg/mL. The solution was prepared once and was stored at approximately -20°C. On each day of dosing (study days 24-27), an aliquot was quickly thawed, kept on ice, and mixed prior to dosing. The formulation was used within approximately 3 hours of thawing.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change (Analyses performed by WIL Research)	For test substance and the vehicle control groups: A comparison of each group with the vehicle control group was performed using DUNNETT's test for the hypothesis of equal means For the vehicle and positive control groups: A comparison of the positive control group with the vehicle control group was performed using the Student's t-test for the hypothesis of equal means

Statistics of clinical pathology

Parameter	Statistical test
AFC assay parameters (Analyses performed at ImmunoTox® Inc)	Data were first tested for homogeneity of variances using the Bartlett's Chi Square test (Bartlett, 1937). Homogeneous data were evaluated using a parametric one-way ANOVA (Kruskal and Wallis, 1952). When significant differences occurred, the treatment groups were compared to the vehicle control group using Dunnett's test (Dunnett, 1955; Dunnett, 1964). Non-homogenous data was evaluated using a nonparametric ANOVA (Wilson, 1956). When significant differences occurred, the treatment groups were compared to the vehicle control group using the Gehan-Wilcoxon test, when appropriate (Gross and Clark, 1975). The Jonckheere's test (Hollander and Wolfe, 1973) was used to test for dose-related trends across the vehicle control and treatment groups. The positive control data was evaluated using the Student's t-Test (Sokal and Rohlf, 1981) and compared to the vehicle control group.

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality twice daily. Observation for overt clinical signs of toxicity was performed at least once daily and included, but were not limited to, changes in the skin, fur, eyes and mucous membranes; respiratory, circulatory, autonomic and central nervous systems function; somatomotor activity and behavior patterns.

Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. The findings were ranked according to the degree of severity, if applicable.

2. Body weight:

The body weight of the animals was determined before the start of the administration period (approx. 1 week before in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter, ending just prior to the scheduled necropsy.

3. Food consumption and compound intake:

Food consumption was determined once weekly on a cage basis and calculated as mean food consumption in grams per animal and day.

The mean daily intake of test substance (mg/kg/day, group means) was calculated based upon individual values for body weight and food consumption (g/kg/day) and the appropriate target concentration of test substance in the food (mg/kg).

4. Water consumption:

Not performed in the study.

5. Hematology (IgM antibody analysis):

At the scheduled necropsy blood was drawn from carbon dioxide anesthetized animals via the inferior vena cava. Blood was collected into tubes containing no anticoagulant, allowed to clot, and processed to serum. Serum samples were transferred to cryovials and stored frozen at approximately -70°C. Serum samples were not analyzed and were discarded upon issuance of the final report.

6. Sacrifice and pathology:

The animals were sacrificed by decapitation under carbon dioxide anesthesia. The exsanguinated animals were necropsied. The following tissues and organs were collected and –except for spleen - placed in 10% neutral-buffered formalin for potential further histopathology):

- Spleen (placed in EBSS/HEPES buffer)
- Thymus
- Lymph nodes (mandibular, mesenteric)
- Peyer's Patches

Individual spleens were placed into individual tubes containing EBSS with 15 mM HEPES and supplemented with gentamicin as a bacteriostat. After weighing the spleen samples were placed on crushed ice and shipped to ImmunoTox®, Inc. for the conduct of the Splenic antibody-forming cell (AFC) assay assay.

7. Splenic antibody-forming cell (AFC) assay:

The spleen samples were processed into single-cell suspensions. The cell suspensions were centrifuged and resuspended in EBSS with HEPES. Spleen cell counts were performed using a Model Z1 Coulter Counter®. Viability of splenocytes was determined using propidium iodide and the Coulter® EPICS® XL-MCL Flow Cytometer.

The primary IgM response to sheep erythrocytes was measured using a modification of the hemolytic plaque assay. After isolation and resuspension of the spleen cells, 1:30 and 1:120 dilutions were prepared. A 0.1 mL aliquot of spleen cells from each suspension was added to separate test tubes, each containing 25 µL of guinea pig complement, 25 µL of sRBC, and 0.5 mL of warm agar (0.5%). After thoroughly mixing, each test tube mixture was plated onto a separate petri dish, covered with a microscope cover slip, and incubated at approximately 36-38°C for 3 hours. The plaques that developed were counted using a Bellco plaque viewer. A plaque, occurring from the lysis of sRBC, is elicited as a result of the interaction of complement and antibodies (produced in response to the IV immunization of the animals) directed against sRBC. Each plaque is generated from a single IgM antibody-producing B cell (plasma cell), permitting the number of AFC present in the whole spleen to be calculated. The data are expressed as Specific Activity (AFC/10^6 Spleen Cells) and Total Spleen Activity (AFC/Spleen).

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

Treatment-related clinical signs were restricted to the high dose level (750 ppm) and consisted of decreased defecation, feces smaller than normal and yellow material around the urogenital area.

Additionally, on study day 1, due to poor body condition and/or body weight loss, all high dose mice were given a water bottle.

2. Mortality

No mortality was observed in this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Statistically significant and treatment-related effects on body weight development were only observed at the high dose level. During the first week of treatment a body weight loss was observed. During the following 3 weeks, high dose animals gained weight again [see Table 5.8.2-16, Figure 5.8.2-5].

No impaired body weight development was observed in CPA treated mice.

Figure 5.8.2-5: Body weight development of female mice administered pyraclostrobin or cyclophosphamide (CPS) for 28 days

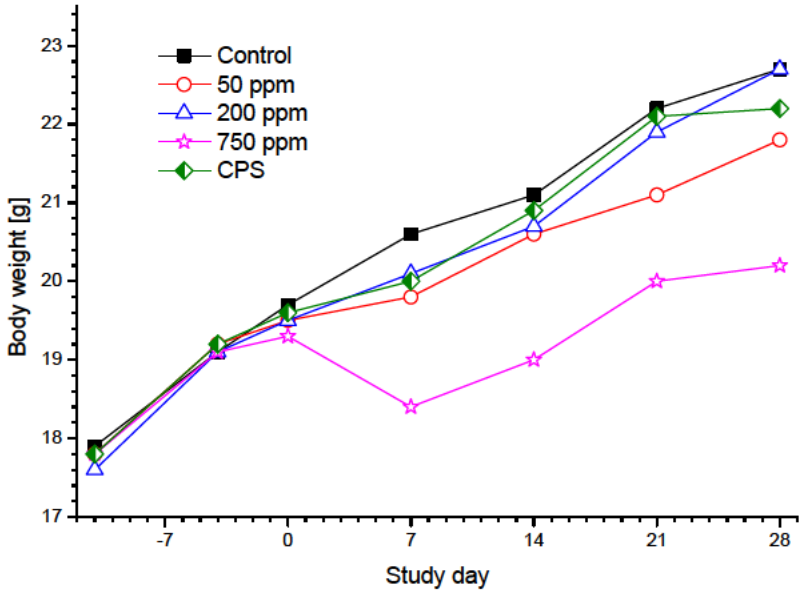


Table 5.8.2-16: Mean body weight of mice administered pyraclostrobin or cyclophosphamide (CPS) for 28 days

Treatment	BAS 500 F				CPS
Dose level	0 ppm	50 ppm	200 ppm	750 ppm	50 mg/kg
Body weight [g]					
- Day -11	17.9	17.8	17.6	17.8	17.8
- Day -4	19.1	19.2	19.1	19.1	19.2
- Day 0	19.7	19.5	19.5	19.3	19.6
- Day 7	20.6	19.8	20.1	**18.4***	20.0
- Day 14	21.1	20.6	20.7	**19.0***	20.9
- Day 21	22.2	21.1	21.9	**20.0***	22.1
- Day 28	22.7	21.8	22.7	**20.2***	22.2
Δ% (compared to control) #		-4.0	0.0	**-11.0***	-2.2
Overall body weight gain [g]	3.0	2.3	3.3	**0.9***	2.6
Δ% (compared to control) #		-23.3	10	-70	13.3

Values may not calculate exactly due to rounding of figures
* $p \leq 0.01$ (Dunnett's test)

Occasionally, statistically significant differences of body weight gain were observed in low dose females. However, these changes were not dose dependent and therefore nor considered to be treatment-related.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

Statistically significant lower food consumption was observed at 200 and 750 ppm for the intervall day 0 to 7. Whereas the decrease at 750 ppm was accompanied by substantial effects on body weight, no such effect was observed at 200 ppm [see Table 5.8.2-17]. Therefore, the effect in mid dose females was not considered to be of adverse nature. Food consumption of CPA treated animals was not significantly altered.

The approximate mean daily test substance intake in the groups with a diet containing 50, 200 and 750 ppm test substance corresponded to 13, 50 and 202 mg/kg body weight/day, respectively.

Table 5.8.2-17: Mean food consumption of mice administered BAS 500 F or cyclophosphamide (CPS) for 28 days

Treatment	BAS 500 F				CPS
Dose level	0 ppm	50 ppm	200 ppm	750 ppm	50 mg/kg
Cumulative food consumption [g/animal]					
- Day 0 to 7	5.6	5.0	4.7*	**4.2****	5.2
- Day 7 to 14	5.4	5.0	4.9	4.9	5.1
- Day 14 to 21	6.7	6.7	5.6	6.9	5.9
- Day 21 to 28	6.1	5.4	5.7	5.0	6.1

* $p \leq 0.05$ (Dunnett's test)
** $p \leq 0.01$ (Dunnett's test)

D. Necropsy

1. Gross pathology

There were no macroscopic findings noted at the scheduled necropsy.

2. Terminal body and organ weight

Treatment-related and statistically significant decreases of absolute and relative terminal body, thymus and spleen weights were observed in the high dose animals [see Table 5.8.2-18]. There were no test substance-related effects on spleen and thymus weights in the 50 and 200 ppm groups. The lower spleen weights correlated with the lower number of spleen cells. No histopathological evaluation of the thymus was performed. However, based on the substantial effect on body weight, a stress related reduction of thymus weights can be assumed. In a subchronic toxicity a similar decrease of thymus weights was associated with thymus atrophy.

Table 5.8.2-18: Selected mean absolute and relative organ weights in mice treated with BAS 500 F or cyclophosphamide for 28 days

Organ weight [mg]	Dose [ppm]	Absolute weight [mg]	Δ%	Relative weight [% of bw]	Δ%
Terminal weight. [g]	0	22.7			
BAS 500 F	50	21.8	(-4.0)		
	200	22.7	(0.0)		
	750	20.2*	(-11.0)		
CPS (pos. control)	50 mg/kg	22.2	(-2.2)		
Spleen	0	92.4		0.41	
BAS 500 F	50	85.2	(-7.8)	0.39	(-4.9)
	200	82.6	(-10.6)	0.37	(-9.8)
	750	**55.4***	**(-40.0*)**	**0.27***	**(-34.1*)**
CPS (pos. control)	50 mg/kg	**46.3***	**(-49.9*)**	**0.21***	**(-48.8*)**
Thymus	0	41.3		0.18	
BAS 500 F	50	42.1	(1.9)	0.19	(5.6)
	200	39.9	(-3.4)	0.18	(0.0)
	750	**25.5***	**(-38.3*)**	**0.12***	**(-33.3*)**
CPS (pos. control)	50 mg/kg	**13.1***	**(-68.3*)**	**0.06***	**(-66.7*)**

* $p \leq 0.01$ (Dunnett's test)
Values may not calculate exactly due to rounding of figures

No decrease of terminal body weight was observed in the animals treated with the positive control substance CPA [see Table 5.8.2-18]. However, absolute and relative spleen and thymus weights were significantly decreased within this group.

E. IMMUNOLOGICAL ANALYSES

Test substance-related significantly lower spleen cell numbers (-45%) were noted in the 750 ppm dose group compared to the vehicle control. As expected, a statistically significant decrease in the spleen cell number (-64%) was observed in the positive control group (CPS) [see Table 5.8.2-19].

An apparent, statistically significant suppression of the humoral immune response was observed in all treated groups when evaluated as Specific Activity (AFC/10^6 Spleen Cells) or total Spleen Cell Activity (AFC/Spleen) [see Table 5.8.2-19].

However, the overall functional responses of the vehicle control and the pyraclostrobin-exposed groups were enhanced compared to historical control data from vehicle animals of the same sex and strain. In the vehicle animals from the pyraclostobin study, the Specific Activity was more than twice the mean of 1206 AFC/10^6 Spleen Cells observed in the historical control data. Similarly, the mean responses of all of the pyraclostobin-exposed animals ranged from 1210-1559 AFC/10^6 Spleen Cells, while historical control values averaged 1206 AFC/10^6 Spleen Cells (historical control minimum/maximum of study means: 1116/1499 AFC/10^6 Spleen Cells), i.e. the responsed of the pyraclostrobin treated animals were well within the historical control range. The reason for the increased AFC activity in controls could not be determined. Thus, the results of this assay can not be conclusively interpreted.

The positive control group displayed statistically significant decreases in specific activity (-100%) and total spleen activity (-100%), when compared to the vehicle control group.

Table 5.8.2-19: Spleen Antibody-Forming Cell responses to the T-dependent antigen, Sheep Erythrocytes, in female mice treated for 28 days with pyraclostrobin or cyclophosphamid

	pyraclostrobin				CPS
Dose	0 ppm	50 ppm	200 ppm	750 ppm	50 mg/kg bw
Spleen Cells (x 10^7)	14.03 ± 0.86	12.32 ± 0.54	12.12 ± 0.60	**7.71 ± 0.35****	**5.07 ± 0.16****
IgM AFC/10^6 Spleen Cells	2939 ± 343	**1559 ± 219****	**1523 ± 94***	**1210 ± 126***	**0 ± 0****
IgM AFC/Spleen (x 10^3)	419 ± 47	**189 ± 24****	**184 ± 14****	**93 ± 9****	**0 ± 0****

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett's Test for homogenous data, the positive control was compared to the vehicle control using Student's t Test)

III. CONCLUSION

Dietary administration of pyraclostrobin to female B6C3F1 mice for 28 consecutive days resulted in decreased body weight gain and reduced absolute and relative spleen and thymus weights at the highest dose of 750 ppm. No adverse effects were noted at 50 and 200 ppm.
Test substance-related lower spleen cell numbers were noted in the 750 ppm group (-45%). An apparent suppression of the humoral component of the immune system was noted in all test substance-treated groups when evaluated using the concurrent control of this AFC assay. However, the functional response of the vehicle control was substantially above the historical control mean and the pyraclostrobin-exposed groups were within or close to the historical control range of the same sex and strain of mice. Therefore, the results of the humoral immune response of this study are not conclusively interpretable. Thus, the test needed to be repeated [see BASF DocID 2012/1084176].

Report:	CA 5.8.2/5 [redacted] 2012d A 28-day oral (dietary) antibody forming cell immunotoxicity study of BAS 500 F in female B6C3F1 mice 2012/1084176
Guidelines:	EPA 870.7800
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The immunotoxic potential of pyraclostrobin (batch: COD-001236; purity: 99.02%) in female B6C3F1 mice was analyzed using dietary dose levels of 0, 50, 200 and 750 ppm (corresponding to mean intake levels of 14, 55 and 191 mg/kg body weight/day, respectively) for 28 days. This is the second assay to assess the IgM mediated immune response, as the first assay [see BASF DocID 2012/1035857] was not interpretable as to immunotoxicity due to a higher activity of the vehicle control, which exceeded the historical control control range by a factor of two.

Treatment-related signs of systemic toxicity were restricted to the high dose level (750 ppm) as assessed by clinical signs, reduced food consumption and impaired body weight development. Furthermore, a statistically significant reduction of absolute and relative spleen and thymus weights and spleen cell numbers were observed. However, the number of antibody forming cells (AFC) per spleen was not affected in high dose animals or at lower dose levels. To the contrary, the specific AFC activity (AFC/10^6 spleen cells) was statistically significant increased at the high dose level, while it was comparable to the control at the lower dose levels. In the positive control a total loss of IgM mediated immune response was noted.

While a decrease in the AFC response has been recognized as an indicator that a test substance has the potential of being immunosuppressive; at the present time, the consequences of an increase in the specific AFC response have not been established and thus the adversity of this change cannot be assessed.

Based on the results of this study, the NOEL (No Observed Effect Level) for general toxicity and overall effect on the various parameters of the immune system was 200 ppm.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin

Description:	Semi-solid / orange
Lot/Batch #:	COD-001236
Purity:	99.02%
Stability of test compound:	Stable: Expiry date Oct. 1, 2015

2. Vehicle control: Rodent diet

3. Positive control: Cyclophosphamide monohydrate (CPS)

Description:	Solid / white
Lot/Batch #:	079K1569
Purity:	100.5% (according to supplier)
Stability of test compound:	Stable Expiry date July 01, 2012).
Vehicle for CPA:	Phosphate buffered saline (PBS); sterile water for injection

4. Test animals:

Species:	Mouse
Strain:	B6C3F1
Sex:	Female
Age:	Approx. 38 days at receipt; approx. 51 days at start of administration
Weight at dosing:	18.0 - 21.4 g
Source:	Charles River Laboratories, Inc., Raleigh, NC, USA
Acclimation period:	13 days
Diet:	PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002 (meal), ad libitum
Water:	Reverse osmosis-treated (on-site) drinking water, ad libitum
Housing:	Single housing in clean, stainless steel, wire-mesh cages suspended above cageboard.
Environmental conditions:	
Temperature:	71 ± 5°F (22 ± 3°C)
Humidity:	50 ± 20%
Air changes:	10/hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN

1. Dates of experimental work: 01-Nov-2011 - 09-Feb-2012
In life dates: 14-Nov-2011 (start of administration) to 12-Dec-2011 (necropsy)

The in-life part of the study was performed at WIL Research, Ashland, OH, USA. The spleens sampled at necropsy were shipped packed in ice to ImmunoTox® Inc, Richmond, VA, USA for the conduct of the SBRC or t-cell dependent antibody response (TDAR) assay.

2. Animal assignment and treatment:

Pyraclostrobin was administered to groups of 10 female mice at dietary concentrations of 0, 200 and 750 ppm for 28 days (the first day of diet administration was study day 0.). Additionally, 10 female mice were administered cyclophosphamide monohydrate (CPS; positive control substance) via intraperitoneal injection once daily during study days 24-27 at a dose level of 50 mg/kg/day and a dose volume of 10 mL/kg/day.

The animals were assigned to the treatment groups by means of computer generated randomization list based on body weights.

On study day 24 all animals were immunized via an intravenous tail vein injection with 0.2 mL of 7.5 x 10^7 sRBC in EBSS with HEPES (If an intravenous injection could not be administered, the volume of sRBC antigen was adjusted and an intraperitoneal injection was performed).

3. Test substance preparation and analysis:

For the test diets (groups 2-4), a sufficient amount of test substance was placed in a metal container and covered, frozen (approximately -20ºC), and then pulverized into a powder and placed in jars. An appropriate amount of acetone was then added to the pulverized test substance and the mixture was sonicated until the test substance was dissolved. The dissolved test substance was then added to a predetermined amount of basal diet and mixed for approximately 10 minutes using a Hobart mixer. The remaining basal diet was added to the pre-mixture to bring the batch size to the final weight and was mixed in a V-blender for approximately 15 minutes using an intensifier bar during the first and last 5 minutes. Diet preparations were performed approx. weekly and stored at room temperature.

For the vehicle and positive control groups (Groups 1 and 5), the same volume of acetone/kg of diet used for preparation of the test diets was added to a predetermined amount of basal diet and mixed for 5 min using a Hobart mixer.

The control and test diets were placed in storage containers which remained open for approximately 18-24 hours with the exhaust system on to allow venting off of the acetone in the diet.

The positive control substance, CPS, was prepared in a PBS solution at a concentration of 5 mg/mL. The solution was stored in aliquots at approximately -20°C. On each day of dosing (study days 24-27), an aliquot was quickly thawed, kept on ice, and mixed prior to dosing. The formulation was used within 3 hours of thawing.

The stability of the test item in the diet for 10 days was proven prior to the start of the study Homogeneity and concentration analyses of the diet preparations were performed twice by sampling diet from the top, middle and bottom of the diet containers prepared for use during study weeks 0 and 3 (i.e. the first and last week of treatment).

Nominal Dose level [ppm]	Sampling	Concentration [ppm] Mean ± SD[#]	Relative standard deviation [%]	Mean % of nominal concentration
50	Nov. 11, 2011	43.3 ± 3.8	8.8	86.5
200	"	181.6 ± 5.6	3.1	90.8
750	"	801.5 ± 0.5	0.1	106.9
50	Dec. 02, 2011	44.4 ± 3.4	7.6	88.8
200	"	181.9 ± 9.8	5.4	90.0
750	"	812.7 ± 8.8	1.1	108.4

Values may not calculate exactly due to rounding of figures

Relative standard deviations of the homogeneity of the pyraclostrobin samples were in the range of 0.1 to 8.8%, which indicate the homogenous distribution of pyraclostrobin in the diet preparations (RSD ≤ 10%). The actual (mean) average test-substance concentrations were in the range of 86.5 to 106.9% and 88.8 to 108.4% of the nominal concentrations. For the lowest concentration these values were slightly below the specification limit of 90%, but mean found concentrations within ± 15% of the target concentration can be regarded acceptable for complex matrices like diet. Furthermore, as the mid dose was identified as NOAEL in this study, the slighty lower dietary concentration does not affect the validity of the study.

The positive control Substance, CPS, was prepared in a PBS solution at a concentration of 5 mg/mL. The solution was prepared once and was stored at approximately -20°C. On each day of dosing (study days 24-27), an aliquot was quickly thawed, kept on ice, and mixed prior to dosing. The formulation was used within approximately 3 hours of thawing.

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change (Analyses performed by WIL Research)	For test substance and the vehicle control groups: A comparison of each group with the vehicle control group was performed using DUNNETT's test for the hypothesis of equal means For the vehicle and positive control groups: A comparison of the positive control group with the vehicle control group was performed using the Student's t-test for the hypothesis of equal means

Statistics of clinical pathology

Parameter	Statistical test
AFC assay parameters (Analyses performed at ImmunoTox® Inc)	Data were first tested for homogeneity of variances using the Bartlett's Chi Square test (Bartlett, 1937). Homogeneous data were evaluated using a parametric one-way ANOVA (Kruskal and Wallis, 1952). When significant differences occurred, the treatment groups were compared to the vehicle control group using Dunnett's test (Dunnett, 1955; Dunnett, 1964). Non-homogenous data was evaluated using a nonparametric ANOVA (Wilson, 1956). When significant differences occurred, the treatment groups were compared to the vehicle control group using the Gehan-Wilcoxon test, when appropriate (Gross and Clark, 1975). The Jonckheere's test (Hollander and Wolfe, 1973) was used to test for dose-related trends across the vehicle control and treatment groups. The positive control data was evaluated using the Student's t-Test (Sokal and Rohlf, 1981) and compared to the vehicle control group.

C. METHODS

1. Observations:

The animals were examined for morbidity or mortality twice daily. Observation for overt clinical signs of toxicity was performed at least once daily and included, but were not limited to, changes in the skin, fur, eyes and mucous membranes; respiratory, circulatory, autonomic and central nervous systems function; somatomotor activity and behavior patterns.

Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. The findings were ranked according to the degree of severity, if applicable.

2. Body weight:

The body weight of the animals was determined before the start of the administration period (approx. 1 week before in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter, ending just prior to the scheduled necropsy.

3. Food consumption and compound intake:

Food consumption was determined once weekly on a cage basis and calculated as mean food consumption in g/animal/day.

The mean daily intake of test substance (mg/kg/day, group means) was calculated based upon individual values for body weight and food consumption (g/kg/day) and the appropriate target concentration of test substance in the food (mg/kg).

4. Water consumption:

Not performed in the study.

5. Hematology (IgM antibody analysis):

At the scheduled necropsy blood was drawn from carbon dioxide anesthetized animals via the inferior vena cava. Blood was collected into tubes containing no anticoagulant, allowed to clot, and processed to serum. Serum samples were transferred to cryovials and stored frozen at approximately -70°C. As the serum samples were not analyzed they were discarded upon issuance of the final report.

6. Sacrifice and pathology:

The animals were sacrificed by decapitation under carbon dioxide anesthesia. The exsanguinated animals were necropsied. The following tissues and organs were collected and –except for spleen - placed in 10% neutral-buffered formalin for potential further histopathology):

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- Thymus
- Lymph nodes (mandibular, mesenteric)
- Peyer's Patches

Individual spleens were placed into individual tubes containing EBSS with 15 mM HEPES and supplemented with gentamicin as a bacteriostat. After weighing the spleen samples were placed on crushed ice and shipped to ImmunoTox®, Inc. for the conduct of the Splenic antibody-forming cell (AFC) assay.

7. Splenic antibody-forming cell (AFC) assay:

Spleen samples from Group 1-4 animals were randomized and coded for AFC analysis. The spleen samples were processed into single-cell suspensions. The cell suspensions were centrifuged and resuspended in EBSS with HEPES. Spleen cell counts were performed using a Model Z1 Coulter Counter®. Viability of splenocytes was determined using propidium iodide and the Coulter® EPICS® XL-MCL Flow Cytometer.

The primary IgM response to sheep erythrocytes was measured using a modification of the hemolytic plaque assay. After isolation and resuspension of the spleen cells, 1:30 and 1:120 dilutions were prepared. A 0.1 mL aliquot of spleen cells from each suspension was added to separate test tubes, each containing 25 µL of guinea pig complement, 25 µL of sRBC, and 0.5 mL of warm agar (0.5%). After thoroughly mixing, each test tube mixture was plated onto a separate petri dish, covered with a microscope cover slip, and incubated at approximately 36-38°C for 3 hours. The plaques that developed were counted using a Bellco plaque viewer. A plaque, occurring from the lysis of sRBC, is elicited as a result of the interaction of complement and antibodies (produced in response to the IV immunization of the animals) directed against sRBC. Each plaque is generated from a single IgM antibody-producing B cell (plasma cell), permitting the number of AFC present in the whole spleen to be calculated. The data are expressed as Specific Activity (AFC/10^6 Spleen Cells) and Total Spleen Activity (AFC/Spleen).

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs indicative of toxicity were observed throughout the study. Secondary to the decreased food consumption the hig dose animals showed decreased defecation, which was observed most frequently beginning on study day 5 and continued throughout the remainder of the study.

2. Mortality

No test-substance related lethality was observed during this study. Two unscheduled deaths of 200 ppm animal # 2509 and 750 ppm animal #2493 were considered to be accidental. Gross necropsy of the former animal revealed no cause of death, whereas the latter animal displayed a fractured mandible.

B. BODY WEIGHT AND BODY WEIGHT GAIN

Statistically significant and treatment-related effects on body weight development were only observed at the high dose level. During the two weeks of treatment a body weight loss was observed. During the following 2 weeks, high dose animals gained weight at a rate comparable to the other groups [see Table 5.8.2-20, Figure 5.8.2-6].

Figure 5.8.2-6: Body weight development of female mice administered pyraclostrobin or cyclophosphamide (CPS) for 28 days

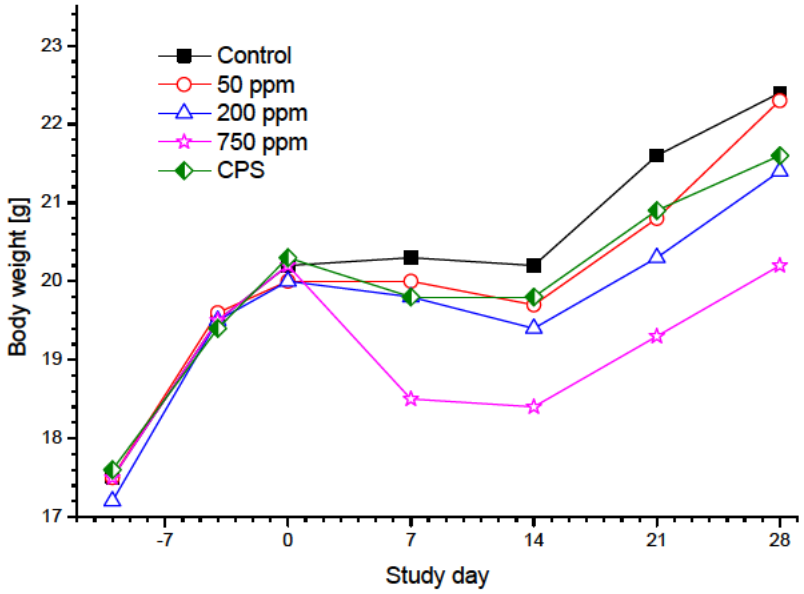

Table 5.8.2-20: Mean body weight of mice administed BAS 500 F or cyclophosphamide (CPS) for 28 days

Treatment	BAS 500 F				CPS
Dose level	0 ppm	50 ppm	200 ppm	750 ppm	50 mg/kg
Body weight [g]					
- Day -10	17.5	17.5	17.2	17.5	17.6
- Day -4	19.5	19.6	19.5	19.5	19.4
- Day 0	20.2	20.0	20.0	20.2	20.3
- Day 7	20.3	20.0	19.8	**18.5****	19.8
- Day 14	20.2	19.7	19.4	**18.4****	19.8
- Day 21	21.6	**20.8***	**20.3****	**19.3****	20.9
- Day 28	22.4	22.3	**21.4***	**20.2****	21.6
Δ% (compared to control) #		-0.4	-4.5	-9.8	-3.6
Overall body weight gain [g]	2.2	2.3	1.3	**0.2****	1.3
Δ% (compared to control) #		4.5	-40.9	-90.9	-40.9

Values may not calculate exactly due to rounding of figues
* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's test)

Statistically significant decreases of absolute body weights and body weight gain were also occasionally observed at 50 and 200 ppm. This was however not considered to be treatment-related, but rather an expression of biological variability. This is because in the two previously conducted immunotoxicity studies [see BASF DocID 2012/1020986 and 2012/1035857] no effects on body weight development were observed at these dose levels. Furthermore, body weight gain in control and in the - at that time still untreated - positive control animals was zero respectively -0.5 grams. This is in contrast to the above mentioned studies where a body weight gain of 0.9 to 1.4 grams was observed during the first two study weeks. No impaired body weight development was observed in CPA treated mice.

C. FOOD AND COMPOUND INTAKE

Test substance-related lower food consumption was observed in the 750 ppm group throughout the study compared to the vehicle control, reaching statistical significance from study days 7 to 14 [see Table 5.8.2-21].

Table 5.8.2-21: Mean food consumption of mice administed BAS 500 F or cyclophosphamide (CPS) for 28 days

Treatment	BAS 500 F				CPS
Dose level	0 ppm	50 ppm	200 ppm	750 ppm	50 mg/kg
Mean food consumption [g/animal]					
- Day 0 to 7	4.6	4.8	4.7	3.8	4.7
- Day 7 to 14	5.4	4.9	5.2	**4.4***	5.3
- Day 14 to 21	5.7	5.8	5.5	5.1	5.4
- Day 21 to 28	7.6	7.2	6.6	6.2	**6.2****

* $p \leq 0.05$ (Dunnett's Test)
** $p \leq 0.05$ (Student's t Test)

The approximate mean daily test substance intake in the groups with a diet containing 50, 200 and 750 ppm test substance corresponded to 14, 55 and 191 mg/kg body weight/day, respectively.

D. NECROPSY

1. Gross pathology

There were no test substance-related macroscopic findings noted at the scheduled necropsy. The only finding was a small thymus in a Cyclophosphamid treated animal. In addition to the fracuted mandible, the high dose animal displayed red discolored skin (nasal buccal) and muscle(s) (neck), which were probably secondary to the fracture.

2. Terminal body and organ weight

Treatment-related and statistically significant decrease of absolute and relative terminal body, thymus and spleen weights were observed in the 750 ppm group animals [see Table 5.8.2-22]. The statistically lower terminal body weight of mid dose (200 ppm) animals was considered incidental for the reasons discussed above. There were no test substance-related effects on spleen and thymus weights in the 50 and 200 ppm groups.

The lower spleen weights correlated with the lower number of spleen cells. No histopathological evaluation of the thymus was performed. However, based on the substantial effect on body weight, a stress related reduction of thymus weights can be assumed. In a subchronic toxicity a similar decrease of thymus weights was associated with thymus atrophy.

No statistically significant decrease of terminal body weight was observed in the animals treated with the positive control substance Cyclophosphamid [see Table 5.8.2-22]. However, absolute and relative spleen and thymus weights were significantly decreased.

Table 5.8.2-22: Mean absolute and relative organ weights in mice treated with pyraclostrobin or cyclophosphamide for 28 days

Organ weight [mg]	Dose [ppm]	Absolute weight [mg]	Δ%	Relative weight [% of bw]	Δ%
Terminal weight. [g]	0	22.4			
BAS 500 F	50	22.3	(-0.4)		
	200	**21.4***	(-4.5)		
	750	**20.2****	(-9.8)		
CPS (pos. control)	50 mg/kg	21.6	(-3.6)		
Spleen	0	94.1		0.42	
BAS 500 F	50	89.4	(-5.0)	0.40	(-4.8)
	200	87.5	(-7.0)	0.41	(-2.4)
	750	**67.5****	**(-28.3**)**	**0.33****	**(-21.4**)**
CPS (pos. control)	50 mg/kg	**44.7****	**(-52.5**)**	**0.21****	**(-50.0**)**
Thymus	0	45.0		0.20	
BAS 500 F	50	46.0	(2.2)	0.21	(5.0)
	200	43.6	(-3.1)	0.20	(0.0)
	750	**29.1****	**(-35.3**)**	**0.14***	**(-30.0*)**
CPS (pos. control)	50 mg/kg	**15.6****	**(-65.3**)**	**0.07****	**(-65.0**)**

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's test)
Values may not calculate exactly due to rounding of figures

E. IMMUNOLOGICAL ANALYSES

A treatment-related lower number of spleen cells (-40%) was noted at the high dose level (750 ppm). An even more pronounced decrease (-74%) was noted for the Cyclophosphamid treated positive control group (CPS).

The statistically significant lower spleen cell number at the low dose level (50 ppm; -17%) was not considered treatment related, because the values were identical to those of the 200 ppm group and statistical significance was most likely due to the decreased variability within the group [see Table 5.8.2-23].

In contrast to the lower spleen cell number, the number of antibody forming cells (AFC) per spleen was not affected by treatment. Accordingly, at the high dose level a statistically significant higher (47%) specific activity (AFC/10^6 spleen cells) was noted when compared with the vehicle control group. There were no effects on the absolute and specific activity at the mid and low dose level.

As anticipated, in the positive control group the total absence of antibody forming cells (AFC) resulted in a 100% decrease of total and specific AFC activity.

Table 5.8.2-23: Spleen Antibody-Forming Cell responses to the T-dependent antigen, Sheep Erythrocytes, in female mice treated for 28 days with pyraclostrobin or cyclophosphamid

	BAS 500 F				CPS
Dose	0 ppm	50 ppm	200 ppm	750 ppm	50 mg/kg bw
Spleen Cells (x 10^7)	15.01 ± 0.92	12.52 ± 0.48*	12.54 ± 0.71	**9.01 ± 0.64****	**3.85 ± 0.22****
IgM AFC/10^6 Spleen Cells	990 ± 66	1003 ± 84	1001 ± 75	**1459 ± 132****	**0 ± 0****
IgM AFC/Spleen (x 10^3)	148 ± 13	125 ± 11	122 ± 5	129 ± 11	**0 ± 0****

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett's Test for homogenous data, the positive control was compared to the vehicle control using Student's t Test)

III. CONCLUSION

Dietary administration of pyraclostrobin to female B6C3F1 mice for 28 consecutive days resulted in decreased body weight and body weight gain and - secondary to the lower food consumption - a decreased defecation at the high dose level (750 ppm). Furthermore, reduced absolute and relative spleen and thymus weights were observed. Secondary to the lower spleen weight / size a lower number of spleen cells was recorded, which however did not result in a decrease in the number of antibody forming cells (AFC). To the contrary, the specific AFC activity was statistically significant increased. No adverse effects were noted at the lower dose levels, whereas the positive control resulted in a total loss of IgM mediated immune response.

While a decrease in the AFC response has been recognized as an indicator that a test substance has the potential of being immunosuppressive; at the present time, the consequences of an increase in the AFC response have not been established and thus the adversity of this change cannot be assessed.

Based on the results of this study, the NOEL (No Observed Effect Level) for general toxicity and overall effect on the various parameters of the immune system was 200 ppm.

Additional toxicological information found in public literature (CA 5.8.2/6 - CA 5.8.2/9)

Report: CA 5.8.2/6
Houck K.A. et al., 2009a
Profiling Bioactivity of the ToxCast Chemical Library Using BioMAP Primary Human Cells
2009/1130882
Guidelines: none
GLP: no
(literature study; part of the ToxCast Program)

Executive Summary

The authors use the BioMAP human cell system to characterize effects relevant to human tissue and inflammatory disease biology as part of EPA's ToxCast program. They show a similar pattern for pyraclostrobin, trifloxystrobin and a reference compound for mitochondrial activity, myxathiazol. As the fungicidal mode of action for stobilurins is on mitochondria activity, this is to be expected. Interestingly the underlying data indicates significant mitochondrial toxcicities with AC_{50}'s for different endpoint of 0.39 to 49.6 µM. Mitochondrial mass and membrane potential are discussed to be hallmarks of cytotoxicity, which is in indeed observer at an AC_{50} of 3.32 µM at the 72 hour time point.

Thus the described data confirm the pesticidal mode of action of pyraclostrobin.

Classification of study: Supplementary information

Report: CA 5.8.2/7
Shah, I. et al, 2011a
Using nuclear receptors activity to stratify hepatocarcinogens
2011/1295091
Guidelines: none
GLP: no
(literature study; part of the ToxCast Program)

Executive Summary

This study is part of the EPA ToxCast program. The authors investigated the effects of 309 environmental pesticides on nuclear receptors using primary human hepatocytes, HepG2 cells transfected with a multiple reporter transcription unit (MRTU) library consisting of 48 transcription factor binding sites, cis reporter gene assays and cell free and cell based cytochrome P450 assays. This data was used to calculate an aggregate scaled activity scores for different nuclear receptors (CAR, PXR, PPAR, AhR, SR, RXR), which was then correlated to lesion progression data extracted from EPA's pesticide database.
Pyraclostrobin, together with cyprodinil, etoxazole, flumetralin, haxaconazole, methoxyfenozide, phosalone, and tebufenozide was grouped into category VIII A. Group A indicated, that the compound can activate the nuclear receptor AhR, and PXR and to a lesser degree CAR. The rationale for this grouping is largely based on the activation of the reporter genes. In case of AhR these are Cyp1a1 genes in various forms and Cyp1b1. For PXR this consists of the PXR gene, several variants of Cyp3a4, PXRE_Cis, nPXR, human PXR agonist and PXR trans. For CAR the situation gets even more complex, with single target genes in cis and trans form and CAR itself in CIS and trans form as reporter gene assays.

It is interesting to note, that for AhR association the actual AhR is not part of the group, although there are numerous publications indicating that also AhR expression can be regulated by prototypical AhR activators like dioxin and beta naphthoflavone, albeit on a small scale (2-3 fold), not involving binding to the AhR binding site. Also Cyp1b1 transcript changes have been reported in numerous studies without accompanying transcription of Cyp1a1, indicating further regulatory mechanisms at least for this cyp. Also, numerous other known hepatic target genes for each of the receptors have been neglected in the choice for target genes of this assay. Additionally, it is important to note that for example the AhR has a very broad ligand range with more than 400 known endogeneous and exogeneous known ligands. Often AhR activitation is seen at high doses for prototypical ligands for other nuclear receptor agonists. The same holds true for other nuclear receptors, like PXR or PPAR.

It is also important to note that many of the Cyps have a all or nothing or at least a very steep dose response curve. E.g. Cyp1a1 is often considered to be a switch gene with binding of an AhR ligand inducing > 100-fold gene expression changes. In case of pyraclostrobin only a 1.5-fold increase (50%) of activity is seen for one of the multiple Cyp1a1 reporter gene transcripts. This indicates that the apparent AhR activation is only weak in comparison to strong AhR ligands.

When looking at the AC_{50} values for nuclear receptor activation that is scored with a positive association for pyraclostrobin (active activity call; taken from the concurrent EPA dashboard), they are in the range of 0.54 to 2.48, with NVS_MP_hPBR activity at 0.9 and NVS_MP_rPBR at 1.5 µM at the low end and NVS_ENZ_hBACE at 13.6 µM at the upper end of the scale. Apart from PBR, all activity calls fall well within the range of cytotoxicity indicated by Houck et al (see CA 5.8.2/6) and other available data from mammalian cells (e.g. Orton EC_{20} = 0.089 µM; see CA 5.8.3/1) or in vitro mutagenicity assays. Recent presentations by EPA indicate that positive associations from any of the ToxCast assays derived at cytotoxic concentration can be at best used as supplementary information.

Association to group VIII is indicative of a lack of cancer lesion progression and indeed pyraclostrobin shows indications of hepatic enzyme induction (increased liver weight, and hepatocyte hypertrophy) but no progression to neoplastic lesions in mice or rats.

It is interesting to note, that other ADME assays within the ToxCast program do not show an activation of any phase I enzymes, including the ones identified in this publication. Therefore, this study can only be viewed as supplemental information.

Classification of study: Supplementary information

Report: CA 5.8.2/8
Kleinstreuer N.C., 2011a
Environmental impact on vascular development predicted by high-throughput screening
2011/1296591
Guidelines: none
GLP: no
(literature study; part of the ToxCast Program)

Executive Summary

This study is part of the EPA ToxCast program. The authors investigated the effects of 309 chemicals using 467 high-throughput screening tests (HTS). The assays measured direct interactions between chemicals and molecular targets (receptors, enzymes), as well as downstream effects on reporter gene activity or cellular consequences. From this assays individual bioactivity scores were determined. Primary pathways of interest, associated to these scores, were inflammatory chemokine signaling, the vascular endothelial growth factor pathway, and the plasminogen-activating system. Figure 2 of the publication shows ToxPi visualizations for the 50 top ranking chemicals in the assay. The visualization is based on the activity on the assay for µPAR up- or down-regulation, PAI-1 up- or down regulation, CCL2 down-regulation, CXCL10 up-regulation, TIE2 binding and VEGFR2 down regulation. It is interesting to note that for µRAR and PAI-1 the direction of regulation was not taken into account, albeit an up-regulation is likely based on fundamentally different molecular events than a down-regulation. Also consequences will marketly differ as shown for multiple signaling pathways.

Pyraclostrobin is depicted to regulate PAI-1 (without direction of change), weak to medium regulation of µPAR (without direction of change) and to down-regulate CCL2. Please note that the original assay database contains assays for rats and humans, and that from this visualization it is not clear, whether the human or rat assay is investigated. This is also not indicated in the text.
We have querried the values for each of the implicated assays in the concurrent version of the data on EPA's dashboard (http://actor.epa.gov/dashboard/). Pyraclostrobin is scored negative for each of the implicated assays, be it in the rodent or human version. The actual AC_{50} values are 1000 µM each. It is interesting to note, that indeed none of the genes/assay implied in the above pathways, including many additional cytokines and chemokines, as well as numerous other factors were not positively associated with pyraclostrobin up to 1000 µM each. Therefore, we consider that pyraclostrobin is not positively correlated with any of the risk factors for vascular development.

Report: CA 5.8.2/9
Sipes N.S. et al., 2013a
Profiling 976 toxcast chemicals across 331 enzymatic and receptor signaling assays
2013/1371960
Guidelines: none
GLP: no

Executive Summary of the Literature

Summary report on the ToxCast program. The publication makes some general statements on the progress of the ToxCast program. The data provided for pyraclostrobin is the same as in Shah et al. (see CA 5.8.2/7). No linkage between pyraclostobin and other endpoints is provided.

Classification of study: Supplementary information

General comment to the evaluation of the ToxCast literature:

ToxCast was originally designed to evaluate more than 600 in vitro and cell free assays for their potential of hazard identification, respectively prioritarization for further testing of chemicals. The initial expectation was very high, in particular due to the massive number of test systems and due to the fact that multiple assays were targeting similar endpoints. The underlying principle was that one could combine the information from multiple members of a pathway, use a weight of evidence approach and give a reasonably precise prediction for hazard identification.

This expectation however was not met. In a seminal effort Russel Thomas et al (2012, Toxicological Science 128(2); 398-417), working for the Hamner Institute at that time, did a comprehensive statistical analysis of the whole ToxCast database. For this he teamed up with the statistical software company SAS and more than 300 prediction models were generated, in particular comparing the predictivity of the in vitro approaches with in silico prediction models. In general, the balanced accuracy of the prediction was <0.55 or smaller than 55%. This is largely based on two influencing factors. 1. Better values for more balanced endpoints and due to the high specificity associated with low sensitivity or high sensitivity with low specificity for each endpoint. The only assay with a predictivity > 0.8 or 80% was cholinesterace inhibition, both for QSAR and in vitro. The underlying database is very data rich for this endpoint as carbamate and organophosphate insecticides make up a significant amount of the 309 ToxCast chemicals. Therefore, it was to be expected, that respective high quality in vitro and QSAR assays would be available.

For the rest of the > 600 assays, the predictivity is little better than tossing a coin. This publication had significant impact on the ToxCast program and indeed Russel Thomas has meanwhile been named the new lead scientist for it.

In a personal presentation given by Richard Judson at BASF, EPA stated that they currently plan to use the ToxCast data only in a weight of evidence approach. The underlying decision tree will consist of the formulation of adverse outcome pathways (AOP). The ToxCast data will be projected onto the AOP and EPA will use the spatial information in the pathway, as well as the order of events to characterize, whether any given chemical will be associated with a pathway. For example, if a chemical is implicated in one pathway a single hit anywhere in the pathways will not be sufficient. However, if a chemical is associated with multiple endpoints in the pathway, this will give the prediction a higher status. E.g., a chemical that hits nuclear receptor binding, receptor localization to the nucleus, receptor transactivation, and target gene expression will be considered positive for this pathway. Currently EPA has not yet published a list of AOPs and assays associated with AOPs. Respective scoring systems for the positive or negative association to any given pathways are also still lacking.

When one applies this AOP principle to the data presented above on pyraclostrobin, it is nonetheless obvious, that no positive assotiation to any given tox endpoint can be drawn.

In the same presentation Judson stated that positive activity calls seen in any given assay have to be evaluated with great care if the concentration range with the positive association is withing the range of the cytotoxicity of the compound. Most assays were no longer predictive at cytotoxic concentration and gave multiple false associations due to e.g. endogeneous substances released from the damaged cells. One could argue, that for most of the assay systems cytotoxicity information is laking. In such cases the mean cytotoxic concentration of a compound should be taken into account, unless the cytotoxicity is mediated by a cell type specific mode of action. In case of pyraclostrobin the cytotoxicity is expected to be based on the mitochondrial activity. As mitochondrial signaling pathways are evolutionary conserved, it is to be expected that mitochondrial toxicity will be similar in most test systems. Therefore, a mean cytotoxicity can be assumed. Positive activity calls as taken from the concurrent version of the data accessible in the EPA dashboard are mostly seen at cytotoxic concentrations or above.

In summary,

1) The balanced accuracy for ToxCast endpoints is < 0.55.
2) Pyraclostrobin in not positively associated with any known AOP, with the exception of hepatic enzyme induction.
3) Most activity calls seen in the ToxCast dataset occure at cytotoxic concentration, reducing the predictivity of the respective assays.
4) No positive association of pyraclostrobin to any endpoint has been raised in the public literature apart from the ToxCast database.

No further point of concern was raised from the public literature. Pyraclostrobin has been adequately tested.

CA 5.8.3 Endocrine disrupting properties

The evaluation of potential endocrine disruption was not a data-requirement at the time of Annex I inclusion of pyraclostrobin. However, this endpoint is considered to be intrinsically covered by the respective pivotal toxicity studies on pyraclostrobin.

The data package of pyraclostrobin neither indicate a potential to affect the estrogen or androgen system nor the pituitary-thyroid axis.

The organ weight changes of male (testes, epididymis (dogs only)), and female sexual organs (ovaries) and endocrine glands (adrenals, thyroid (dogs only)) observed in subchronic studies at higher dose levels were secondary to the observed decrease of terminal body weights and typically not accompanied by histopathological findings. The decrease of absolute and relative adrenal weights in female mice was accompanied by a decrease of lipid deposition in the x-zone, which is specific to mice and of unknown function. The decreased lipid content is considered secondary to the impaired body weight development. No histopathological effects on the thyroid were observed in rodents and dogs. Likewise, long-term administration of pyraclostrobin did not indicate treatment-related effects on neoplastic or non-neoplastic findings in sexual organs or endocrine tissues. In the 2-generation study administration of pyraclostrobin had no effect on the estrous cycle; the number, morphology and motility of sperm as well as on male or female fertility.

The only potential indication for a sex-hormone mediated effect is the slight delay of vaginal opening in the 2-generation study (BASF DocID 1999/11869). In this study a slight, but statistically significant delay of vaginal opening was observed. The mean time of vaginal opening was 31.7, 32.1, 32.4 and 33.3** ($p < 0.01$) days at 0, 25, 75 and 300 ppm. However, the mean day of vaginal opening at the high dose level was well within the historical control range of 29.9 to 34.9 days (see Table 5.8.3-1). There is only a limited number of studies (4) in the historical control because a) determination of vagnial opening was only started in 1998 and b) the (sub-) strain and source of rats used in the laboratory was changed from Boehringer Ingelheim, Biberach/Riss, Germany (Chbb.THOM(SPF)) to Charles River Laboratories, Sulzfeld, Germany (CrlCLxBrHan:WI) in the year 2000. Nonetheless, it is interesting to note that for animals delivered to the laboratory within a 3 week period, the mean day of vaginal opening in control females varied between 31.7 days (study 93175 (pyraclostrobin)) and 34.9 days (study 96173), indicating a relatively high biological variability of this parameter.

Table 5.8.3-1: Historical control data base for vaginal opening in Chbb.Thom Wistar rats

Delivery date of animals	Study identification	Mean day of vaginal opening
16.03.1998	98010	33.0
26.05.1998	96172 (BAS 500 F)	31.7
16.06.1998	96173	34.9
01.09.1998	97136	30.6
22.09.1998	97139	29.9

As already discussed in the study report as well as in the 2003 DAR, the slight delay in vaginal opening (VO) is most probably related to the slight delay in physical development secondary to the slightly lower body weights observed at weaning (45.2 g ± 3.04 vs. 51.3 g ± 3.99 in controls; -11.9%, $p < 0.01$) and in F1 parental females at week 0 (73.4 g ± 7.67 vs. 80.9 g ± 7.50; - 9.3%, $p < 0.05$), week 1 (111.4 g ± 9.16 vs. 118.5 g ± 9.71; - 6.0%, $p < 0.05$) and week 2 (140.9 g ± 9.64 vs. 145.5 g ± 10.22; -3.2%, n.s.).

This becomes even more evident if one compares the mean weight of high dose and control females at VO (103.0 g ± 9.3 g vs. 103.0 g ± 10.9 g in control females), which is identical in both groups. As the body weights at VO were not recorded in this study, the body weights were extrapolated for the day of VO by linear extrapolation from the body weight curve in the period of interest for each individual animal.

Overall, there is no indication that pyraclostrobin has a human relevant endocrine related effect. Thus, the conclusion for relevant endpoints for the current renewal was amended as follows:

Other toxicological studies (SANCO/11802 data point 5.8)

Endocrine disrupting properties	No endocrine effects on the oestrogen, androgen or thyroid hormone system.

In addition to the pivotal toxicity studies the following literature was taken into consideration.

Literature data

As many other pesticides and chemicals, pyraclostrobin is part of the US ToxCast program. Several assays react to varying doses of pyraclostrobin, however, no conclusive picture has emerged. In the following two publications related to endocrine effects are discussed.

Report: CA 5.8.3/1
Orton F. et al, 2011a
Widely used pesticides with previously unknown endocrine activity revealed as in vitro antiandrogens
2011/1291251
Guidelines: none
GLP: no

Executive Summary of the Literature

This publication investigated the antiandrogenic activity of pesticides based on the heights of exposure and known or QSAR predicted interaction with the androgen receptor. A total of 37 pesticides were tested in the MDA-kb2 assay using MDA-kb2 human breast cancer cells transfected with a fire-fly kuciferase reporter gene that is driven by an androgen-response element-containing promoter. Selected pesticides were also tested in the YAS-assay.

The QSAR analysis of pyraclostrobin was 'out of domain', i.e. no predicition with regard to anti-androgenic activity was possible. The MDA-kb2 assay was negative, indicating the absence of antiandrogenic activity. The cytotoxicity in the MDA.kb2 assay was relatively high with an EC_{20} of 0.089 µM. This confirms the high cytotoxicity also seen in other assays (e.g. BASF DocID 1998/11421). No YAS assay was performed with pyraclostrobin.

Classification of study: Supplementary information

Report: CA 5.8.3/2
Reif D.M. et al., 2010a
Endocrine profiling and prioritization of environmental chemicals using ToxCast data
2010/1231552
Guidelines: none
GLP: no

Executive Summary of the Literature

This publication illustrates a profiling tool developed on the ToxCast database to prioritize chemicals with regard to endocrine disruption evaluation/testing as a decision support tool. Thus this priorization tool was applied also to pyraclostrobin being part of the ToxCast program. The priorization tool focused on estrogen, androgen and thyroid pathways and thus incorporated those screening assays of the ToxCast program considered relevant for putative endocrine profiles. In addition, it incorporated external molecular pathway databases i.e. Kyoto Encyclopedia of Genes and Genomes (KEGG), Ingenuity software and the Online Mendelian Inheritance in Men repository. The tests in which pyraclostrobin showed an activity are the same as described in the publication of Shah et al. 2011 (see M-CA 5.8.2/7). The current evaluation of the results can be seen on EPA's dashboard (http://actor.epa.gov/dashboard/). The so-called ToxPi profile for pyraclostrobin (supplementary information) indicates medium activity in LogP and KEGG path, and weak activity for Ingenuity path, other NR, other XME/ADME and TR. The publication does not provide an absolute ranking but in visual comparison to activity alerts for other compound the ranking of activities for pyraclostrobin is low. No linkage between pyraclostrobin and other endpoints is provided.

It is important to note that both Ingenuity Pathways and KEGG rely on literature data often generated in completely different cellular setting. In particular Ingenuity pathways are highly curated and quality controlled. However, genes/proteins have linkage to multiple pathways. An estimation from Lockhart et al (Nature 405, 827-836, (15 June 2000)) indicated that a typical protein is associated to 7 other proteins. This is much more profound in key cellular regulators or chaperones (e.g. heat shock proteins). Those are implicated in multiple pathways and often regulation on gene expression level is not associated to any functional change in the pathway, as cells have large pools for those protein and can compensate for changes. A regulation of such an accessory protein thus will indicate a positive association to multiple pathways, without giving any information on a functional change. Current approaches in bioinformatics therefore apply a weight of evidence approach, which means that multiple parameters within a pathway need to be regulated, before this is considered to be affected. Please also note that neither KEGG nor Ingenuity pathway analysis takes the direction of the regulation into account. This is an important limitation, as an up regulation can have fundamentally different consequences than a down regulation. A really meaningful prediction using either KEGG or Ingenuity would require a detailed evaluation of the affected network and prior knowledge of the regulation of this network in the used cell system. On their own, KEGG or Ingenuity pathways can only be seen as indicator or roadsigns pointing a researcher into a direction.

Another factor which needs to be taken into account, is the level of complexity for each pathway. A gouping according to nuclear receptor regulation does not provide any information, which receptor is regulated. The same is true for a group like KEGG path: Which of the more than 500 KEGG pathways is regulated? What does it mean?

Also this analysis does not take into account that multiple assays exist for the same pathway or endpoint within the ToxCast program. In principle a chemical acting on any given pathway should score at multiple assays associated with that endpoint. Individual hits should be disregarded in the context of a weight of evidence. Another factor not taken into account is that assay results achieved at cytotoxic concentrations have low reliability (please see discussion in M-CA 5.8.2). Using the EPA dashboard it is apparent, that positive activity calls were largely seen at cytotoxic concentration. Furthermore, different assays for the indicated outcome did not show concordance. Therefore, we consider this information to be supplementary only.

Classification of study: Supplementary information

CA 5.9 Medical Data

Information evaluated in the draft monograph of Rapporteur Member State Germany of Sep. 12, 2000:

Since pyraclostrobin is a new compound, no data and medical experience regarding possible health effects in humans are available to the rapporteur. All information provided by the sole notifier is presented here.

Thus, the following conclusion was given in the list of endpoints for Annex I listing of pyraclostrobin:

Medical data (SANCO/1420, 8. Sept. 2004, data point 5.9**)**

Medical data	Limited data (new compound); no human health problems identified

Information on medical data obtained since then has been collected and evaluated. In order to extend the evaluation basis a search in the literature and internet databases listed below - restricted to "pps=human" and "ct d human" - has been performed on May 5, 2014 via DIMDI-host for the following terms:
Pyraclostrobin* or F500
CAS 175013-18-0

Literature data basis:
Medline 66 (NLM)
Medline alert (NLM)
Embase 74 (Elsevier)
Embase alert (Elsevier)
Cochrane Library-Central
Biosys (Thomson Reuters)
gms (German Medical Science)
IPA International Pharmaceutical Abstracts (Thomson Reuters)
Deutsches Aerzteblatt (Aerzteverlag)

Internet available databases:
CHEMID (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp)
PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi)
Toxnet (http://toxnet.nlm.nih.gov/)

These searches revealed one relevant document, which is summarized in M-CA 5.9.3.

Considering all available information, the conclusion for relevant endpoints for the current renewal remains as follows:

Medical data	Exposure to humans may result in irritation of skin and eyes. Accidental inhalation of pyraclostrobin containing products was reported to result in symptoms of respiratory irritation.

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of pyraclostrobin. Thus, the medical monitoring program is designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.
The surveillance program includes a general physical examination including neurological status, red and white blood cell counts and liver enzymes. Adverse health effects suspected to be related to pyraclostrobin exposure have not been observed and no monitoring studies are known to the applicant.

CA 5.9.2 Data collected on humans

Some cases of eye irritation deriving from pyraclostrobin in combination with other substances in the course of production, transportation, formulation and packaging have been reported to BASF SE.

CA 5.9.3 Direct observations

Some cases of slight irritation of the skin and mouth and/or intoxication (indisposition, headache, ague, fatigue, aching muscles, vomiting, drowsiness, dizziness, adynamic feet, breathing difficulties) have been registered in the internal clinical incident log at BASF SE in Ludwigshafen for persons exposed to pyraclostrobin containing products in combination with other active ingredients and/or products. It is not clear whether pyraclostrobin was the cause for these effects and whether exposure was according to good agricultural practice.

Gergely et al. described 5 events. All cases were categorized as being of low to moderate severity. The patients had irritation and pain of the upper respiratory tract as well as nausea, headache, eye pain, weakness, dizziness, chest pain. More details are shown below.

Report: CA 5.9.3/1
Gergely R.M. et al., 2008a
Acute pesticide poisoning associated with Pyraclostrobin fungicide - Iowa, 2007
2008/1102036
Guidelines: none
GLP: no

Executive Summary

The information was published in the Morbidity and Mortality Weekly Report (MMWR) series of the US Centers for Disease Control and Prevention (CDC). The MMWR series is the CDC's "primary vehicle for scientific publication of timely, reliable, authoritative, accurate, objective, and useful public health information and recommendations". The data are based on reports sent by state health departments to CDC.
The authors reported 5 events, which were reported to the Iowa Departement of Public Health. 33 persons were exposed to pyraclostrobin. All cases were categorized as being of low to moderate severity.
In all but one incidents the exposure was to spray drift from aerial application. The most severe incident pertained 27 Hispanics. They were exposed to off-target drift from a nearby field while detasseling corn (i.e., removing tassels from corn plants to prevent auto-pollination and enable hybridization). Some workers reported feeling wet droplets on their skin and seeing mist coming from the aircraft. All workers received skin decontamination on-site by a hazardous materials team before being transported to an emergency department for observation until their symptoms resolved. All cases were categorized as being of low severity. According to NIOSH classifications low severity illness or injury includes "illnesses manifested by skin, eye, or upper respiratory irritation". These illnesses might also include fever, headache, fatigue or dizziness. Typically, the illness or injury resolves without treatment, and time lost from work or normal activities is < 3 days.
The most common symptom was upper respiratory tract pain or irritation (26 patients), followed by chest pain (20 patients). Three patients had nausea, and one patient each had pruritis, skin redness, eye pain, weakness, headache, dizziness, and chest pain.
The other incidents pertained single cases with off-target drift of pyraclostrobin from nearby aerial applications. They were exposed by riding a motorcycle near a field or by spray drifting to their home yard. Symptoms reported were headache, eye pain partially associated with conjunctivitis and dizziness. The last case was from a crop-duster pilot who – when his plane crashed during take-off – was exposed to spilling of the liquid fungicide. In this case of moderate severity (non-life-threatening health effects that generally are systemic and require medical treatment), the pilot reported no respiratory symptoms.

Classification of study: Additional information

Based in the above described observations, the known irritant potential of pyraclostrobin as well as the supportive evidence for respiratory irritation observed in subchronic inhalation studies (see M-CA 5.3.3/1 and 5.3.3/2) BASF self-classified pyraclostrobin with STOT SE Category 3 (H335, May cause respiratory irritation).

CA 5.9.4 Epidemiological studies

Data on exposure of the general public or epidemiologic studies are not available to the applicant and no epidemiologic studies performed by third parties are known**.**

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Specific signs of toxicity or specific clinical test methods are not known to the applicant.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

Supportive treatment should be done according to symptoms. No specific antidote is known. Further information can be found in M-CA 3.10, which is reflecting the information given in the safety data sheet.

CA 5.9.7 Expected effects of poisoning

Effects of poisoning are not known. Expected effects may be derived from acute and subacute studies in animals.

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT M-CA, Section 6

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162247 (version 1)
27-Feb-2017	A new residue study in potatoes was added in 6.3.1 (including tier 1 summary) and the text in 6.7.2 was revised accordingly. In 6.9 additional metabolites were added to the assessment. The argumentation on the residue level in maize pollen was elaborated in 6.10.1. New or changed text is marked in yellow.	BASF DocID 2017/1032925 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

Pyraclostrobin (BAS 500 F), a fungicide for use in cereals, maize, potatoes, legumes, fruits, vegetables and various other crops, is registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive No 2004/30/EC and 2009/25/EC. Inclusion entered into force on 10 March 2004. The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011. Approval extension was granted until 31 January 2017 by Regulation No 823/2012/EU.

All relevant information on the first Annex I review and the endpoints used in consumer dietary assessments can be found in the monograph of pyraclostrobin and in the SANCO/1420/2001-Final document (EU Review Report).

For the current renewal of approval under Regulation 1107/2009, a data gap analysis according to new guidelines, new guidance documents and new policies in exposure assessments was performed. New studies / evaluations were initiated where considered necessary. Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality.

Although titles and abstracts of several publications indicated a potential connection to respective consumer safety chapters of this dossier, the detailed evaluation of these publications showed no endpoint of sufficient reliability which could be used for the required risk assessments. Consequently, for consumer safety only a summary of public literature data on pyraclostrobin is provided in this supplemental dossier. Further information on the literature assessment and respective justifications can be found in M-CA 9.

An overview of metabolites identified during consumer safety studies is given below. In addition, two metabolites being identified in the aerobic soil metabolism studies (500M01, 500M02) are included in Table 6-1. Their potential uptake behavior into succeeding crops will be discussed in M-CA 6.7 and M-CA 6.9. The list of metabolites occurring in rats (limited to in-vivo investigations of BASF DocID 1999/11781) is not complete; the complete list can be found in M-CA 5.1. The information in the table allows a comparison between the pathways in different test systems.

The metabolite overview below is including the different code numbers that are available for each metabolite. Due to historic reasons (e.g. use of different metabolite codes in different study reports), it is unfortunately not possible to use always only one and the same metabolite code for a certain metabolite. In the following chapters and study summaries synonym metabolite codes are given in brackets where deemed to be helpful.

Table 6-1: Notations of parent and metabolites of pyraclostrobin

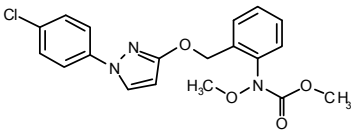
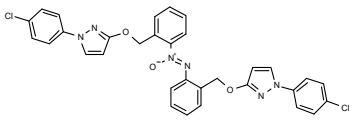
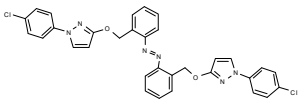
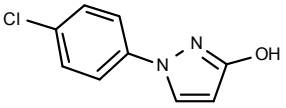
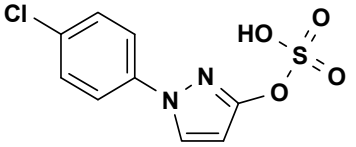
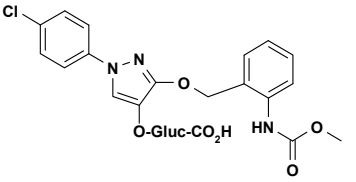
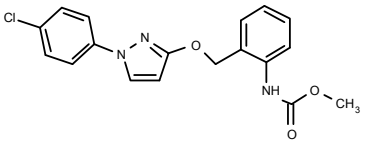
Substance/ Metabolite Code	Reg. No	Synonyms	CAS-No	Compound found in	Structure/Name
BAS 500 F	304428	500M00	175013-18-0		
500M01	364380	BF 500-6	not assigned	soil (aerobic metabolism)	
500M02	369315	BF 500-7	not assigned	soil (aerobic metabolism)	
500M04	298327	BF 500-5	76205-19-1	grapes, Chinese cabbage, potatoes, wheat, hydrolysis (olive oil, high temp.) hen, goat (*in-vitro* and *in-vivo*), cow (*in-vitro*), fish (bioaccumulation) rat	
500M05	not assigned		not assigned	goat rat	
500M06	not assigned		not assigned	hen rat	
500M07	340266	BF 500-3	512165-96-7	grapes, Chinese cabbage, potatoes, rice, wheat, hydrolysis (olive oil, high temp.) hen, goat rat	

Table 6-1: Notations of parent and metabolites of pyraclostrobin

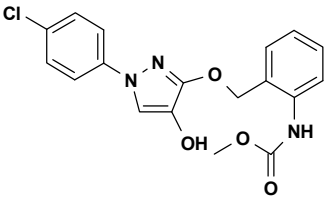
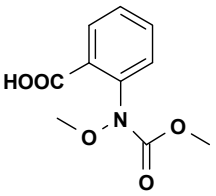
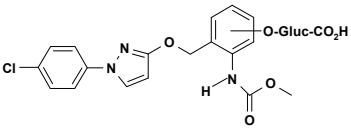
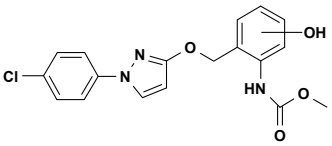
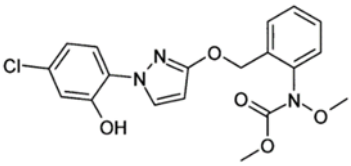
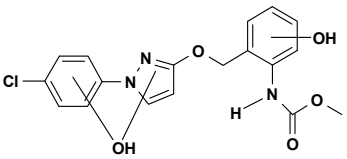
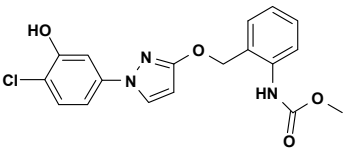
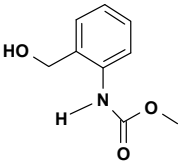
Substance/ Metabolite Code	Reg. No	Synonyms	CAS-No	Compound found in	Structure/Name
500M08	not assigned		not assigned	goat, fish (bioaccumulation)	
500M24	5916421		not assigned	wheat rat	
500M32	not assigned		not assigned	hen rat	
500M34	not assigned		not assigned	wheat goat, cow (*in-vitro*) rat	
500M35	412040		not assigned	goat, cow (*in-vitro*) rat	
500M39	not assigned		not assigned	hen, goat rat	
500M45	not assigned		not assigned	goat, fish (bioaccumulation) rat	
500M49	5916420		not assigned	hydrolysis (olive oil, high temp.) hen	

Table 6-1: Notations of parent and metabolites of pyraclostrobin

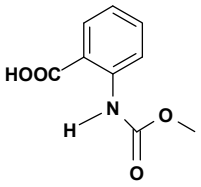
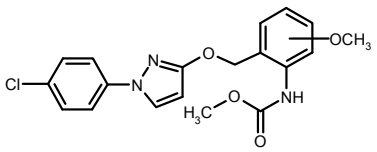
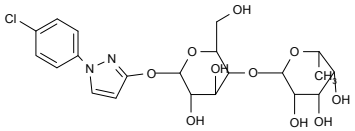
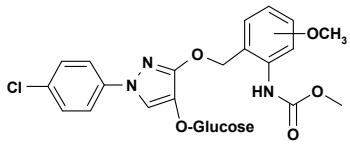
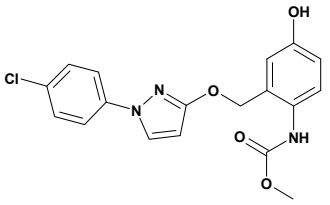
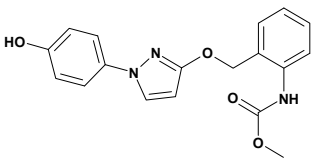
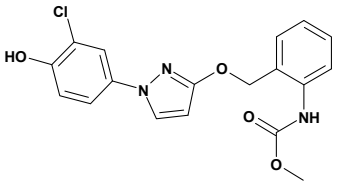
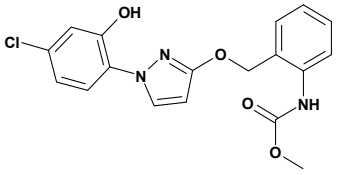
Substance/ Metabolite Code	Reg. No	Synonyms	CAS-No	Compound found in	Structure/Name
500M51	78810		6268-38-8	goat rat	
500M54	not assigned		not assigned	grapes, potatoes, wheat	
500M55	not assigned		not assigned	grapes	
500M56	not assigned		not assigned	grapes	
500M64	not assigned		not assigned	hen, goat	
500M65	not assigned		not assigned	goat	
500M66	not assigned		not assigned	hen, goat	
500M67	not assigned		not assigned	goat	

Table 6-1: Notations of parent and metabolites of pyraclostrobin

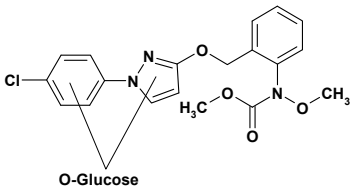
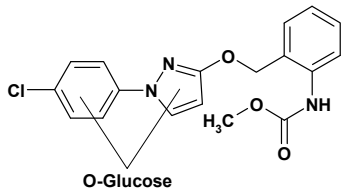
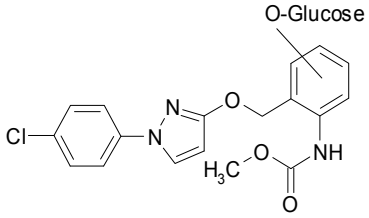
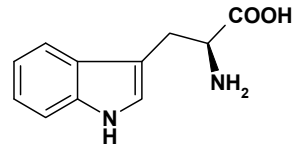
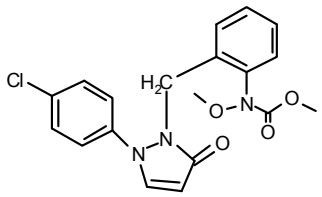
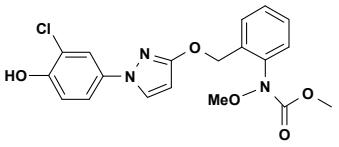
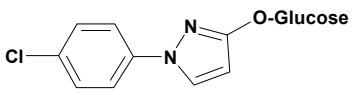
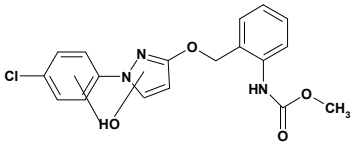
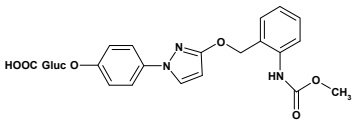
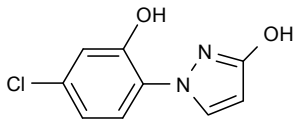
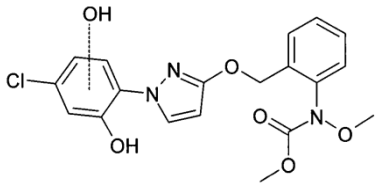
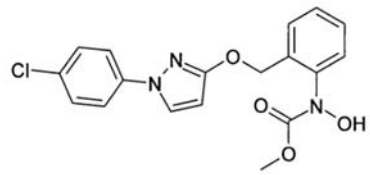
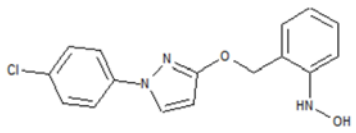
Substance/ Metabolite Code	Reg. No	Synonyms	CAS-No	Compound found in	Structure/Name
500M68	not assigned		not assigned	grapes, potatoes, wheat	
500M70	not assigned		not assigned	wheat	
500M71	not assigned		not assigned	grapes, wheat	
500M72	not assigned	L tryptophan		Chinese cabbage, potatoes, wheat,	
500M76	413038	BF 500-14	not assigned	wheat	
500M77	4001763	BF 500-16	not assigned	hen	
500M79	not assigned		not assigned	grapes, potatoes	
500M80	not assigned		not assigned	hen	

Table 6-1: Notations of parent and metabolites of pyraclostrobin

Substance/ Metabolite Code	Reg. No	Synonyms	CAS-No	Compound found in	Structure/Name
500M83	not assigned		not assigned	hen	
500M85	399530	BF 500-8	not assigned	goat	
500M86 / 500M87	not assigned		not assigned	goat, cow (*in-vitro*)	
500M88	322410	BF 500-1	220897-76-7	goat, cow (*in-vitro*)	
500M89	334089		not assigned	fish metabolism (dietary)	

CA 6.1 Storage stability of residues

Stability of residues during storage of samples

The stability of residues was reviewed during the previous Annex I inclusion process (Annex II, section 4, point 6.1) and no further data are required.

For pyraclostrobin in **plant** matrices, only an interim report was available at the time point of DAR preparation. The storage stability study in plant matrices covering a period of 18 to 25 months is summarised below. It has already been submitted several times during the past years in context of national and zonal registrations, but is not considered as peer reviewed. In order to fulfil the reporting needs of the OECD Guideline 506, the results of the study are additionally presented as measured and not recovery corrected.

In case of **animal** matrices, storage stability data exists covering the period between sample generation and analysis. They were submitted and evaluated during the previous Annex I inclusion process.

The following information is copied from the Draft Assessment Report prepared by RMS Germany (Vol. 1, list of endpoints, 2001):

Stability of residues (Annex IIA, point 6 introduction, Annex IIIA, point 8 introduction)

Food of animal origin: pyraclostrobin stable for 8 month Metabolite BF 500-10 (model compound) with slow degradation but stable enough to evaluate the submitted feeding study (analysed within 6 month). Plant (peanut nutmeat, peanut oil, wheat grain, wheat straw, sugar beet tops, sugar beet roots, tomatoes, grape juice): pyraclostrobin, metabolite BF 500-3 stable for 18 month

Report: CA 6.1/1
Abdel-Baky S., 2001a
Freezer storage stability of BAS 500 F and BF 500-3 in plant matrices including processed commodities
2001/5000232
Guidelines: EPA 860.1380
GLP: yes
(certified by United States Environmental Protection Agency)

Note: This study was erroneously not contained in the application. The reason for submission is described above.

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: active ingredient pyraclostrobin and its metabolite BF 500-3
Description: pyraclostrobin; BF 500-3
Lot/Batch #: 00937-128, BAS 500 F; 00937-272, BF 500-3
Purity: 99.8%, BAS 500 F; 99.0% BF 500-3
CAS#: 175013-18-0, BAS 500 F
Development code: BAS 500 F; BF 500-3 (synonym : 500M07)
Spiking level: 1 mg/kg

2. Test Commodity: peanut, wheat, sugar beet, tomato, grape
Crop: oilseed, cereals, root crop, fruiting vegetables, berries & other small fruit
Type: n/a
Variety: n/a
Botanical name: *Arachis hypogeae, Triticum vulgare, Beta vulgaris, Lycopersicon esculentum, Vitis vivifera*
Crop parts(s) or processed
Commodity: peanut nutmeat, wheat grain, sugar beet tops, sugar beet roots, tomatoes, wheat straw, peanut oil, grape juice
Sample size: 20 g (peanut oil 10 g)

B. STUDY DESIGN AND METHODS

1. Test procedure

The freezer stability of pyraclostrobin and its metabolite BF 500-3 (synonym: 500M07) were determined in a diverse set of commodities. The materials were an oilseed (peanut nutmeat; OECD: high oil content), a non-oily grain (wheat grain; OECD: high starch content) a leafy vegetable (sugar beet tops; OECD: high water content), a root crop (sugar beet roots; OECD: high starch content), a fruit/fruiting vegetable (tomatoes; OECD: high water content), and a dry feed item (wheat straw). In addition, the unique processed commodities of oil (peanut) and juice (grape; OECD: high acid content) are also represented. Control samples of each matrix were fortified at a level of 1.0 mg/kg with each pyraclostrobin and its metabolite BF 500-3. Samples were analysed for recoveries initially after fortification and after 1, 3, 6, 14, 18 - 19 and 25 months frozen storage (< -10°C). The maximum frozen storage interval used for all matrices was 25 months, with the exception of peanut nutmeat and peanut refined oil, which were held a maximum of 19 months. The samples were analysed by method number D9908 (421/0). The limit of quantitation (LOQ) of the method is 0.02 mg/kg for each analyte.

2. Description of analytical procedures

Method Number D9908 (421/0) was used for the analyses of this study. Pyraclostrobin and its metabolite BF 500-3 are extracted from plant matrix samples with a methanol/water/HCl mixture (70/25/5). A 0.5% aliquot of the extract is removed and cleaned by liquid/liquid partition with cyclohexane. For wheat and sugar beet matrices, the extracts are further purified on a Silica Gel Speedisk® column. The final chromatography analysis of pyraclostrobin and its metabolite BF 500-3 are determined by LC/MS-MS. The limit of quantitation (LOQ) for pyraclostrobin and BF 500-3 residues is 0.02 mg/kg for each analyte. The ranges of the procedural recoveries at typically 1 mg/kg are shown in Table 6.1-1.

Table 6.1-1: Ranges of procedural recoveries

Matrix	Storage intervals [months]	Range of mean procedural recoveries [%]	
		BAS 500 F	BF 500-3 (500M07)
Peanut nutmeat	0, 1, 3, 6, 14, 19	65 – 101	59 – 104
Peanut oil	0, 1, 3, 6, 14, 19	77 – 107	72 – 107
Wheat grain	0, 1, 3, 6, 14, 19, 25	74 – 100	66 – 99
Wheat straw	0, 1, 3, 6, 14, 19, 25	82 – 112	73 – 111
Sugar beet tops	0, 1, 3, 6, 14, 19, 25	80 – 105	64 – 119
Sugar beet roots	0, 1, 3, 6, 14, 19, 25	56 – 109	50 – 137
Tomatoes	0, 1, 3, 6, 14, 19, 25	80 – 107	72 – 101
Grape juice	0, 1, 3, 6, 14, 19, 25	75 – 125	71 – 110

II. RESULTS AND DISCUSSION

The results obtained from the stored fortified samples indicate that pyraclostrobin and BF 500-3 residues are stable when frozen in peanut nutmeat and oil for at least 19 months and in wheat (grain and straw), sugar beets (tops and roots), tomatoes and grape juice for at least 25 months. The table below presents a summary of the recoveries from the stored fortified samples, corrected for procedural recoveries. A comparison between the recovery corrected and the uncorrected values indicate that lower recoveries in stored samples are rather caused by variability of the method than actual instability of pyraclostrobin residues in sample matrices.

Table 6.1-2: Storage stability of BAS 500 F and BF 500-3 in plant matrices

Matrix	Average Relative Recovery [1] (%)						
	0-Month	1-Month	3-Months	6-Months	14-Months	18-Months	25-Months
BAS 500 F							
Peanut nutmeat	92	96	90	91	95	88	n.a. [2]
Peanut oil	105	92	102	118	101	106	n.a. [2]
Wheat grain	105	92	87	91	82	88	90
Wheat straw	93	96	96	113	71	99	100
Sugar beet tops	103	100	97	100	91	98	99
Sugar beet roots	92	94	92	96	78	91	79
Tomatoes	104	98	96	90	91	96	95
Grape juice	91	96	94	96	69	88	80
BF 500-3 (synonym: 500M07)							
Peanut nutmeat	94	96	112	92	92	84	n.a. [2]
Peanut oil	104	92	102	122	103	120	n.a. [2]
Wheat grain	101	90	86	86	79	89	83
Wheat straw	90	91	99	104	63	97	100
Sugar beet top	103	101	99	99	93	99	107
Sugar beet roots	87	97	97	94	78	91	76
Tomatoes	103	98	97	85	85	92	89
Grape juice	92	95	92	94	80	93	89

[1] Average Relative Recovery = Average Stored Recovery / Average Procedural Recovery *100
[2] For peanut nutmeat and oil samples, the longest storage interval used was 19 months.

Table 6.1-3: Storage stability of BAS 500 F and BF 500-3 in plant matrices

Matrix	Average Recovery (%) in stored samples as measured						
	0-Month	1-Month	3-Months	6-Months	14-Months	18-Months	25-Months
BAS 500 F							
Peanut nutmeat	72	97	82	62	61	77	n.a. [1)]
Peanut oil	87	99	109	91	90	92	n.a. [1)]
Wheat grain	90	89	80	84	61	88	91
Wheat straw	80	108	76	93	36	90	82
Sugar beet tops	108	94	97	87	69	86	79
Sugar beet roots	100	90	89	74	44	82	70
Tomatoes	100	97	103	84	73	90	79
Grape juice	114	78	96	72	55	86	68
BF 500-3 (synonym: 500M07)							
Peanut nutmeat	67	99	89	66	54	72	n.a. [1)]
Peanut oil	80	98	94	91	77	86	n.a. [1)]
Wheat grain	88	82	69	79	52	88	70
Wheat straw	98	101	67	84	33	87	72
Sugar beet top	122	93	88	85	59	84	79
Sugar beet roots	119	91	88	68	39	82	68
Tomatoes	97	96	98	77	62	87	73
Grape juice	101	69	93	74	57	90	84

[1)] For peanut nutmeat and oil samples, the longest storage interval used was 19 months.

III. CONCLUSION

This storage stability study demonstrates that residues of pyraclostrobin and BF 500-3 are stable for up to 25 months in the following frozen raw agricultural and processed commodities: wheat grain and straw, sugar beet roots and tops, tomatoes and grape juice. In addition, the frozen stability of pyraclostrobin and its metabolite have been demonstrated for up to 19 months in peanut nutmeat and oil; peanut matrices were not analysed at the 25-months sampling interval. The pyraclostrobin and BF 500-3 residues remained consistent from time 0 up to the last analysis time period in all plant matrices.

Stability of residues in sample extracts

For the active substance pyraclostrobin, investigations were performed using sample extracts out of ^{14}C-metabolism studies and fortified samples during the validation of the residue analytical methods. In none of the extracts investigated any degradation was observed. From the data available it can be concluded that pyraclostrobin is stable in sample extracts or solutions when stored during residue analysis.

CA 6.2 Metabolism, distribution and expression of residues

CA 6.2.1 Metabolism, distribution and expression of residues in plants

In context of the previous submission for Annex I inclusion, plant metabolism studies have been submitted in grapes, potatoes and wheat covering the crop categories of fruits, root & tuber vegetables and cereals (all foliar application).

According to the results of these studies, pyraclostrobin is intensively metabolized by mainly three key transformation steps:

- N-Desmethoxylation and O-desmethylation of the side chain
- Hydroxylation of the aromatic ring systems
- Cleavage between the ring systems

The contribution of these reactions followed by subsequent conjugation leads to a large number of metabolites. The studies are considered as suitable and valid for describing the degradation behaviour of pyraclostrobin in plants.

The assessment was recently confirmed by EFSA during the re-evaluation of the established MRLs according to Reg. 396/2005, Art. 12.

The following conclusion is directly copied from the Reasoned Opinion (see: EFSA Journal 2011;9(8):2344):
The relevant residue in grapes consisted of parent pyraclostrobin (55.7 - 66% TRR) and its desmethoxy metabolite 500M07 (11.2 - 15.3% TRR). In potatoes the highest TRR was identified in green matter (41.2 - 57.9 mg/kg) in both studies. Parent pyraclostrobin was the main component of the TRR in green matter and potato tubers in studies with [chlorophenyl-U-14C]-pyraclostrobin, amounting for 55% and 29.4% of the TRR, respectively. In the green matter desmethoxy metabolite 500M07 was identified in levels > 20% of the TRR in both studies. In the tolyl study the major component of the TRR in potato tubers was identified as natural amino acid L-tryptophan (29.2% TRR). In cereals, the lowest TRR was found in grains, varying between 0.098 mg/kg in the chlorophenyl labelled and 0.441 mg/kg in the tolyl labelled matrix. The highest TRR was identified in wheat straw, amounting for up to 37.76 mg/kg (chlorophenyl study) and 40.46 mg/kg (tolyl study). The major component of the TRR in straw and grain in the chlorophenyl study was parent pyraclostrobin and its desmethoxy metabolite (500M07). In the tolyl study the major component of the TRR in grain was L-tryptophan (23% TRR), any other components being below 10% of the TRR. L-tryptophan is an essential natural amino acid therefore it is of no toxicological relevance (EFSA, 2010).
Generally it was concluded in the peer review (EC, 2002) that the metabolic pathway is similar in all crop groups investigated. Results from the supervised residue trials indicated that desmethoxy metabolite 500M07 occurs in crops in small amounts compared to parent pyraclostrobin; therefore in the peer review it was concluded that a general residue definition for risk assessment and enforcement should be set as parent pyraclostrobin.

For covering a broader use spectrum and a different application type, two new crop metabolism studies have been conducted. New crop metabolism studies were performed in wheat (seed treatment application) and in paddy rice. Besides these new studies, a cabbage metabolism study is newly submitted in the EU. This study was performed for achieving the Japanese registration. Furthermore, a supplementary document (non-GLP) to the grape metabolism study was prepared upon request of Japan. Purpose of the document was to clarify the way of metabolite identification which is based on extracts from grape leaves. In order to provide a comprehensive picture in grapes, an overall summary of all relevant documents is provided.

The studies summarized below were performed using the tolyl-^{14}C and chlorophenyl-^{14}C labeled pyraclostrobin.

CA 6.2.1.1 Grapes (category: fruits)

Report:	CA 6.2.1/1 Hamm R.T., 1998a Metabolism of BAS 500 F in grapes 1998/10988
Guidelines:	EPA 165-1, BBA IV 3-2
GLP:	yes (certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)
Report:	CA 6.2.1/2 Hamm R.T., 2000a Report Amendment No. 1 to final report: Metabolism of BAS 500 F in grapes 2000/1000201
Guidelines:	EPA 860.1300, BBA IV 3-2
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)
Report:	CA 6.2.1/3 Bross M., 2004a Pyraclostrobin (BAS 500 F) - Grape metabolism: Additional information on the investigations of grape leaves 2004/1000758
Guidelines:	see above
GLP:	no

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	Chlorophenyl-U-C^{14}- pyraclostrobin and tolyl-U-C^{14}
	Description:	Carbon-14 chlorophenyl ring labelled (4.34 MBq/mg) and carbon-14 tolyl ring labelled (4.50 MBq/mg) pyraclostrobin
	Lot/Batch #:	^{14}C-BAS 500 F (chlorophenyl): 579-1017 ^{14}C-BAS 500 F (tolyl): 566-1047
	Purity:	^{14}C-BAS 500 F (chlorophenyl): >99% (radiochemical) ^{14}C-BAS 500 F (tolyl): >99% (radiochemical)
	CAS#:	not applicable
	Development code:	not applicable
	Stability of test Compound:	The residues in grapes, treated with pyraclostrobin were stable under the chosen experimental conditions (-20°C, 188 days).
2.	**Test Commodity**:	Berries & small fruits
	Crop:	Grapevine
	Type:	not reported
	Variety:	Müller-Thurgau
	Botanical name:	*Vitis vinifera L.*
	Crop parts(s) or processed Commodity:	Grapes and leaves
	Sample size:	Grapes: 849 g, leaves: >1507 g
3.	**Soil:**	not reported

B. STUDY DESIGN

1. Test procedure

In the plant-uptake part of the study, ^{14}C-BAS 500 F was applied in the form of a pyraclostrobin EC-formulation to grapevines. In total, six post-emergence applications at an intended use rate of 1.500 kg as/ha and vegetation period were performed. The first application was performed at growth stage BBCH 53 – 55 (inflorescences visible to fully developed). The application was repeated 5 times approximately every 16 to 19 days thereafter. The last application was done at growth stage 81 (beginning of ripening), 40 days before harvest.

Figure 6.2.1-1: Structural formula of ^{14}C-BAS 500 F labelled at the chlorophenyl ring

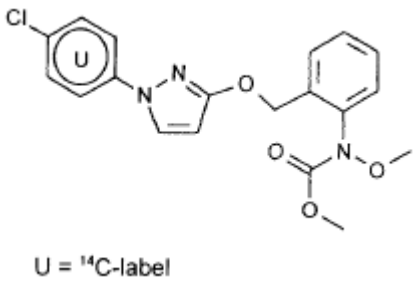
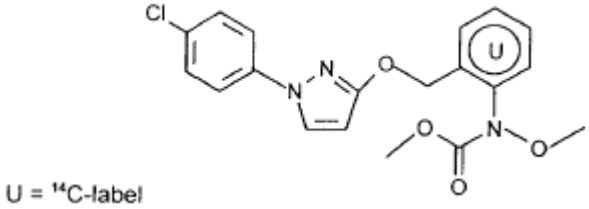


Figure 6.2.1-2: Structural formula of ^{14}C-BAS 500 F labelled at the tolyl ring



The grapevines were cultivated at a field site which belongs to the BASF Agricultural Center Limburgerhof, BASF Aktiengesellschaft (today: BASF SE). During the study, leaves were sampled to take care for good growing conditions (e.g. high light intensity and low humidity) 12 days after the last application. Brunches were also harvested 12 days after the last application and young brunches were sampled 14 days after the 4th application. At harvest, 40 days after the last application (=PHI), grapes and leaves were harvested. Samples were taken for analysis only at harvest, 40 days after the last application (= PHI).

2. Description of analytical procedures

Grape and leaf samples were extracted three times with methanol. The extractable radioactivity was characterized and quantified by HPLC and radio-assayed by LSC. In addition, liquid/liquid partitioning experiments using cyclohexane and ethyl acetate were carried out. The metabolites were identified by comparison with reference substances. If possible, they were isolated by HPLC and their structures elucidated by LC-MS/MS.

For characterisation of the Residual Radioactive Residue (RRR) after solvent extraction, the radioactive residues were further extracted with ammonia solution (tolyl-label) or water (chlorophenyl-label), followed by an extraction step (reflux) with NaOH.

In order to get information on the storage stability of the grape fruit samples, the extractability and the HPLC metabolite profiles were investigated at the beginning and at the end of the study.

The residual radioactive residue of grape leaf sampled was determined by combustion analysis.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Grapes:

The total radioactive residues (TRRs) from the grape samples at harvest time (40 DALA) treated either with [tolyl-label]- or [chlorophenyl-label]- labelled pyraclostrobin are shown in Table 6.2.1-1. Due to the high water content of the samples, TRRs were calculated from the sum of ERRs and RRRs. In spite of 6 applications with a total application amount of 1.500 kg/ha, the total radioactive residue level in the fruits from both labels is relatively low. It ranges from 0.951 mg/kg (chlorophenyl-label) to 1.559 mg/kg (tolyl-label). One reason for this low residue level could be that fruits are partly protected by the leaf canopy.

Table 6.2.1-1: Total radioactive residues (TRR) after treatment of grape fruits with ^{14}C-BAS 500 F

Total radioactive residues (TRR)[1] [mg/kg]		
Label position	**Tolyl label**	**Chlorophenyl label**
Grape fruits (at harvest)	1.559	0.951

[1] Calculation as sum of ERR+RRR

Leaves:

The total radioactive residues (calc.) were almost identical for both labels. A TRR of 40.029 mg/kg was found for the chlorophenyl label whereas 41.243 mg/kg were detected in the tolyl label.

The total radioactive residue data in grape leaves are shown in Table 6.2.1-2.

Table 6.2.1-2: Total radioactive residues (TRR) after treatment of grape leaves with ^{14}C-BAS 500 F

Total radioactive residues (TRR)[1] [mg/kg]		
Label position	**Chlorophenyl label**	**Tolyl label**
Grapes leaves (at harvest)	40.029	41.243

[1] Calculation as sum of ERR+RRR

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

1. Extraction of residues

Grapes:

In grape samples, the extractability with methanol was high; it ranged from 84 to 88% of the TRR. To increase the extractability, ammonia/water extraction was applied.

Extractability in grapes is shown in Table 6.2.1-3.

Table 6.2.1-3: Extraction of radioactivity after treatment of grapes with ^{14}C-BAS 500 F

Extraction of radioactivity [mg/kg] (% TRR)	
Solvent	**Grapes**
Tolyl label	
Methanolic extract	1.314 (84.3)
Residue after solvent extraction (RRR)	0.245 (15.7)
Ammonia extract	0.023 (1.5)
Chlorophenyl label	
Methanolic extract	0.835 (87.8)
Residue after solvent extraction (RRR)	0.166 (12.2)
Aqueous extracts	0.006 (0.6)

In order to classify metabolites into organo-soluble and water-soluble compounds, the methanolic extracts of the tolyl- and the chlorophenyl-label were partitioned. In Table 6.2.1-4 the values are summarized. In all samples, the organo-soluble metabolites significantly predominated. Their amounts ranged from 73% TRR (tolyl-label) to 78% TRR (chlorophenyl-label).

Table 6.2.1-4: Partition characteristics of Extractable Radioactivity (grape fruit samples)

Label	Cyclohexane/water Partition				Ethyl acetate/water Partition				Sum			
	organo-soluble		water-soluble		organo-soluble		water-soluble		organo-soluble		water-soluble	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Chlorophenyl	0.663	69.7	-	-	0.077	8.1	0.075	7.8	**0.740**	**77.8**	**0.075**	**7.8**
Tolyl	1.011	65.5	-	-	0.119	7.7	0.096	6.2	**1.130**	**73.2**	**0.096**	**6.2**

Leaves:

Both labels showed comparable extraction behaviour. Around 70% of the TRR could be extracted with methanol. Around 30% of the TRR remained in the residual radioactive residue.

Extractability data in grape leaves are shown in Table 6.2.1-5.

Table 6.2.1-5: Total radioactive residue and extraction behaviour in grape leaves, 40 DAT

Matrix	Label	TRR combusted	ERR		RRR	TRR[1] calc.
		[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[mg/kg]
Leaves (at harvest)	Chlorophenyl label	49.673	28.327	70.8	11.702	40.029
	Tolyl label	40.266	28.866	70.0	12.377	41.243

[1] Calculation as sum of ERR+RRR

The methanol extract of the chlorophenyl label was partitioned between organic solvents and water. Most of the radioactivity was found to be organo-soluble. About 14% of the TRR was detected in the aqueous phase.

Partition behaviour data in grape leaves are shown in Table 6.2.1-6.

Table 6.2.1-6: Partition behaviour of the MeOH extract (Chlorophenyl label) - grape leaves

Matrix	ERR		Cyclohexane phase[1]		Ethyl acetate phase		Aqueous phase		Recovery
	[mg/g]	[%TRR]	[mg/g]	[%TRR]	[mg/g]	[%TRR]	[mg/g]	[%TRR]	[%]
Leaves	28.327	70.8	12.326	30.8	9.086	22.8	5.615	14.1	95

[1] Calculation as sum of ERR+RRR

2. Identification, characterization and quantitation of extractable residues

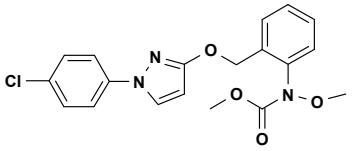
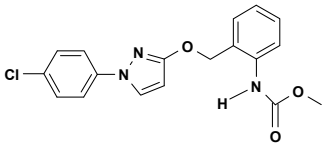
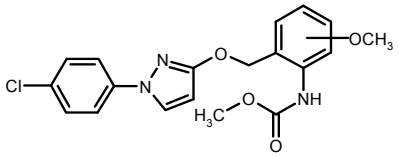
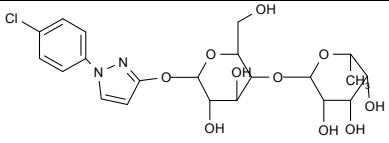
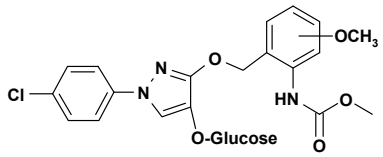
Grapes:

Extractable radioactive residues:
In the methanol extracts only parent (56-62% TRR) and one main metabolite, 500M07 or synonym BF 500-3 (11% - 17% TRR) could be isolated and analyzed (LC-MS).
Three minor metabolites could be further characterized by co-chromatography with isolated and identified metabolites from leaves, designated as 500M54, 500M55 and 500M56 (each single peak was less or equal to 4.01% TRR). Their concentrations in the berries were < 0.05 mg/kg. Compound 500M54 is a derivative of the desmethoxy metabolite BF 500-3 and the two other metabolites are glycosides of the chlorophenyl-OH-pyrazol.

Residual radioactive residues:
Approximately half of the Residual Radioactive Residues (RRR) after solvent extraction could be characterized as bound to or associated to cellulose and lignin. The rest of the RRR was not further characterized because of low concentrations. It is postulated that the remaining residues are likely to be also lignins of a lower molecular weight.

The quantification of the individual metabolites present in grapes fruits is summarized in Table 6.2.1-7.

Table 6.2.1-7: Summary of identified components in grape fruit samples after treatment with ^{14}C-BAS 500 F

Metabolite code (Reg. No. of reference substance)	Metabolite structure	Grape fruits	
		Tolyl label mg/kg (% TRR)	Chlorophenyl label mg/kg (% TRR)
BAS 500 F (304428)		0.860 **(55.7)**	0.588 **(61.79)**
500M07 (BF 500-3) (340266)		0.170 **(11.02)**	0.159 **(16.68)**
500M54 (not assigned)		0.045 **(2.9)**	0.015 **(1.55)**
500M55 (not assigned)		n.d.	0.038 **(4.01)**
500M56 (not assigned)		0.048 **(3.11)**	0.016 **(1.69)**

n.d. not detected

Leaves:

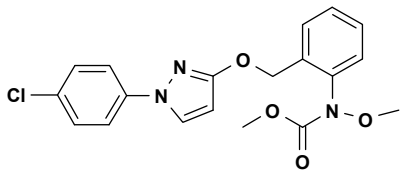
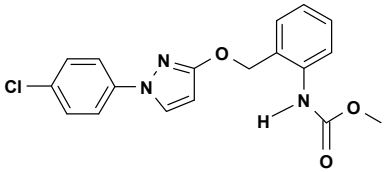
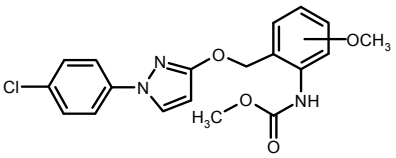
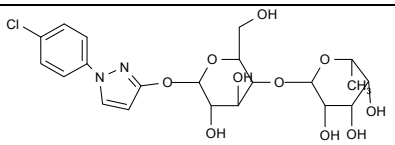
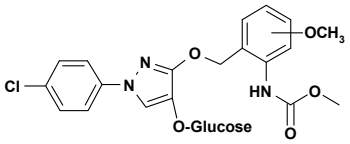
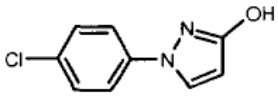
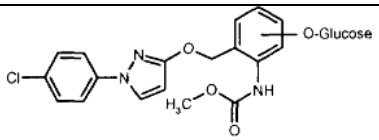
Pyraclostrobin and its desmethoxy metabolite **500M07** (BF500-3) formed the major part of the radioactivity in the MeOH extracts. Additionally, some polar and medium polar peaks were detected. Since in case of the tolyl labelled sample, the individual metabolites were only present in trace amounts, no additional work-up was performed. The MeOH extract of the chlorophenyl label was partitioned between water and organic solvents. Despite no HPLC quantitation was performed, the amounts can be roughly estimated by considering the radioactivity being present in the relevant partition phases.

The **cyclohexane phase** containing 12.326 mg/kg (corresponding to 30.8% TRR) consisted of pyraclostrobin and 500M07; only one minor additional peak was detected. The radioactivity present in the **ethyl acetate phase** (corresponding to 9.086 mg/kg, 22.8% TRR) was distributed among at least 10, partly overlapping metabolites. The ethyl acetate phase was fractionated and the individual fractions passed to MS for structural identification. Fraction 2 consisted at least of three metabolites. One of them was characterized as saccharose conjugate of the hydroxy parent molecule. Based on its fragmentation pattern, hydroxylation and subsequent conjugation is more likely in the chlorophenyl-pyrazole moiety. In fraction 4, two metabolites were detected. The first one was characterized as saccharose conjugate; according to the fragmentation pattern, hydroxylation and subsequent conjugation took place in the pyrazole ring system. The second peak was identified as 500M56. Further fractionation of fraction 5 resulted in two samples. In one sample, four metabolites were found. For none of them a clear identification was possible. In the other sample, the metabolite present was identified as 500M04. Fraction 6 consisted of at least three metabolites. One of them was characterized as glucose conjugate of the desmethoxy metabolite. Most likely, hydroxylation and conjugation took place in the tolyl ring system resulting in structure 500M71. In fraction 8, a glucose conjugate was present. Based on the fragmentation pattern, hydroxylation and subsequent conjugation is more likely in the chlorophenyl pyrazole moiety. In fraction 9, MS was identified as 500M54.

The remaining **aqueous phase** containing 5.615 mg/kg and 14.1% TRR is containing one predominant peak plus 4 additional components in very low amounts. The peak was identified as the metabolite 500M55. It is clearly present in amounts greater than 10% TRR.

The structure of the metabolites present in grapes leaves is summarized in Table 6.2.1-8.

Table 6.2.1-8: Summary of components in grape leaf samples after treatment with ^{14}C-BAS 500 F

Metabolite code (Reg. No. of reference substance)	Structure
BAS 500 F (304428)	
500M07 (BF 500-3) (340266)	
500M54 (not assigned)	
500M55 (not assigned)	
500M56 (not assigned)	
500M04 (BF 500-5) (not assigned)	 500M04
500M71 (not assigned)	 500M71or isomer

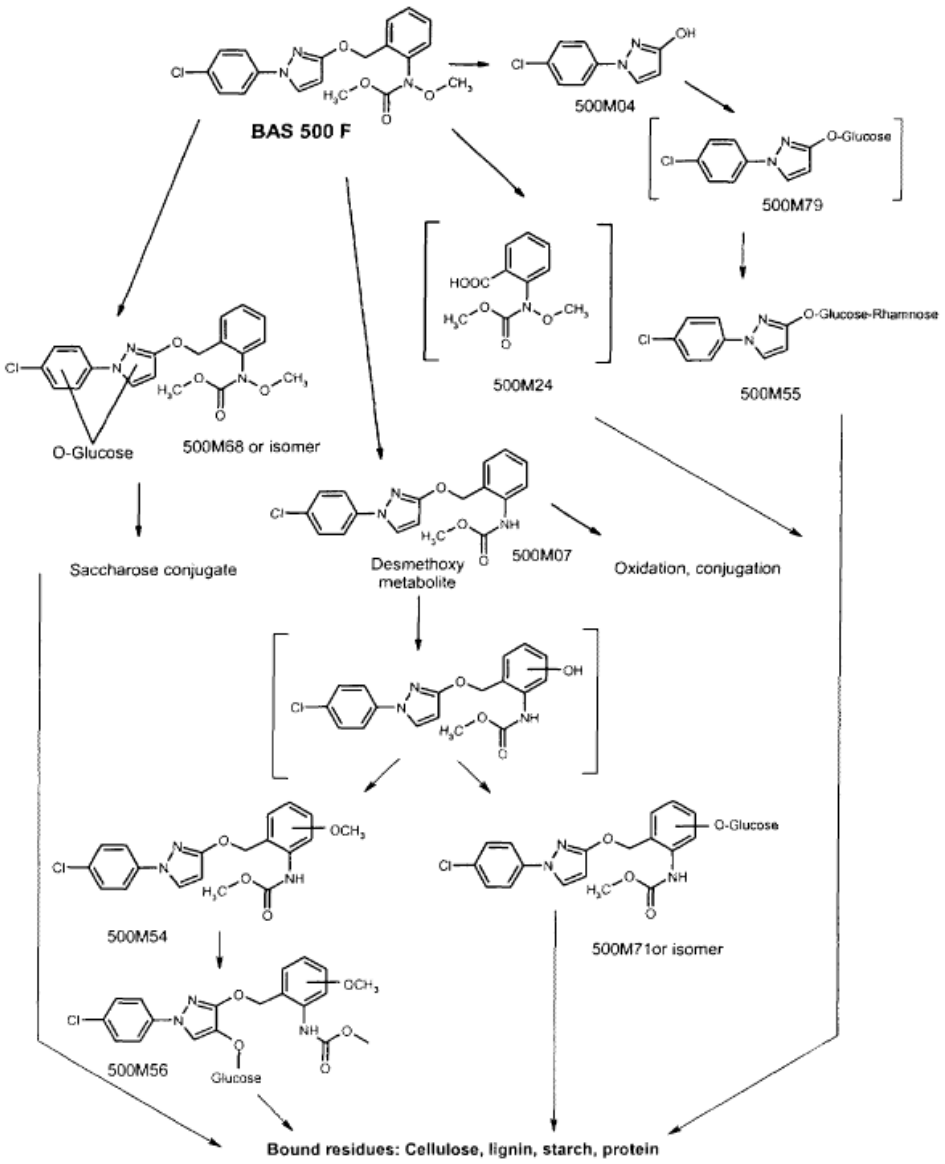
3. Proposed metabolic pathway

The metabolic pathway, presented in Figure 6.2.1-3, shows that the metabolization takes places on two sites of the molecule. One is the tolyl-moiety, were a desmethoxylation takes place in the side chain and a methoxylation in the aromatic ring. At the second site, the chlorophenyl-pyrazol, a glucosidation after hydroxylation takes place. Later, this molecule moiety is cleaved from the tolyl-moiety. In the next step a glycosidation by scillabiose (disaccharide of glucose and rhamnose) is performed. Only smaller amounts of the radioactivity could be found in the bound residues (RRR). They could be assigned to the biopolymers lignin and cellulose.

In grapes, ^{14}C- pyraclostrobin is metabolized by three key transformation steps:

(1) desmethoxylation at the oxime ether bond,
(2) hydroxylation of the tolyl and the chlorophenyl ring systems followed by glucosylation or methylation, and
(3) cleavage between both ring systems and subsequent transformation of the resulting intermediates by glucosylation.

Figure 6.2.1-3: Metabolic pathway of BAS 500 F in grapes



4. Storage stability

In order to examine the storage stability, samples were triple extracted with methanol at the beginning of the metabolism work and at the end of the study. The methanol extracts were analyzed by Radio-HPLC, and both resulting metabolic patterns compared with each other.

An estimate of the storage stability of the metabolite pattern was carried out with MeOH extracts of grapes treated with both ^{14}C-labels of pyraclostrobin and stored at about -20°C until further work up. The MeOH extracts from the first and the last extraction were compared by radio-HPLC.
At the end of the study, 97 days after the 1st extraction, the chromatogram from the tolyl-label corresponds considerably with that of the 1st extraction. Only a slight change from 74.1% TRR to 73.5% TRR for pyraclostrobin and from 19.5% to 20.7% for the metabolite 500M07 (BF 500-3) could be observed. Grapes which were stored at -20°C for 188 days were extracted three times with MeOH. By HPLC analysis, the resulting extract was compared with the 1st extract, which had been stored for the same time at -20°C. The results showed that in the MeOH extract pyraclostrobin slightly decreased when compared to the active substance content in the stored grapes (73% TRR to 70% TRR). The concentration of the metabolite 500M07 slightly increased from 17 to 19% TRR in the MeOH extract within the same time interval.

These results indicate that the residues in grapes, treated with pyraclostrobin were stable under the chosen experimental conditions.

III. CONCLUSION

The investigation of the metabolism of pyraclostrobin in grapes using material labelled either in the tolyl or the chlorophenyl ring leads to the conclusion that the predominant residue in grapes consists of the parent compound pyraclostrobin and its desmethoxy metabolite 500M07 (BF 500-3). Some other compounds were identified as products formed by cleavage of the molecule. The cleavage reaction resulting in the metabolites 500M04 (BF 500-5) and 500M55 is more expressed in leaves than in fruits. The metabolization by O-glucosylation or methoxylation is a minor degradation pathway; the metabolites 500M54, 500M56 and 500M71 are present in amounts clearly below 10% of the TRR.

CA 6.2.1.2 Chinese cabbage (category: leafy crops)

Report: CA 6.2.1/4
Sato K., 2000a
Metabolic fate of BAS 500 F in Chinese cabbage
2000/1018512
Guidelines: JMAFF
GLP: yes
(certified by Japan Ministry of Agriculture, Forestry and Fisheries, Japan)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: chlorophenyl-U-C14-BAS 500 F, tolyl-U-C14-BAS 500 F, pyrazole-3-C13-BAS 500 F

Description:	carbon-14 chlorophenyl ring labelled (5.06 MBq/mg) carbon-14 tolyl ring labelled (6.79 MBq/mg)
Lot/Batch #:	^{14}C-BAS 500 F (chlorophenyl): 579-2012 ^{14}C-BAS 500 F (tolyl): 566-3016
Purity:	^{14}C-BAS 500 F (chlorophenyl): >99% (radiochemical), >98% (chemical) ^{14}C-BAS 500 F (tolyl): >99% (radiochemical), >97% (chemical)
CAS#:	175013-18-0
Stability of test compound:	not reported

2. Test Commodity: Leafy brassica

Crop:	Chinese cabbage
Type:	not reported
Variety:	Shin-Kyoto No. 3
Botanical name:	*Brassica campestris L.*
Crop parts(s) or processed Commodity:	outer leaves, leaf balls (edible portion)
Sample size:	not reported

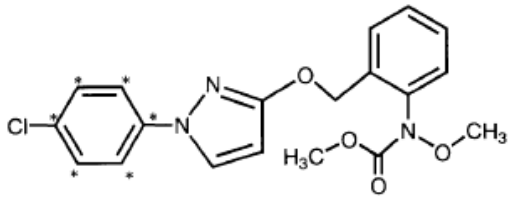
3. Soil: Tachikawa soil

B. STUDY DESIGN AND METHODS

1. Test procedure

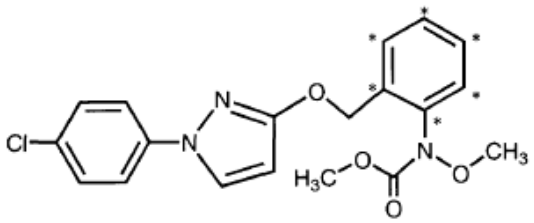
Two different ^{14}C-radiolabeled test items of pyraclostrobin uniformly labelled at either chlorophenyl ring (C-ring label) or tolyl ring (T-ring label) were formulated as 18.8% DF, and then diluted 3000 times with water to prepare a spray solution of each label. Three potted Chinese cabbage plants were treated three times with either radiolabeled test substance by a spray application on the day of 17, 10 and 3 days before mature harvest at a nominal rate of ca. 0.130 kg as/ha. Three days after the final application, the treated Chinese cabbage plants were harvested and separated to a leaf-ball (as edible portion) and outer leaves.

Figure 6.2.1-4: Structural formula of ^{14}C-BAS 500 F labelled at the chlorophenyl ring (C-ring)



* Labeling position

Figure 6.2.1-5: Structural formula of ^{14}C-BAS 500 F labelled at the tolyl ring (T-ring)



* Labeling position

The cabbage plants were grown in pots filled with Tachikawa soil to which small amounts of calcium hydroxide and commercially available fertilizer (N:P:K = 8:8:8) were added. Physical and chemical properties of Tachikawa soil are given in Table 6.2.1-9.

Table 6.2.1-9: Physical and chemical properties of Tachikawa soil

Information from Agricultural Experimental Station of Tokyo Megalopolis	
Type	Volcanic ash
Dominant clay mineral	Allophane
Classified by ISSA method	
Texture	CL
Soil parameters	
Clay content [%]	18.2
pH (H_2O)	5.7
pH (KCl)	5.2
Organic carbon content [%]	5.58
CEC [meq/100g dry soil]	34.7

2. Description of analytical procedures

Leaf-ball and outer leaves samples of both labels were mainly extracted with methanol. The extractable radioactivity was characterized and quantified by radio HPLC. In addition, liquid/liquid partitioning experiments using benzene and methanol were carried out. The metabolites were identified by comparison with reference substances. Where possible, they were isolated by HPLC and their structures elucidated by LC-MS/MS.

The residual radioactive residue (RRR) after extraction was air-dried at ambient temperature (ca. 25°C) for more than 1 day and then weighed. Triplicate aliquots of the dried residues were then subjected to oxidative combustion.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The outer leaves or the leaf ball of Chinese cabbage was pulverized and then the TRR in each sample was determined by oxidative combustion. The results are reported in Table 6.2.1-10. The TRR levels of chlorophenyl ring label in the outer leaves and the leaf ball were 2.75 and 1.12 mg/kg, respectively. The TRR levels of tolyl ring label in the outer leaves and the leaf ball were 3.72 and 1.20 mg/kg, respectively. There was no significant difference for the TRR levels in plant samples between both labels. The distributions of radioactive residues among the outer leaves and the leaf-ball of Chinese cabbage plants were approximately 60% and 40%, respectively. There was also no significant difference for the distributions of radioactive residues between the labels indicating that cleavage of the molecule is not an important degradation pathway.

Table 6.2.1-10: Total radioactive residues (TRR) after treatment of Chinese cabbage with ^{14}C-BAS 500 F

Total radioactive residues (TRR) [mg/kg]		
Label position	**Chlorophenyl label**	**Tolyl label**
Outer leaves*	2.7484	3.7219
Leaf-ball*	1.1161	1.2013

* determined by direct combustion analysis

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

1. Extraction of residues

In outer leaves and leaf ball samples, the solvent extractability was high; it ranged from 89 to 109% of the TRR. In outer leaves, no difference between the extractabilities of the two labels could be observed with values of 106% (chlorophenyl label) and 109% (tolyl label). In leaf ball samples, a slight difference between the extractabilities of the two labels could be observed. In the case of the tolyl label, 109% of the TRR could be extracted whereas the extractable portion from the leaf ball samples treated with the chlorophenyl label was lower (89%).
Residues after solvent extraction were less than 5% of the TRR, indicating that there was no appreciable formation of unextractable bound residues.

The extraction behaviour is summarized in Table 6.2.1-11.

Table 6.2.1-11: Extraction of radioactive residues after treatment of Chinese cabbage with ^{14}C-BAS 500 F

Extraction of radioactivity [mg/kg] (% TRR)		
Solvent	**Outer leaves**	**Leaf ball**
Tolyl label		
ERR	4.0166 **(109.40)**	1.2911 **(108.95)**
RRR	0.1551 **(4.22)**	0.0414 **(3.49)**
TRR	4.1716 **(113.63)**	1.3324 **(112.44)**
Chlorophenyl label		
ERR	2.9260 **(106.46)**	0.9889 **(88.60)**
RRR	0.0978 **(3.56)**	0.0284 **(2.54)**
TRR	3.0238 **(110.02)**	1.0172 **(91.14)**

In order to classify the metabolites into organo-soluble and water-soluble ones, solid phase extraction (SPE) experiments were carried out. In the case of the samples treated with the chlorophenyl and tolyl label most of the radioactivity was found in the benzene eluate (81.5% - 102%). The polar components in the methanol eluate amounted for only 6.0-8.8% of the TRR. The amounts of highly polar fractions eluted in aqueous eluate were negligible (less than 0.5% of the TRR for all samples).
For both labelled samples, the behavior of outer leaves and leaf ball was very similar. The solid phase extraction behavior is summarized in Table 6.2.1-12.

Table 6.2.1-12: Solid phase extraction of radioactive residues in extract after treatment of Chinese cabbage with ^{14}C-BAS 500 F

Partition of radioactivity [mg/kg] (% TRR)		
Solvent	**Outer leaves**	**Leaf ball**
Tolyl label		
Initial ^{14}C in extract	4.0166 **(109.40)**	1.2911 **(108.95)**
Aqueous eluate	0.0116 **(0.32)**	0.0058 **(0.49)**
Benzene eluate	3.7343 **(101.71)**	1.1831 **(99.84)**
Methanol eluate	0.3128 **(8.52)**	0.0785 **(6.63)**
Total recovery	4.0587 **(110.55)**	1.2674 **(106.95)**
Chlorophenyl label		
Initial ^{14}C in extract	2.9260 **(106.46)**	0.9889 **(88.60)**
Aqueous eluate	0.0046 **(0.17)**	0.0030 **(0.27)**
Benzene eluate	2.6982 **(98.17)**	0.9097 **(81.51)**
Methanol eluate	0.2421 **(8.81)**	0.0672 **(6.02)**
Total recovery	2.9448 **(107.15)**	0.9798 **(87.79)**

2. Identification, characterization and quantitation of extractable residues

Unchanged pyraclostrobin was the principal radioactive component in both outer leaves and the leaf-ball. This compound accounted for 82.9% (2.28 mg/kg) and 82.5% (3.03 mg/kg) of the TRR in outer leaves of C-ring label and T-ring label treated plants, respectively. For the leaf-ball, BAS 500 F represented 74.2% (0.83 mg/kg) and 85.1% (1.01 mg/kg) of the TRR in the C-ring label and the T-ring label treated plants, respectively. The principal metabolite was found to be 500M07 (BF 500-3). The residue levels of this metabolite in the outer leaves of the C-ring label and the T-ring label treated plants was 8.5% (0.23 mg/kg) and 11.9% (0.44 mg/kg), respectively. The metabolite also accounted for 5.6% (0.06 mg/kg) and 10.6% (0.13 mg/kg) of the TRR in the leaf-ball of the C-ring label and the T-ring label treated plants, respectively.
In addition to metabolite 500M07, several minor radioactive metabolites were found in the extracts of the C-ring label and/or the T-ring label treated plants. None of these metabolites, however, matched with available reference standards by HPLC and TLC co-chromatography. Two minor metabolites were found to contain only C-ring label, whereas other several minor metabolites contained both C-ring moiety and T-ring moiety. The residue levels of these minor metabolites were extremely low. For example, the most abundant minor metabolite, Unknown CT1, accounted for only 2.6% (0.07 mg/kg) and 3.7% (0.13 mg/kg) of the TRR in the outer leaves of the C-ring label and the T-ring label treated plants, respectively. For the leaf-ball, this metabolite represented 1.6% (0.02 mg/kg) and 1.7% (0.02 mg/kg) of the TRR in the C-ring label and the T-ring label treated plants, respectively.

The quantification of the individual metabolites present in the different plant matrices is summarized in Table 6.2.1-13 (^{14}C-tolyl-BAS 500 F) and Table 6.2.1-14 (^{14}C-chlorophenyl-BAS 500 F).

Table 6.2.1-13: Summary of identified components in Chinese cabbage samples after treatment with ^{14}C-tolyl-BAS 500 F

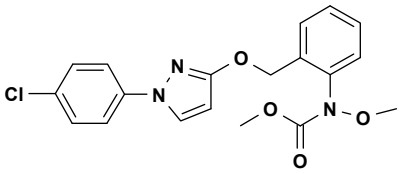
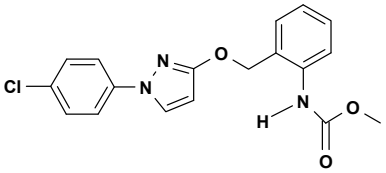
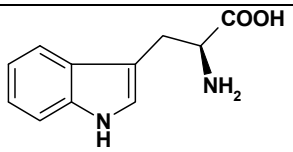
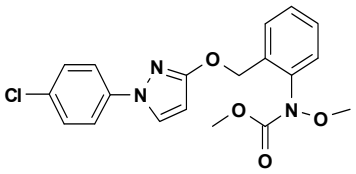
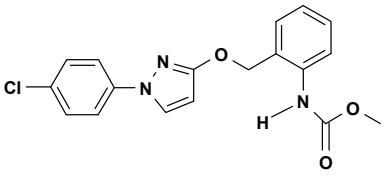
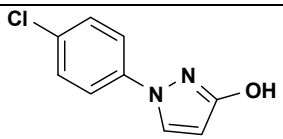
Metabolite code (Reg. No. of reference substance)	Metabolite identity	Outer leaves	Leaf ball
		mg/kg (% TRR)	mg/kg (% TRR)
BAS 500 F (304428)		3.0279 **(82.47)**	1.0086 **(85.11)**
500M07 (BF 500-3) (340266)		0.4355 **(11.86)**	0.1254 **(10.58)**
Unknown CT1	-	0.1339 **(3.65)**	0.0203 **(1.71)**
500M72 (not assigned)		0.0076 **(0.21)**	<0.0018 **(<0.16)**

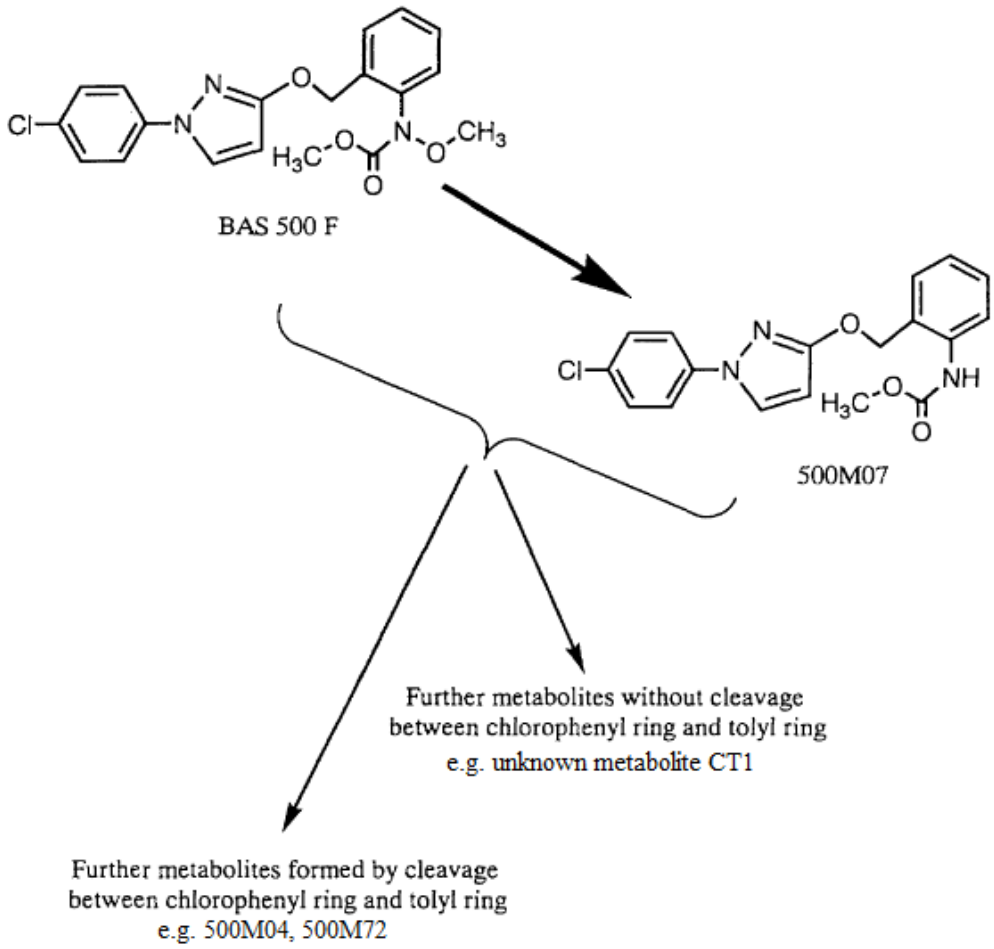
Table 6.2.1-14: Summary of identified components in Chinese cabbage samples after treatment with ^{14}C-chlorophenyl-BAS 500 F

Metabolite Code (Reg. No. of reference substance)	Metabolite identity	Outer leaves	Leaf ball
		mg/kg (% TRR)	mg/kg (% TRR)
BAS 500 F (304428)		2.2789 **(82.92)**	0.8281 **(74.20)**
500M07 (340266)		0.2335 **(8.49)**	0.0624 **(5.59)**
Unknown CT1	-	0.0708 **(2.58)**	0.0173 **(1.55)**
Unknown C2	-	0.0574 **(2.09)**	0.0119 **(1.07)**
Unknown C1	-	0.0540 **(1.96)**	0.0078 **(0.070)**
500M04 (298327)		0.0440 **(1.60)**	0.0077 **(0.69)**

3. Proposed metabolic pathway

In Chinese cabbage, pyraclostrobin is metabolized by desmethoxylation which resulted in the formation of the metabolite 500M07 (BF 500-3). Cleavage of the molecule resulting in the metabolites 500M04 (BF 500-5) and 500M72 (tryptophan) is occurring to a minor extent. Besides these reactions, metabolites being considerably more polar than parent / 500M07 were found in both labels, but in low amounts (clearly below 5% TRR). No incorporation into bound residues was observed.

Figure 6.2.1-6: Proposed metabolic pathway of BAS 500 F in Chinese cabbage



III. CONCLUSION

The proposed metabolic pathway of pyraclostrobin in Chinese cabbage is presented in Figure 6.2.1-6**. The investigation of the metabolism of BAS 500 F in Chinese cabbage using material labeled in either the tolyl or the chlorophenyl ring leads to the conclusion that the predominant residue in outer leaves and leaf ball consists of the parent compound pyraclostrobin and its metabolite 500M07. Some other components which were identified as products formed by cleavage of the molecule, O-glucosylation or methoxylation turned out to be of minor importance, because their respective amount is far below 10% of the TRR (amounts ranged between <0.16 and 3.65% of the TRR).**

CA 6.2.1.3 Paddy rice

Report:	CA 6.2.1/5 Rabe U.,Kloeppner U., 2014a Metabolism of 14C-Pyraclostrobin in rice 2013/1134958
Guidelines:	EPA 860.1000, EPA 860.1300: Nature of the Residue in Plants Livestock, PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada), Lundehn III: 7028/VI/95 rev. 3 Appendix A (EU) Metabolism and distribution in plants (draft), JMAFF 59 NohSan No 4200, OECD 501 - Metabolism in crops (adopted January 8 2007)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: chlorophenyl-U-C14-BAS 500 F, tolyl-U-C14-BAS 500 F, pyrazole-3-C13-BAS 500 F

Description:	carbon-14 chlorophenyl ring labelled (6.57 MBq/mg) carbon-14 tolyl ring labelled (7.32 MBq/mg) carbon-13 pyrazole labelled pyraclostrobin
Lot/Batch #:	^{14}C-BAS 500 F (chlorophenyl): 579-6103 ^{14}C-BAS 500 F (tolyl): 566-4201 ^{13}C-BAS 500 F (pyrazole): 1026-1018
Purity:	^{14}C-BAS 500 F (chlorophenyl): 98.3% (radiochemical), 97.6% (chemical) ^{14}C-BAS 500 F (tolyl): 99.4% (radiochemical), 97.8% (chemical) ^{13}C-BAS 500 F (pyrazole): 99.8% (chemical)
CAS#:	175013-18-0
Stability of test compound:	The test item was stable for at least 11 month

2. Test Commodity: Cereals

Crop:	Rice
Type:	Paddy rice
Variety:	Thaibonnet
Botanical name:	*Oryza sativa L.*
Crop parts(s) or processed Commodity:	Forage, spelts & straw (combined), grain
Sample size:	Forage: 673.1 g (chlorophenyl) and 792.4 g (tolyl) Straw: 1675.7 g (chlorophenyl) and 1576.0 g (tolyl) Grain: 945.4 g (chlorophenyl) and 974.3 g (tolyl)

3. Soil: A sandy loam soil was used. The soil physicochemical properties are described below (see Table 6.2.1-15).

B. STUDY DESIGN AND METHODS

1. Test procedure

Two different application solutions were used for the investigations. For the chlorophenyl label chlorophenyl-U-C14-labelled and pyrazole-3-C13-labelled test item were mixed in a ratio of 2:1. The tolyl label comprised a mixture of tolyl-ring-U-C14-labelled, pyrazole-3-C13-labelled and unlabelled test item in a ratio of 50:33:17 (14C:13C:12C).

Figure 6.2.1-7: Structural formula of ^{14}C-BAS 500 F labelled at the chlorophenyl ring

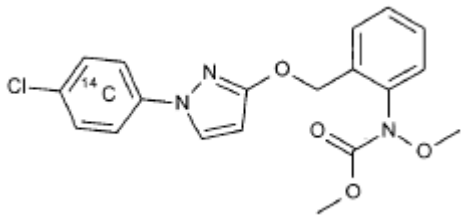


Figure 6.2.1-8: Structural formula of ^{14}C-BAS 500 F labelled at the tolyl ring

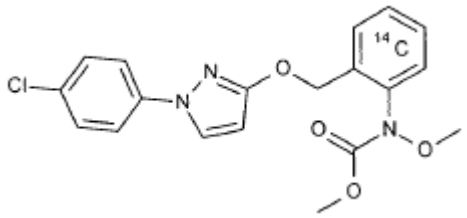


Figure 6.2.1-9: Structural formula of pyrazole-3-C13-labelled pyraclostrobin

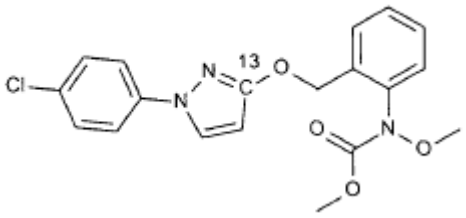
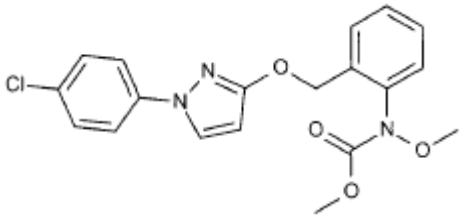


Figure 6.2.1-10: Structural formula of unlabelled pyraclostrobin



For each test item mixture, two foliar spray applications were performed with BAS 500 GG F (CS formulation), each with a nominal application rate of 100 g a.s./ha. The first application was carried out at growth stage BBCH 39, the second at BBCH 69. Forage samples of both labels were taken one day before the second application. Straw, grain and spelts were sampled from mature rice plants at BBCH 89. The spelts were combined with straw.
All samples were stored at -18°C or below until analysis. Subsamples of rice straw and grain were extracted with methanol and water 69 - 71 days after sampling, forage samples were extracted 126 / 127 days after sampling.

The rice was sown into two multicells filled with sandy loam soil. Physical and chemical properties of sandy loam soil are given in Table 6.2.1-15.

Table 6.2.1-15: Soil characteristics: soil used for sowing

USDA classification	
clay	9.3%
silt	20.6%
sand	70.1%
soil class	sandy loam
DIN classification	
clay	9.3%
silt	23.9%
sand	66.8%
soil class	loamy sand (SL3)
Other soil parameters	
total nitrogen	0.14%
total organic carbon	1.40%
total carbon	3.57%
pH ($CaCl_2$)	7.2
pH (H_2O)	7.9
effective cation exchange capacity	11.8 cmol/kg
max. water holding capacity	27.7 g/100 g dry soil
microbial biomass	38.7 mg C/100 g dry soil
microbial C / organic C	2.8%
bulk density	1269 g/L
dry matter	91.9%

2. Description of analytical procedures

All samples (forage, straw and grain) were homogenized with a knife mill along with dry ice. After sublimation of the dry ice, the samples were weighed, mixed, divided into aliquots and radioassayed.
The total radioactive residues (TRR) were determined by direct combustion analysis of small aliquots of homogenized sample material. The sample material was combusted by means of an automatic sample oxidizer. The $^{14}CO_2$ was trapped by an absorption and scintillation liquid, and the collected radioactivity was measured by liquid scintillation counting.
Aliquots of liquid samples were mixed with a sufficient volume of a suitable scintillator prior to measurement.

The homogenized samples (rice forage, straw and grain) were extracted with methanol and water. *Methanol extracts* were concentrated and investigated by radio HPLC. The column used was a Phenomenex Synergi Polar RP, the eluent system consisted of 2 mobile phases (A: water/acetonitrile/formic acid, 950/50/2.5, v/v/v & B: water/acetonitrile/formic acid, 50/950/2.5, v/v/v) which were used applying gradient elution. *Water extracts* were concentrated, centrifuged and also investigated by HPLC.

The residual radioactive residue (RRR) after solvent extraction was extracted with ammonia and successively solubilized with mixtures of macerozyme / cellulase and tyrosinase / laccase and for grain also with amylase / amyloglucosidase. Residues in solubilizates of the straw specimens (both labels) were concentrated, if necessary centrifuged and analyzed by HPLC using method LC05. Due to the low residue levels, the solubilizates of the grain and forage specimens (both labels) were not analyzed by HPLC.

In case of the tolyl label, after extraction with cyclohexane, some further purification steps were applied using acetonitrile. Finally, the sample was resuspended in water/acetonitrile and subsequently fractionated by HPLC. The fractions were concentrated and analyzed by HPLC-MS.

In case of the chlorophenyl label, after extraction with cyclohexane, some further purification steps were applied using SPE (MegaBondElut, C18) and an eluent system consisting of water and methanol with increasing concentration of methanol. Finally, the obtained sample was fractionated. The fractions were concentrated and analyzed by HPLC-MS.

The active substance pyraclostrobin (BAS 500 F) and its desmethoxy metabolite 500M07 were identified by HPLC-MS analysis of purified fractions from rice grain methanol extracts (both labels). Peak assignment in forage, straw and grain methanol extracts was additionally supported by HPLC co-chromatography experiments and retention time comparison with reference items of BAS 500 F and 500M07 or well-characterized extracts.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Aliquots of the homogenized samples were successively extracted with methanol and water. The Total Radioactive Residues (TRR) was determined by summarizing the Extractable Radioactive Residues (ERR) and the Residual Radioactive Residues (RRR) after solvent extraction. The total radioactive residues in forage sampled 1 day before the second treatment accounted for 1.924 mg/kg and 1.622 mg/kg (chlorophenyl label and tolyl label, respectively). The highest radioactive residues were found in rice straw samples accounting for 8.564 mg/kg (chlorophenyl label) and 10.503 mg/kg (tolyl label). In rice grain, lower residue levels were found for both labels (chlorophenyl label: 1.948 mg/kg, tolyl label: 2.112 mg/kg).

Additionally, the TRR was measured by direct combustion analysis of homogenized sample material. The measured TRR showed no major differences to the calculated TRR values. Slightly larger deviations found for rice straw of the chlorphenyl label may be caused by inhomogeneity of the sample material and the low amounts used for combustion.

The levels of total radioactive residues (TRR) of pyraclostrobin found in rice forage, straw and grain are reported in Table 6.2.1-16.

Table 6.2.1-16: Total radioactive residues in rice samples

Rice matrix	Sampling Interval (DAT[1])	TRR determined by direct combustion [mg/kg]	TRR calculated[2] [mg/kg]
Chlorophenyl Label			
Forage[3]	-1	2.021	1.921
Straw	57	7.271	8.564
Grain	57	2.075	1.948
Tolyl Label			
Forage[3]	-1	1.557	1.622
Straw	57	10.776	10.503
Grain	57	2.031	2.112

[1] DAT = Days After Treatment
[2] TRR was calculated as the sum of ERR + RRR
[3] Forage was sampled one day before the second application, thus the samples of forage had received only one application

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

1. Extraction of residues

For the investigations of metabolic patterns, subsamples of forage, straw and grain were extracted with methanol and water. The extractability with methanol and water was high in rice forage (chlorophenyl label: 86.5% TRR, tolyl label: 83.5% TRR). For rice straw, the extractability was lower with 68.5% TRR (chlorophenyl label) and 64.8% TRR (tolyl label). From rice grain 75.6% TRR (chlorophenyl label) and 71.4% TRR (tolyl label) were extracted by solvent extraction. The major part of the radioactivity was extracted with methanol, while water released only minor portions (1.9 – 7.8% TRR) from rice forage, straw and grain.

Table 6.2.1-17: Extractability of radioactive residues in rice samples

Matrix	TRR Calculated [1] mg/kg	Combined Methanol Extract		Combined Water Extract		ERR [2]		RRR [3]	
		mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
Chlorophenyl Label									
Forage [4]	1.921	1.604	83.5	0.058	3.0	1.662	86.5	0.259	13.5
Straw	8.564	5.193	60.6	0.672	7.8	5.865	68.5	2.699	31.5
Grain	1.948	1.436	73.7	0.037	1.9	1.473	75.6	0.476	24.4
Tolyl Label									
Forage [4]	1.622	1.317	81.2	0.037	2.3	1.354	83.5	0.268	16.5
Straw	10.503	6.038	57.5	0.762	7.3	6.801	64.8	3.702	35.2
Grain	2.112	1.435	68.0	0.073	3.4	1.508	71.4	0.604	28.6

[1] TRR was calculated as the sum of ERR + RRR (ERR and RRR see below)
[2] ERR = Extractable Radioactive Residue (extraction with methanol and water)
[3] RRR = Residual Radioactive Residue (after solvent extraction with methanol and water)
[4] Forage was sampled one day before the second application, thus the samples of forage had received only one application

2. Identification, characterization and quantitation of extractable residues

The active substance pyraclostrobin (BAS 500 F) and its desmethoxy metabolite 500M07 (BF 500-3) were identified by HPLC-MS, co-chromatography and retention time comparison.
The results of the analysis of rice forage, straw and grain are summarized in Table 6.2.1-18 to Table 6.2.1-23.

TRR in rice forage (chlorophenyl label) accounted for 1.921 mg/kg, whereby pyraclostrobin and metabolite 500M07 were identified in ERR with 1.165 mg/kg (60.7% TRR) and 0.312 mg/kg (16.3% TRR), respectively. In total, 1.960 mg/kg were identified and characterized (ERR and RRR, including the final residue), representing 102.0% of TRR.
Further components in the methanol and water extracts were characterized by their chromatographic properties which each single peak less or equal to 5.9% TRR. The solubilization steps released altogether further portions of 4.9% from forage.

Table 6.2.1-18: Summary of identified and characterized residues of rice forage (chlorophenyl label)

Designation	Solvent Extracts				Sum of Extracts	
	Methanol Extract[1)]		Water Extract[2)]			
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Total Radioactive Residues in Rice Forage					***1.921***	***100.0***
Identified from ERR						
BAS 500 F	1.165	60.7	n.d.	n.d.	1.165	60.7
500M07	0.312	16.3	n.d.	n.d.	0.312	16.3
Total Identified from ERR					**1.478**	**76.9**
Characterized from ERR						
Up to 16 peaks characterized (below or equal to 0.112 mg/kg or 5.9% TRR)	0.230	12.0	0.043	2.2	0.273	14.2
Total Characterized by HPLC from ERR					**0.273**	**14.2**
Residue after Solvent Extraction / RRR					*0.259*	*13.5*
Ammonia Solubilizate 1					0.038	2.0
Ammonia Solubilizate 2					0.021	1.1
Macerozyme / Cellulase Solubilizate					0.023	1.2
Tyrosinase / Laccase Solubilizate					0.013	0.7
Total Characterized (ERR and RRR)					**0.368**	**19.2**
Total Identified and Characterized (ERR and RRR)					**1.846**	**96.1**
Final Residue					**0.114**	**5.9**
Total Identified and Characterized (ERR and RRR) + Final Residue					**1.960**	**102.0**

[1)] HPLC run LC05
[2)] HPLC run LC07
n.d. not detected

TRR in rice forage (tolyl label) accounted for 1.622 mg/kg, whereby pyraclostrobin and metabolite 500M07 were identified in ERR with 1.189 mg/kg (73.3% TRR) and 0.140 mg/kg (8.6% TRR), respectively. In total, 1.648 mg/kg were identified and characterized (ERR and RRR, including the final residue), representing 101.6% of TRR.
Further components in the methanol and water extracts were characterized by their chromatographic properties which each single peak less or equal to 1.7% TRR. The solubilization steps released altogether further portions of 6.7% from forage.

Table 6.2.1-19: Summary of identified and characterized residues of rice forage (tolyl label)

Designation	Solvent Extracts				Sum of Extracts	
	Methanol Extract[1)]		Water Extract[2)]			
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Total Radioactive Residues in Rice Forage					***1.622***	***100.0***
Identified from ERR						
BAS 500 F	1.188	73.3	0.001	< 0.1	1.189	73.3
500M07	0.140	8.6	n.d.	n.d.	0.140	8.6
Total Identified from ERR					**1.329**	**82.0**
Characterized from ERR						
Up to 14 peaks characterized (below or equal to 0.028 mg/kg or 1.7% TRR)	0.049	3.0	0.027	1.7	0.076	4.7
Total Characterized by HPLC from ERR					**0.076**	**4.7**
Residue after Solvent Extraction / RRR					*0.268*	*16.5*
Ammonia Solubilizate 1					0.027	1.7
Ammonia Solubilizate 2					0.019	1.2
Macerozyme / Cellulase Solubilizate					0.031	1.9
Tyrosinase / Laccase Solubilizate					0.032	1.9
Total Characterized from ERR and RRR					**0.185**	**11.4**
Total Identified and Characterized from ERR and RRR					**1.514**	**93.4**
Final Residue					**0.134**	**8.3**
Total Identified and Characterized (ERR and RRR) + Final Residue					**1.648**	**101.6**

[1)] HPLC run LC05
[2)] HPLC run LC07
n.d. not detected

TRR in rice straw (chlorophenyl label) accounted for 8.564 mg/kg, whereby pyraclostrobin and metabolite 500M07 were identified in ERR with 3.794 mg/kg (44.3% TRR) and 1.477 mg/kg (17.3% TRR), respectively. In total, 8.067 mg/kg were identified and characterized (ERR and RRR, including the final residue), representing 94.2% of TRR.
Further components in the water extracts were characterized by their chromatographic properties which each single peak less or equal to 2.8% TRR.
The solubilization steps released altogether further portions of 14.9% from straw.
HPLC analyses of the ammonia and enzyme solubilizates from the RRR of straw revealed similar to the water extracts, several components eluting in the range between 15 min and 30 min (both labels, HPLC method LC05). These components were characterized by their elution behavior (each below or equal to 2.8% TRR).

Table 6.2.1-20: Summary of identified and characterized residues of rice straw (chlorophenyl label)

Designation	Solvent Extracts				Sum of Extracts	
	Methanol Extract[1)]		Water Extract[2)]			
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Total Radioactive Residues in Rice Straw					***8.564***	***100.0***
Identified from ERR						
BAS 500 F	3.794	44.3	n.d.	n.d.	3.794	44.3
500M07	1.477	17.3	n.d.	n.d.	1.477	17.3
Total Identified from ERR					**5.271**	**61.5**
Characterized from ERR						
Up to 8 peaks characterized (below or equal to 0.239 mg/kg or 2.8% TRR)	n.d.	n.d.	0.470	5.5	0.470	5.5
Total Characterized by HPLC from ERR					**0.470**	**5.5**
Residue after Solvent Extraction / RRR					*2.699*	*31.5*
Ammonia Solubilizate 1 [3)]					0.569	6.6
Ammonia Solubilizate 2 [3)]					0.324	3.8
Macerozyme / Cellulase Solubilizate [4)]					0.249	2.9
Tyrosinase / Laccase Solubilizate [5)]					0.138	1.6
Total Characterized from ERR and RRR					**1.750**	**20.4**
Total Identified and Characterized from ERR and RRR					**7.021**	**82.0**
Final Residue					**1.046**	**12.2**
Total Identified and Characterized (ERR and RRR) + Final Residue					**8.067**	**94.2**

[1)] HPLC run LC05
[2)] HPLC run LC07
[3)] ammonia solubilizates 1 and 2 were pooled, concentrated and HPLC analyzed (LC05)
[4)] HPLC run of the concentrated macerozyme / cellulase solubilizate (LC05)
[5)] HPLC run of the concentrated and centrifuged tyrosinase / laccase solubilizate (LC05)
n.d. not detected

TRR in rice straw (tolyl label) accounted for 10.503 mg/kg, whereby pyraclostrobin and metabolite 500M07 were identified in ERR with 4.356 mg/kg (41.5% TRR) and 0.839 mg/kg (8.0% TRR), respectively. In total, 10.138 mg/kg were identified and characterized (ERR and RRR, including the final residue), representing 96.5% of TRR.
Further components in the methanol and water extracts were characterized by their chromatographic properties which each single peak less or equal to 8.0% TRR.
The solubilization steps released altogether further portions of 16.7% from straw.
HPLC analyses of the ammonia and enzyme solubilizates from the RRR of straw revealed similar to the water extracts, several components eluting in the range between 15 min and 30 min (both labels, HPLC method LC05). These components were characterized by their elution behavior (each below or equal to 0.8% TRR).

Table 6.2.1-21: Summary of identified and characterized residues of rice straw (tolyl label)

Designation	Solvent Extracts				Sum of Extracts	
	Methanol Extract[1)]		Water Extract[2)]			
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Total Radioactive Residues in Rice Straw					***10.503***	***100.0***
Identified from ERR						
BAS 500 F	4.347	41.4	0.009	0.1	4.356	41.5
500M07	0.839	8.0	n.d.	n.d.	0.839	8.0
Total Identified from ERR					**5.196**	**49.5**
Characterized from ERR						
Up to 10 peaks characterized (below or equal to 0.839 mg/kg or 8.0% TRR)	1.074	10.2	0.569	5.4	1.643	15.6
Total Characterized by HPLC from ERR					**1.643**	**15.6**
Residue after Solvent Extraction / RRR					*3.702*	*35.2*
Ammonia Solubilizate 1 [3)]					0.741	7.1
Ammonia Solubilizate 2 [3)]					0.475	4.5
Macerozyme / Cellulase Solubilizate [4)]					0.378	3.6
Tyrosinase / Laccase Solubilizate [5)]					0.162	1.5
Total Characterized from ERR and RRR					**3.399**	**32.4**
Total Identified and Characterized from ERR and RRR					**8.595**	**81.8**
Final Residue					**1.543**	**14.7**
Total Identified and Characterized (ERR and RRR) + Final Residue					**10.138**	**96.5**

[1)] HPLC run LC05
[2)] HPLC run LC07
[3)] ammonia solubilizates 1 and 2 were pooled, concentrated and HPLC analyzed (LC05)
[4)] HPLC run of the concentrated macerozyme / cellulase solubilizate (LC05)
[5)] HPLC run of the concentrated and centrifuged tyrosinase / laccase solubilizate (LC05)
n.d. not detected

TRR in rice grain (chlorophenyl label) accounted for 1.948 mg/kg, whereby pyraclostrobin and metabolite 500M07 were identified in ERR with 1.060 mg/kg (54.4% TRR) and 0.288 mg/kg (14.8% TRR), respectively. In total, 1.854 mg/kg were identified and characterized (ERR and RRR, including the final residue), representing 95.2% of TRR.
Further components in the methanol and water extracts were characterized by their chromatographic properties which each single peak less or equal to 2.0% TRR. The solubilization steps released altogether further portions of 7.5% from grain.

Table 6.2.1-22: Summary of identified and characterized residues of rice grain (chlorophenyl label)

Designation	Solvent Extracts				Sum of Extracts	
	Methanol Extract[1)]		Water Extract[2)]			
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Total Radioactive Residues in Rice Grain					***1.948***	***100.0***
Identified from ERR						
BAS 500 F	1.059	54.3	0.001	0.1	1.060	54.4
500M07	0.288	14.8	n.d.	n.d.	0.288	14.8
Total Identified from ERR					**1.348**	**69.2**
Characterized from ERR						
Up to 16 peaks characterized (below or equal to 0.040 mg/kg or 2.0% TRR)	0.079	4.1	0.021	1.0	0.100	5.1
Total Characterized by HPLC from ERR					**0.100**	**5.1**
Residue after Solvent Extraction / RRR					*0.476*	*24.4*
Ammonia Solubilizate 1					0.047	2.4
Ammonia Solubilizate 2					0.026	1.3
Amylase / Amyloglucosidase Solubilizate					0.029	1.5
Macerozyme / Cellulase Solubilizate					0.033	1.7
Tyrosinase / Laccase Solubilizate					0.011	0.6
Total Characterized from ERR and RRR					**0.245**	**12.6**
Total Identified and Characterized from ERR and RRR					**1.593**	**81.8**
Final Residue					**0.261**	**13.4**
Total Identified and Characterized (ERR and RRR) + Final Residue					**1.854**	**95.2**

[1)] HPLC run LC05
[2)] HPLC run LC07
n.d. not detected

TRR in rice grain (tolyl label) accounted for 2.112 mg/kg, whereby pyraclostrobin and metabolite 500M07 were identified in ERR with 1.091 mg/kg (51.6% TRR) and 0.218 mg/kg (10.3% TRR), respectively. In total, 1.934 mg/kg were identified and characterized (ERR and RRR, including the final residue), representing 91.6% of TRR.
Further components in the methanol and water extracts were characterized by their chromatographic properties which each single peak less or equal to 2.6% TRR. The solubilization steps released altogether further portions of 8.8% from grain.

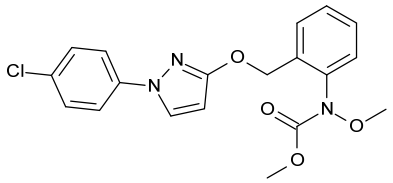
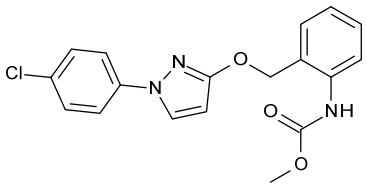
Table 6.2.1-23: Summary of identified and characterized residues of rice grain (tolyl label)

Designation	Solvent Extracts				Sum of Extracts	
	Methanol Extract[1)]		Water Extract[2)]			
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Total Radioactive Residues in Rice Grain					***2.112***	***100.0***
Identified from ERR						
BAS 500 F	1.091	51.6	n.d.	n.d.	1.091	51.6
500M07	0.218	10.3	n.d.	n.d.	0.218	10.3
Total Identified from ERR					**1.309**	**62.0**
Characterized from ERR						
Up to 12 peaks characterized (below or equal to 0.054 mg/kg or 2.6% TRR)	0.054	2.6	0.037	1.7	0.091	4.3
Total Characterized by HPLC from ERR					**0.091**	**4.3**
Residue after Solvent Extraction / RRR					*0.604*	*28.6*
Ammonia Solubilizate 1					0.052	2.5
Ammonia Solubilizate 2					0.028	1.3
Amylase / Amyloglucosidase Solubilizate					0.032	1.5
Macerozyme / Cellulase Solubilizate					0.056	2.7
Tyrosinase / Laccase Solubilizate					0.017	0.8
Total Characterized from ERR and RRR					**0.275**	**13.0**
Total Identified and Characterized from ERR and RRR					**1.584**	**75.0**
Final Residue					**0.350**	**16.6**
Total Identified and Characterized (ERR and RRR) + Final Residue					**1.934**	**91.6**

[1)] HPLC run LC05
[2)] HPLC run LC07
n.d. not detected

A summary of all identified components in rice matrices after foliar treatment with ^{14}C-pyraclostrobin (chlorophenyl label and tolyl label) is given in Table 6.2.1-24.

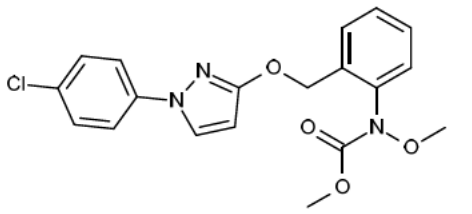
Table 6.2.1-24: Summary of identified components in rice matrices after foliar treatment with ^{14}C-BAS 500 F (chlorphenyl label and tolyl label)

Metabolite	Structure	Forage		Straw		Grain	
		Chloro-phenyl Label mg/kg (% TRR)	Tolyl Label mg/kg (% TRR)	Chloro-phenyl Label mg/kg (% TRR)	Tolyl Label mg/kg (% TRR)	Chloro-phenyl Label mg/kg (% TRR)	Tolyl Label mg/kg (% TRR)
BAS 500 F		1.165 (60.7)	1.189 (73.3)	3.794 (44.3)	4.356 (41.5)	1.060 (54.4)	1.091 (51.6)
500M07 (BF500-3)		0.312 (16.3)	0.140 (8.6)	1.477 (17.3)	0.839 (8.0)	0.288 (14.8)	0.218 (10.3)

3. Proposed metabolic pathway

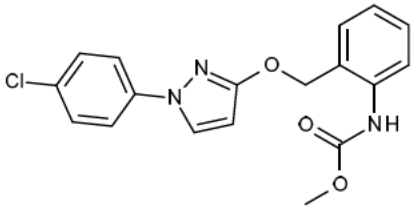
Metabolism of pyraclostrobin in rice occurred mainly by N-desmethoxylation. Unchanged parent compound was the main constituent in all matrices of both labels, representing 41.5 - 73.3% TRR. The N-desmethoxy metabolite 500M07 (BF 500-3) represented the second most abundant component accounting for 8.0% - 17.3% TRR. Some further polar and medium polar components were characterized by their HPLC elution behavior and were all clearly below 10% TRR. Parts of non-extractables found in straw and grain, are partially incorporated in or associated with cellulose, lignin, starch or protein.

Figure 6.2.1-11: Proposed metabolic pathway of BAS 500 F in rice



BAS 500 F

N-desmethoxylation



500M07

Bound Residues:
Cellulose, lignin, starch

4. Extractability of residues according to residue analytical methods

Rice matrices of the chlorophenyl label were extracted following the protocol of BASF residue method 535/1 and QuEChERS method. Compared to the solvent extractions of the metabolism investigations, similar amounts of the parent compound and 500M07 were extracted using BASF residue method 535/1. The concentrations of both metabolites ranged from 83%-105% compared to the values found during metabolism investigations. QuEChERS method extracted somewhat lower amounts of parent compound and 500M07 from forage and straw (46%-92%). From grain it extracted 75% of pyraclostrobin and 114% of metabolite 500M07.

Table 6.2.1-25: Amounts of BAS 500 F and 500M07 extracted from rice samples with different extraction methods (chlorophenyl label)

Matrix	**Forage**			**Straw**			**Grain**		
Extraction Method	Metabolism Investigation	BASF Method 535/1	QuEChERS Method	Metabolism Investigation	BASF Method 535/1	QuEChERS Method	Metabolism Investigation	BASF Method 535/1	QuEChERS Method
Metabolite	[mg/kg]								
BAS 500 F	1.165 (100%)	1.228 (105%)	0.697 (59.8%)	3.794 (100%)	3.331 (87.8%)	1.728 (45.5%)	1.059 (100%)	0.875 (82.6%)	0.799 (75.4%)
500M07	0.312 (100%)	0.319 (102%)	0.287 (92.0%)	1.477 (100%)	1.355 (91.7%)	0.713 (48.3%)	0.288 (100%)	0.285 (99.0%)	0.329 (114%)

5. Storage stability

Storage stability investigations were performed in the rice extracts at the beginning and at the end of the study. For all matrices a reanalysis of stored extracts was performed. Initial analyses of rice forage, straw and grain for quantification were carried out within a maximum of 176 days after sampling for the methanol extracts and within 262 days for the water extracts. The stored extracts were reanalyzed approximately 11 month after extraction. In all cases, the chromatograms obtained from the stored extracts were in very good accordance with the initial analyses.

III. CONCLUSION

The highest levels of total radioactive residues (TRR) were found in rice straw (8.564 mg/kg chlorophenyl label and 10.503 mg/kg tolyl label). In rice grain, lower levels were detected for both labels (1.948 mg/kg chlorophenyl label, 2.112 mg/kg tolyl label). The lowest values were found in rice forage, sampled one day before the second application, amounting to 1.921 mg/kg (chlorophenyl label) and 1.622 mg/kg (tolyl label).

The extractability with methanol and water was high in rice forage (chlorophenyl label: 86.5% TRR, tolyl label: 83.5% TRR). For rice straw, the extractability was lower with 68.5% TRR (chlorophenyl label) and 64.8% TRR (tolyl label). From rice grain 75.6% TRR (chlorophenyl label) and 71.4% TRR (tolyl label) were extracted by solvent extraction. From all matrices the major part of the radioactivity was extracted with methanol.

The nature of the residues was examined by HPLC analysis of the methanol and water extracts. Metabolites were identified by mass spectrometric analysis and/or co-chromatography experiments. The predominant residues of ^{14}C-pyraclostrobin in rice consisted of the unchanged parent compound and its desmethoxy metabolite 500M07 (BF 500-3). In rice forage, straw and grain of both labels pyraclostrobin was the main component identified, ranging from 41.5% - 73.3% TRR. Metabolite 500M07 was found in all matrices as the second most prominent compound (from 8.0% to 17.3% TRR). Some further polar and medium polar components were characterized by their HPLC elution behavior and were all clearly below 10% TRR.

The Residual Radioactive Residue (RRR) after solvent extraction was characterized using a sequential solubilization procedure with aqueous ammonia solution and enzymes that released further portions of 4.9% - 16.7% TRR. The ammonia and enzyme solubilizates of straw (both labels) were analyzed by HPLC. Like in the water extracts several components eluting in the range between 15 min and about 30 min were detected and characterized by their HPLC elution behavior. Higher levels of non-extractables found in straw and grain, are partially incorporated in or associated with cellulose, lignin, starch or protein.

CA 6.2.1.4 Wheat (seed treatment application, category: cereal)

Report:	CA 6.2.1/6 Birk B., Kloeppner U., 2013a Metabolism of 14 C-Pyraclostrobin (14C-BAS 500 F) in wheat after seed treatment 2012/1158148
Guidelines:	EPA 860.1300: Nature of the Residue in Plants Livestock, EPA 860.1000: Background - PMRA Section 97.2 (Canada): Residue Chemistry Guidelines: Plants and Lifestock (June 1997), JMAFF 59 NohSan No 4200, Lundehn III: 7028/VI/95 rev. 3 Appendix A (EU) Metabolism and distribution in plants (draft), OECD 501 - Metabolism in crops (adopted January 8 2007)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: chlorophenyl-U-C14- pyraclostrobin and tolyl-U-C14

Description:	carbon-14 chlorophenyl ring labelled (6.8 MBq/mg) and carbon-14 tolyl ring labelled (7.09 MBq/mg) pyraclostrobin
Lot/Batch #:	^{14}C-BAS 500 F (chlorophenyl): 579-4007 ^{14}C-BAS 500 F (tolyl): 566-4101
Purity:	^{14}C-BAS 500 F (chlorophenyl): 98.6% (radiochemical) ^{14}C-BAS 500 F (tolyl): 99.8% (radiochemical)
CAS#:	175013-18-0
Development code:	BAS 500 F
Stability of test compound	Storage stability investigations were not performed

2. Test Commodity: Cereals

Crop:	Wheat
Type:	Spring wheat
Variety:	WS Thasos 661
Botanical name:	*Triticum aestivum L.*
Crop parts(s) or processed	
Commodity:	Forage, Hay, Straw, Grain
Sample size:	Forage: 500.7 g (chlorophenyl) and 508.8 g (tolyl) Hay: 297.9 g (chlorophenyl) and 260.3 g (tolyl) Straw: 1957.6 g (chlorophenyl) and 2894.7 g (tolyl) Grain: 2917.1 g (chlorophenyl) and 1823.6 g (tolyl)

3. Soil: A sandy loam soil was used. The soil physicochemical properties are described below (see Table 6.2.1-26).

B. STUDY DESIGN AND METHODS

1. Test procedure

Two experiments were performed using either chlorophenyl-^{14}C-labelled or tolyl-^{14}C-labelled pyraclostrobin. The active substance was applied to the seeds using a blank formulation (BAS 662 XI F), for each label at a nominal rate of 1 x 0.010 kg as/ha.

Figure 6.2.1-12: Structural formula of ^{14}C-BAS 500 F labelled at the chlorophenyl ring

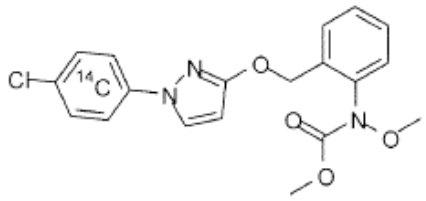
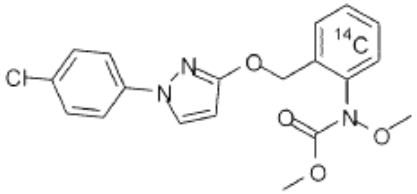


Figure 6.2.1-13: Structural formula of ^{14}C-BAS 500 F labelled at the tolyl ring



The treated seeds were sown into plastic containers and filled with loamy sand soil (Bruch West; German scheme DIN 4220: loamy sand, USDA scheme sandy loam). Physical and chemical properties of sandy loam soil are given in Table 6.2.1-26.

Table 6.2.1-26: Soil characteristics: soil used for sowing

USDA classification	
clay	11.9%
silt	21.7%
sand	66.4%
soil class	sandy loam
DIN classification	
clay	11.9%
silt	25.8%
sand	62.3%
soil class	loamy sand
Other soil parameters	
total nitrogen	0.13%
total organic carbon	1.48%
total carbon	3.33%
pH ($CaCl_2$)	7.2
pH (H_2O)	7.8
effective cation exchange capacity	11.8 cmol/kg
max. water holding capacity	30.2 g/100 g dry soil
microbial biomass	37.8 mg C/100 g dry soil
microbial C / organic C	2.6%
bulk density	1220 g/L
dry matter	90.6%

Samples of wheat matrices were collected at growth stage 59 (forage), GS 73-75 (hay) and GS 89 (grain and straw) for the chlorophenyl and tolyl label.

2. Description of analytical procedures

All samples (forage, hay, straw and grain) were homogenized with a knife mill along with dry ice. After sublimation of the dry ice, the samples were weighed, mixed, divided into aliquots and radioassayed.

The total radioactive residues (TRR) were determined by direct combustion analysis of small aliquots of homogenized sample material. The sample material was combusted by means of an automatic sample oxidizer. The $^{14}CO_2$ was trapped by an absorption and scintillation liquid, and the collected radioactivity was measured by liquid scintillation counting. Aliquots of liquid samples were mixed with a sufficient volume of a suitable scintillator prior to measurement.

Due to the low radioactivity levels of all wheat matrices, a solvent extraction was only performed for straw samples to get information about potential metabolites formed.

The homogenized straw samples were extracted with methanol and water. Subsequently the methanol extracts were partitioned with cyclohexane and ethyl acetate. The cyclohexane phases were fractionated using a SPE column and the fractions containing the highest radioactivity amount were investigated by HPLC using two different HPLC methods.

For metabolite assignment, the retention times of peaks in the HPLC runs of the isolated straw fractions were compared to those of authentic reference compounds that were analyzed with the same HPLC methods.

For straw the TRR was also calculated by summarizing the Extractable Radioactive Residues (ERR) and the Residual Radioactive Residues (RRR) after solvent extraction.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The levels of total radioactive residues (TRR) of pyraclostrobin found in wheat forage, hay, straw and grain are reported in Table 6.2.1-27.

Table 6.2.1-27: Total radioactive residues in wheat samples

Wheat matrix	Sampling Interval (DAT[1])	TRR determined [mg/kg]	TRR calculated [2] [mg/kg]
Chlorophenyl Label			
Forage	65	0.0008 [3]	n.p.
Hay	76	0.0015 [3]	n.p.
Straw	104	0.0051 [3]	0.0043
Grain	104	0.0008	n.p.
Tolyl Label			
Forage	63	0.0005	n.p.
Hay	74	0.0014	n.p.
Straw	103	0.0045	0.0038
Grain	103	0.0011	n.p.

[1)] DAT = Days After Treatment (sowing of the treated seed)
[2)] TRR was calculated as the sum of ERR + RRR
[3)] mean value of the two measurements
[4)] n.p. = not performed

All residue levels were below 0.010 mg/kg in all matrices. The TRR values of forage accounted for 0.0008 mg/kg (chlorophenyl label) and 0.0005 mg/kg (tolyl Label). In wheat hay the TRR amounted to 0.0015 mg/kg (chlorophenyl label) and 0.0014 mg/kg (tolyl label). The highest radioactivity levels were found in straw with 0.0051 mg/kg (chlorophenyl label) and 0.0045 mg/kg (tolyl label). In grain the TRR amounted to 0.0008 mg/kg (chlorophenyl label) and 0.0011 mg/kg (tolyl label).
For straw the TRR was also calculated by summarizing the Extractable Radioactive Residues (ERR) and the Residual Radioactive Residues (RRR) after solvent extraction. The values amounted to 0.0043 mg/kg for the chlorophenyl label and to 0.0038 mg/kg for the tolyl label. The calculated TRR values of wheat straw were in good accordance to those obtained by direct combustion analysis. The calculated TRR values of straw samples were set to 100% TRR in the workups for further sample calculations.

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

1. Extraction of residues

Since the amount of radioactive residues was below 0.010 mg/kg in all matrices, no further investigations regarding identification, characterization and quantification were performed for forage, hay and grain.
In spite of the also very low radioactive residues, straw samples were extracted with methanol and water to obtain information about potential metabolites formed.
The extractability of the radioactive residues from straw with methanol and water is summarized in Table 6.2.1-28. Altogether, 63.3% TRR were extracted for the chlorophenyl label and 46.4% TRR for the tolyl label. The major part of the residues was extracted with methanol (chlorophenyl label: 44.1% TRR, tolyl label: 37.4% TRR).

Table 6.2.1-28: Extractability of radioactive residues in wheat samples

Matrix	Combined methanol extracts		Combined water extracts		ERR		RRR		TRR calculated [1]
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	
Chlorophenyl Label									
Straw	0.0019	44.1	0.0008	19.3	0.0027	63.3	0.0016	36.7	0.0043
Tolyl Label									
Straw	0.0014	37.4	0.0003	9.0	0.0018	46.4	0.0021	53.6	0.0038

[1)] TRR was calculated as the sum of ERR + RRR

2. Identification, characterization and quantitation of extractable residues

Due to the low amounts of radioactive residues, the background noise of the chromatograms was comparatively high. Nevertheless, one peak was detected for both labels when analyzed according to both HPLC methods.
Retention time comparison with reference items suggested that this peak represents the parent compound pyraclostrobin and / or the metabolite 500M07 (BF 500-3). Since pyraclostrobin and 500M07 have similar retention times in both HPLC methods, the peak may contain one or both of the compounds. The identical retention times in the second HPLC method were confirmed by a co-chromatography experiment performed with the unlabelled reference items pyraclostrobin and 500M07. The isolated SPE fractions probably containing pyraclostrobin and / or 500M07 represented about 0.0005 mg/kg or 12.7% TRR for the chlorophenyl label and about 0.0004 mg/kg or 9.6% TRR for the tolyl label. The results of the analysis of wheat straw are summarized in Table 6.2.1-29 (chlorophenyl label) and Table 6.2.1-30 (tolyl label).

Table 6.2.1-29: Summary of characterized residues of wheat straw and final residue (chlorophenyl label)

Designation	[mg/kg]	[% TRR]
Total radioactive residues in wheat straw	0.0043	100
Characterized in the methanol extract		
Radioactive residues in the cyclohexane phase	0.0009	20.4
Characterized in the cyclohexane phase by SPE fractionation		
Fraction 1	<0.0001	<0.1
Fraction 2	0.0001	1.2
Fraction 3	<0.0001	0.4
Fraction 4	<0.0001	0.8
Fraction 5: Containing BAS 500 F and / or 500M07	0.0005	12.7
Fraction 6	0.0002	4.5
Sum of characterized residues in the cyclohexane Phase (sum fractions 1-6)	0.0008	19.6
Ethyl acetate phase	0.0007	17.1
Water phase	0.0004	8.2
Total characterized in the methanol extract (sum fractions 1-6 + ethyl acetate and water phase)	**0.0019**	**44.9**
Total characterized in the water extract	**0.0008**	**19.3**
Total characterized from ERR	**0.0028**	**64.1**
Final residue (RRR)	0.0016	36.7
Total characterized (ERR) + final residue	**0.0043**	**100.8**

In the ERR of the chlorophenyl label, 12.7% of the total radioactive residues were characterized by HPLC analysis of the isolated SPE fraction of the methanol extract (Fraction 5, as described above), most probably containing pyraclostrobin and / or 500M07. Additionally, 51.4% TRR were characterized by means of their extractability and elution properties. The RRR amounted to 0.0016 mg/kg or 36.7% TRR and was not further investigated due to the low residue concentration.

Table 6.2.1-30: Summary of characterized residues of wheat straw and final residue (tolyl label)

Designation	[mg/kg]	[% TRR]
Total radioactive residues in wheat straw	0.0038	100
Characterized in the methanol extract		
Radioactive residues in the cyclohexane phase	0.0006	15.1
Characterized in the cyclohexane phase by SPE fractionation		
Fraction 1	<0.0001	0.1
Fraction 2	<0.0001	0.3
Fraction 3	<0.0001	0.6
Fraction 4	<0.0001	1.1
Fraction 5: Containing BAS 500 F and / or 500M07	0.0004	9.6
Fraction 6	0.0001	2.7
Sum of characterized residues in the cyclohexane Phase (sum fractions 1-6)	0.0006	14.4
Ethyl acetate phase	0.0005	13.6
Water phase	0.0003	8.7
Total characterized in the methanol extract (sum fractions 1-6 + ethyl acetate and water phase)	**0.0014**	**36.7**
Total characterized in the water extract	**0.0003**	**9.0**
Total characterized from ERR	**0.0018**	**45.7**
Final residue (RRR)	0.0021	53.6
Total characterized (ERR) + final residue	**0.0038**	**99.3**

In the ERR of the tolyl label, 9.6% of the total radioactive residues were again characterized by HPLC analysis of the isolated SPE fraction of the methanol extract (Fraction 5), most probably containing pyraclostrobin and / or 500M07. Another 36.1% TRR were characterized upon their extractability and elution characteristics. The RRR amounted to 0.0021 mg/kg or 53.6% TRR and was also not further investigated due to its low residue concentration.

3. Proposed metabolic pathway

In all wheat matrices the total radioactive residues were below 0.010 mg/kg. Nevertheless, a solvent extraction was performed to get information about potential metabolites formed and it was possible to isolate one metabolite fraction from the methanol extract of straw from both labels. Retention time comparison with reference compounds suggest, that the isolated fraction contains the unchanged parent compound pyraclostrobin and / or the desmethoxylated metabolite 500M07. These results are in good agreement with those obtained from a wheat metabolism study (Reinhardt, K.: Metabolism of 14C-BAS 500 F in Wheat, BASF DocID 1999/11137) and a rotational crop study (Veit, P.: Confined Rotational Crop Study with 14C-BAS 500 F, BASF DocID: 1999/11829), in which unchanged parent compound and 500M07 were also detected.

4. Storage stability

Storage stability investigations on pyraclostrobin and its metabolites were already performed within a wheat metabolism study (Reinhardt, K.: Metabolism of 14C-BAS 500 F in Wheat, BASF DocID 1999/11137) and within a rotational crop study (Veit, P.: Confined Rotational Crop Study with 14C-BAS 500 F, BASF DocID: 1999/11829). Additionally, the HPLC analyses performed in this study were carried out within two month after extraction. Therefore, concerning the current study no further storage stability investigations were necessary.

III. CONCLUSION

The levels of total radioactive residues (TRR) were very low (<0.010 mg/kg) in all wheat matrices. The highest levels of total radioactive residues (TRR) were found in wheat straw (0.0051 mg/kg and 0.0045 mg/kg, chlorophenyl and tolyl label, respectively) followed by hay (0.0015 mg/kg and 0.0014 mg/kg, chlorophenyl and tolyl label). In wheat forage the TRR amounted to 0.0008 mg/kg (chlorophenyl label) and 0.0005 mg/kg (tolyl label). The TRR values of grain were 0.0008 mg/kg (chlorophenyl label) and 0.0011 mg/kg (tolyl label).
Due to the low radioactivity levels only straw samples were extracted with solvent. The extractability of the radioactive residues from straw with methanol and water was moderate for both labels (63.3% TRR chlorophenyl label and 46.4% tolyl label). For both labels, the major part of the residues (about 40% TRR) was extracted with methanol. Due to low levels of radioactivity in the Residual Radioactive Residues (RRR) after solvent extraction no further solubilization steps were performed.
The nature of the residues was examined by HPLC analyses of fractions isolated from the methanol extracts of straw (both labels). These fractions were obtained from the methanol extracts by liquid /liquid partition and subsequent fractionation of the cyclohexane phases by SPE fractionation on a silica gel column. The isolated fractions were further investigated by HPLC. Retention time comparison with authentic reference compounds suggest that the only significant peak detected most probably represents the parent compound pyraclostrobin and/or its desmethoxylated metabolite 500M07 (BF 500-3), that are co-eluting using the applied HPLC methods.

CA 6.2.2 Poultry

A poultry metabolism study conducted with tolyl-^{14}C and chlorophenyl-^{14}C labeled pyraclostrobin was evaluated during the previous Annex I inclusion process.

Pyraclostrobin (BAS 500 F) is rapidly absorbed, distributed and excreted. In hens dosed with 12 mg/kg feed, the residues in muscle were below 0.010 mg/kg. Consequently, no further investigations were carried out. In fat and eggs, pyraclostrobin and the desmethoxy metabolite 500M07 (BF 500-3) formed the major part of the residue whereas in liver hydroxylation reactions were predominant. As in plant matrices, cleavage products (as 500M04 and 500M49) have been identified in tissues and eggs. The study is still meeting today's requirements. Therefore, no new study has been performed.

CA 6.2.3 Lactating ruminants

A goat metabolism study conducted with tolyl-^{14}C and chlorophenyl-^{14}C labeled pyraclostrobin was evaluated during the previous Annex I inclusion process.

The following conclusion is copied from the EFSA Reasoned Opinion on the re-evaluation of established MRLs:
Studies of the metabolism of pyraclostrobin in goats showed that residues in products of animal origin derive from the parent compound as well as from its desmethoxy metabolite (500M07). After five consecutive daily oral administrations of ^{14}C-pyraclostrobin at a nominal dosage of 12 or 50 mg/kg DM feed, there was rapid absorption from the gastrointestinal tract. Radioactivity was excreted mainly via the faeces. The radiolabel in milk accounted for only 0.1–0.5% of the total applied radioactivity. There was no indication of accumulation of ^{14}C-pyraclostrobin in tissues. The parent compound was found in fat, muscle and, at lower amounts, in liver. Metabolites are formed in liver and kidney by hydroxylation of the chlorophenyl and tolyl rings and by cleavage of the molecule. Little extraction was seen in liver. Pyraclostrobin was present in all tissues and in milk and was the main residue component in muscle and in fat (log Pow = 3.9).

The study is still meeting today's requirements. Therefore, no new study has been performed.

Upon request of the Australian authority, an in-vitro comparison study was performed for goat and cow cell cultures. In order to allow a comprehensive overview, the study summary of these supplemental investigations are provided. Goat and cow microsomes and hepatocytes were incubated with the chlorophenyl-^{14}C labeled test substance.

Report: CA 6.2.3/1
Bross M., Lutz T., 2009a
In vitro investigations of the metabolism of BAS 500 F in goat and cow
2009/1067176
Guidelines: none
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin (unlabelled, 14C-labeled)

Description:	unlabelled BAS 500 F (pyraclostrobin, methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl] oxymethyl}phenyl) N-methoxycarbamate), 14C-labeled BAS 500 F (pyraclostrobin, methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl] oxymethyl}phenyl) N-methoxycarbamate, labeled at chlorophenyl-U-C14); (specific activity: 91.4 MBq/mg)
Lot/Batch #:	unlabelled pyraclostrobin: 01815-183 labelled pyraclostrobin: 579-2202
Purity:	unlabelled pyraclostrobin: 99.9% (HPLC) labelled pyraclostrobin: 99.4% (RHPLC)
CAS#:	unlabelled/labelled pyraclostrobin: 175013-18-0
Development code:	not applicable
Spiking levels:	100 µM (high dose), 10µM (low dose)
Stability of test compound:	no storage stability investigations were necessary

2. Test Commodity:

Cell cultures: Liver microsomes from male goats (purchased from In Vitro Technologies GmbH Leipzig, Germany) and male cows (purchased from In Vitro Technologies GmbH Leipzig, Germany), all preparations contained 20 mg microsomal protein per mL.

In vitro assay: The test solutions (mix of unlabeled and 14C-labeled test item pyraclostrobin) were incubated at approximately 100 µM for high dose or approximately 10 µM for low dose conditions with goat or cow microsomes. The radioactivity per incubation mixture was approximately 100000 dpm. Each sample (2 mL total volume) comprised 1 mg of microsomal protein, a NADPH generating system and potassium phosphate buffer (approximately 90 mM, pH 7.4). The reactions were performed at 37°C for 3 h in a shaker and stopped by addition of 2 mL acetone (resulting in a final volume of 4 mL for each sample). All experiments (goat high dose, goat low dose, cow high dose and cow low dose) were carried out as triplicates. For every condition one additional experiment (not as a triplicate) was done with heat inactivated microsomes (incubated for 10 min in boiling water).

B. STUDY DESIGN AND METHODS

The present study including the analytical phase was performed at the BASF Agricultural Center Limburgerhof, Germany.

1. Test procedure

The aim of the study was to compare the metabolism of BAS 500 F (pyraclostrobin) in cell cultures of the ruminants goat and cow. The *in vitro* metabolism investigations with pyraclostrobin were performed with a mixture of unlabelled and ^{14}C-labeled (chlorophenyl-U-C14) test item. The active substance was incubated at 100 µM (high dose) or 10 µM (low dose) with goat or cow liver microsomes (1 mg microsomal protein per incubation mixture). The incubations were carried out for 3 h at 37°C and stopped by addition of acetone. For each experimental condition a control experiment with heat inactivated microsomes was performed.

Figure 6.2.3-1: Structural formula of unlabelled BAS 500 F

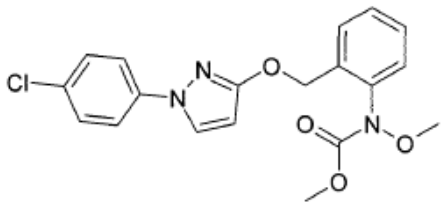
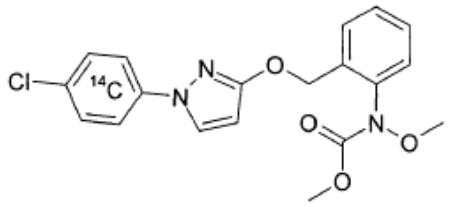


Figure 6.2.3-2: Structural formula of ^{14}C-BAS 500 F labelled at the chlorophenyl ring



2. Description of analytical procedures

2 mL of each stopped incubation mixture were pooled and stored at -18°C until further processing. The samples from the incubations with inactivated microsomes were directly stored at -18°C. An aliquot of the pooled workup sample from the high dose incubations with cow microsomes was concentrated and stored at -18°C until HPLC-MS analysis. For quantitative HPLC analysis, aliquots of each sample were taken and were evaporated with nitrogen. The volume was adjusted with water and the samples were stored at -18°C until HPLC analysis.
For fractionation, an aliquot of the pooled sample from the high dose incubations with goat microsomes was concentrated. Aliquots of the concentrated sample were sequentially fractionated by HPLC (HPLC method LC04). Three fractions per HPLC run were manually cut and the corresponding fractions were pooled. The pooled fractions were adjusted to an end volume of 5 mL for LSC measurement. Then, the solvent was completely evaporated, the residues resuspended in HPLC eluent A (water/acetonitrile/acetic acid, 950/50/1, v/v/v) and stored at -18°C until HPLC-MS analysis.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Total radioactive residues were quantified in stopped incubation mixtures and pooled samples. The total radioactive residues were also measured in the stopped incubates with heat inactivated microsomes, which served as controls. The recoveries of both experiments were compared. The found total residual levels per incubation mixture corresponded approximately to the intended value of 100000 dpm.
Additionally, no significant difference of the total radioactive residues in incubates with active or inactivated microsomes were observed. This finding excluded a possible generation of volatile components in the presence of active microsomal enzymes.

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

1. Extraction and hydrolysis of residues

All control experiments with heat inactivated microsomes yielded 100.0% TRR of the unmodified parent compound pyraclostrobin indicating that no hydrolytical degradation is taking place under the conditions applied.

2. Identification, characterization and quantitation of extractable residues

The identification of the metabolites was based on HPLC-MS analyses of HPLC fractions from high dose incubations with goat microsomes (fraction 1 to 3) and HPLC-MS analysis of the concentrated sample from high dose incubations with cow microsomes. Peak assignment in the other samples was done by comparison of the HPLC retention times and metabolite patterns with those of the fractions investigated by HPLC-MS and reference items.

Goat

In the sample from the **high dose** incubations with goat microsomes, the unchanged parent compound pyraclostrobin (fraction 3) was the most abundant component, accounting for 6.994 mg/kg (43.4% TRR). The metabolite 500M04 (fraction 1) was detected at a concentration of 4.210 mg/kg (26.1% TRR). Additionally, the metabolites 500M35, 500M86, 500M87, 500M34 and 500M88 were identified by HPLC-MS (fraction 2). In total, 16.128 mg/kg (100.0% TRR) of radioactive residues were identified in samples from the high dose incubations with goat microsomes. In the sample from **low dose** incubations with goat microsomes, the metabolite 500M04 was the most abundant component, accounting for 1.473 mg/kg (90.3% TRR). The parent compound pyraclostrobin was found at 0.159 mg/kg (9.7% TRR). Accordingly, 1.632 mg/kg (100.0% TRR) of the radioactive residues were identified.

Table 6.2.3-1: Identified components in the workup samples from incubations with goat microsomes

Designation	[mg/kg]	[µg/mg Protein]	[% TRR]
HIGH dose incubation with goat microsomes			
Total radioactive residue (TRR)	**16.128**	**64.511**	**100.0**
Identified in stopped and pooled incubation mixtures			
500M04	4.210	16.841	26.1
500M88, 500M34, 500M35, 500M86 and 500M87	1.526	6.104	9.5
	1.744	6.976	10.8
	1.653	6.614	10.3
BAS 500 F	6.994	27.975	43.4
Total identified	**16.128**	**64.511**	**100.0**
LOW dose incubation with goat microsomes			
Total radioactive residue (TRR)	**1.632**	**6.529**	**100.0**
Identified in stopped and pooled incubation mixtures			
500M04	1.473	5.894	90.3
BAS 500 F	0.159	0.636	9.7
Total identified	**1.632**	**6.529**	**100.0**

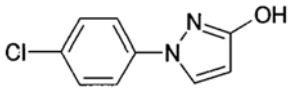
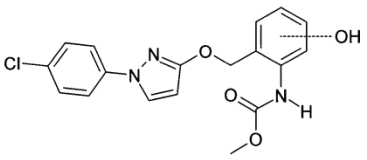
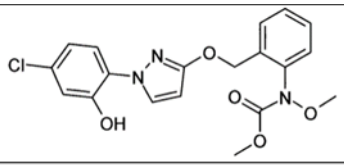
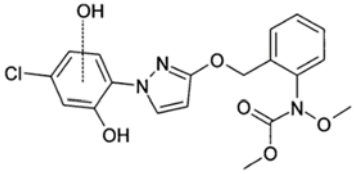
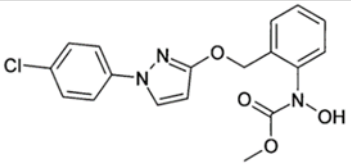
Cow

In the sample from the **high dose** incubations with cow microsomes, the parent compound pyraclostrobin was the most abundant component, accounting for 15.007 mg/kg (90.3% TRR). The metabolite 500M04 was present at a concentration of 1.610 mg/kg (9.7% TRR). Additionally, the metabolites 500M34 and 500M88 were identified by HPLC-MS, but present only in trace amounts. In total, 16.617 mg/kg (100.0% TRR) of the radioactive residues were identified. In the sample from **low dose** incubations with cow microsomes, the parent compound pyraclostrobin was also the most abundant component, accounting for 0.553 mg/kg (29.7% TRR). The metabolite 500M04 was present at a comparable concentration of 0.462 mg/kg (24.8% TRR). Moreover, peaks corresponding to metabolites of fraction 2 (500M35, 500M86, 500M87, 500M34 and 500M88) were detected in the quantitative HPLC chromatogram. Hence, 1.015 mg/kg (54.6% TRR) of radioactive residues were identified and additional 0.845 mg/kg (45.4% TRR) were characterized in samples from the low dose incubations with cow microsomes.

Table 6.2.3-2: Identified components in the workup samples from incubations with cow microsomes

Designation	[mg/kg]	[µg/mg Protein]	[% TRR]
HIGH dose incubation with cow microsomes			
Total radioactive residue (TRR)	**16.617**	**66.467**	**100.0**
Identified in stopped and pooled incubation mixtures			
500M04	1.610	6.439	9.7
500M88	trace amounts		
500M34	trace amounts		
BAS 500 F	15.007	60.027	90.3
Total identified	**16.617**	**66.467**	**100.0**
LOW dose incubation with cow microsomes			
Total radioactive residue (TRR)	**1.860**	**7.440**	**100.0**
Identified in stopped and pooled incubation mixtures			
500M04	0.462	1.847	24.8
BAS 500 F	0.553	2.213	29.7
Total identified	**1.015**	**4.060**	**54.6**
Characterized in stopped and pooled incubation mixtures			
Five further HPLC peaks with retention times comparable to those components identified in concentrated fraction 2	0.332	1.329	17.9
	0.089	0.355	4.8
	0.202	0.810	10.9
	0.148	0.592	8.0
	0.074	0.295	4.0
Total characterized	**0.845**	**3.380**	**45.4**
Total identified and characterized	**1.860**	**7.440**	**100.0**

Table 6.2.3-3: Structures of identified metabolites

Metabolite	Structure
500M04 (BF 500-5)	
500M34	
500M35	
500M86/500M87	
500M88	

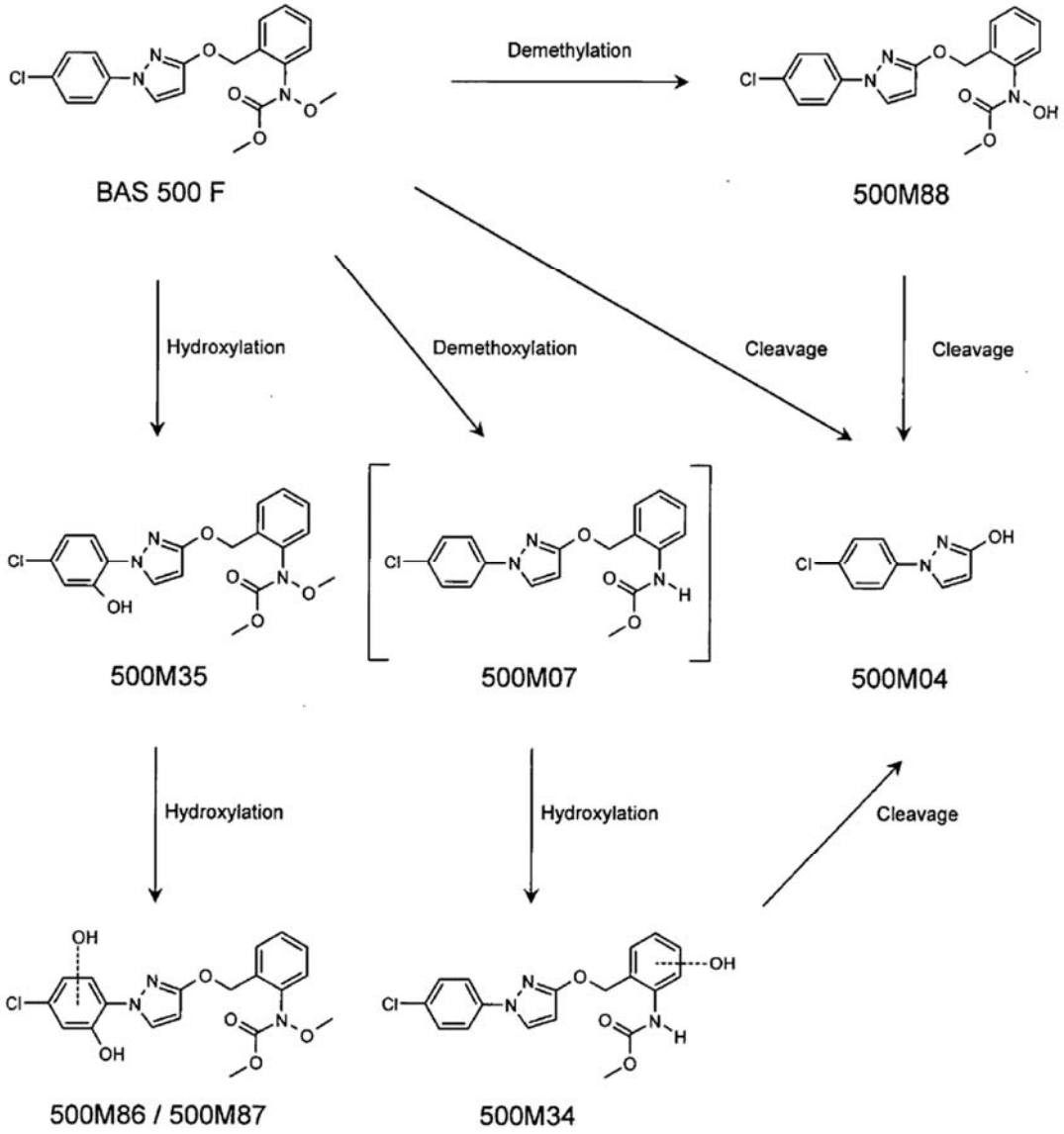
3. Proposed metabolic pathway

Pyraclostrobin was similarly metabolized by goat and cow microsomes. After low dose incubation of pyraclostrobin at 10 µM with goat microsomes the parent compound was detected in portions below 10% TRR. The transformation of pyraclostrobin was not as complete when incubated at 100 µM (portion of parent compound at 43.4% TRR), which indicates that under these conditions the responsible microsomal enzymes were saturated. A similar tendency was observed for the incubations of pyraclostrobin with cow microsomes. Whereas only 29.7% TRR of the parent compound were detected when incubated at 10 µM, a portion of 90.3% TRR of pyraclostrobin remained in samples from high dose incubations at 100 µM.

Pyraclostrobin was metabolized by five transformation reactions:
• Cleavage of the ether bond
• Demethylation of the N-methoxycarbamate group
• Desmethoxylation of the N-methoxycarbamate group
• Hydroxylation of the tolyl moiety
• Hydroxylation of the chlorophenyl moiety

The cleavage product 500M04 (1-(4-chlorophenyl)-1H-pyrazole-3-ol) is the central metabolite in the *in vitro* biotransformation route of pyraclostrobin through goat and cow microsomes. This compound was also found in a previous *in vivo* study in goat as one of the key metabolites of pyraclostrobin. The metabolite 500M04 can be generated by direct cleavage of the parent compound pyraclostrobin, but also by cleavage of the metabolites 500M88 or 500M34, because their chlorophenyl-pyrazole moiety remains unaltered. The latter path is supported by the observation that after low dose incubations with goat microsomes pyraclostrobin was almost completely transformed to 500M04 (>90% TRR), whereas in samples from high dose incubations the metabolites 500M88 and 500M34 were also present.
The metabolite 500M88 results from demethylation of the N-methoxycarbamate group of pyraclostrobin and appeared in samples from incubations with goat and cow microsomes. Desmethoxylation of the N-methoxycarbamate group leads to metabolite 500M07, which was not detected in the course of this study, but previously observed in the goat study mentioned above. 500M07 is the putative precursor of metabolite 500M34, which was found in the present study (goat and cow microsomes) and results from hydroxylation of 500M07 at the benzylic ring. As mentioned above, both metabolites (500M88 and 500M34) were possibly cleaved to the main metabolite 500M04.
The metabolite 500M35 results from hydroxylation and the metabolites 500M86 / 500M87 result from twofold hydroxylation of the chlorophenyl residue of pyraclostrobin. In the previous *in vivo* goat study, both hydroxylation of the benzylic and the chlorophenyl residue was observed.
The metabolic pathway of pyraclostrobin incubated with goat and cow microsomes is shown in Figure 6.2.3-3.

Figure 6.2.3-3: Metabolic pathway of BAS 500 F incubated with goat and cow microsomes



4. Comparison of *in vitro* metabolism in goat and cow

All metabolites identified in samples from incubations with cow microsomes were also found when using goat microsomes. Incubation of pyraclostrobin with microsomes from either origin preferentially led to cleavage of the ether bond, yielding metabolite 500M04. The amount of the cleavage product 500M04 was 90.3% TRR in samples from incubations with goat microsomes and 24.8% TRR in samples from incubations with cow microsomes, both under non-saturated low dose conditions.

Demethylation and desmethoxylation of the N-methoxycarbamate group of pyraclostrobin was also observed with goat and cow microsomes, leading to metabolite 500M88 and the putative precursor 500M07, respectively. Hydroxylation of this precursor at the benzylic ring led to metabolite 500M34, in samples from goat and cow microsomes. The metabolites 500M88 and 500M34 are both precursors of the main metabolite 500M04.

Hydroxylation of the chlorophenyl moiety of pyraclostrobin led to the metabolites 500M35 and 500M86 / 500M87. These metabolites were identified by HPLC-MS in the sample from high dose incubations with goat microsomes and a corresponding peak group was identified by HPLC in the sample from low dose incubations with cow microsomes. However, hydroxylation of the chlorophenyl moiety played only a minor role in the *in vitro* biotransformation route of pyraclostrobin.

5. Storage stability

All samples were stored at approximately -18°C during the course of the study. The pattern and the main peaks in the quantitative HPLC chromatogram of the sample from high dose incubations with goat microsomes and the HPLC chromatogram of the fractionation of the same sample showed no significant difference. Since the quantitative HPLC analyses of all samples in this study were performed six days after sampling, no storage stability investigations were necessary.

III. CONCLUSION

The investigation of the *in vitro* metabolism of pyraclostrobin in goat and cow was conducted by incubation experiments of the test item (mix of unlabeled and ^{14}C-Iabeled pyraclostrobin) at 100 µM (high dose) or 10 µM (low dose) with the corresponding microsomes, followed by qualitative and quantitative analysis of the residues.

Pyraclostrobin was similarly metabolized by goat and cow microsomes. All metabolites identified in samples from incubations with cow microsomes were also found in the experiments with goat microsomes. The appearance of the main metabolite 500M04 (BF 500-5) was independent from the microsome‘s origin and resulted from cleavage of the ether bond. lt was already identified in a previous *in vivo* goat study as one of the key degradation products of pyraclostrobin. The metabolite 500M88 (demethylation of the N-methoxycarbamate group) and 500M34 (desmethoxylation of the N-methoxycarbamate group and hydroxylation of the benzylic ring) were further common metabolites found after the incubation of pyraclostrobin with goat or cow microsomes. Both are putative precursors of the main metabolite 500M04. Hydroxylation of the chlorophenyl moiety played only a minor role in the *in vitro* biotransformation route of pyraclostrobin.

In samples from the control experiments with heat inactivated microsomes only the unmodified parent compound pyraclostrobin was detected, demonstrating that the transformation of pyraclostrobin was dependent on the presence of active microsomal enzymes.

CA 6.2.4 Pigs

According to Reg. 283/2013, pig metabolism studies shall be provided where the plant protection product is used in crops whose parts or products, also after processing, are fed to pigs and where it becomes apparent that metabolic pathways differ significantly in the rat as compared to ruminants and where the intake is expected to exceed 0.004 mg/kg bw/day.

For pyraclostrobin, no metabolism study was performed in pigs, since the metabolite patterns in rodents (rats) and ruminants (goats) did not differ significantly.

CA 6.2.5 Fish

A bioaccumulation study has been performed in 1999. The study has been already submitted in context of the previous Annex I inclusion process. However, in the previous evaluation it has not been assessed within the residue section. In the study the tolyl-^{14}C and chlorophenyl-^{14}C labeled test substance was applied to water. The study includes the identification of metabolites in edible and inedible portions of bluegill sunfish.

As a new data requirement according to Regulation 283/2013, metabolism studies on fish may be required where the plant protection product is used in crops whose parts or products, also after processing, are fed to fish and where residues in feed may occur from the intended applications. Pyraclostrobin is registered in the majority of crops being intended as fish feed item. In most of these crops residue levels above LOQ occur. The working document on the study conduct was published in II/2013. In order to meet these new data requirements, a fish metabolism study (dosing via diet) has been performed.

Report: CA 6.2.5/1
[redacted] 1999a
Bioaccumulation and metabolism of (14C)-BAS 500 F in bluegill sunfish
1999/11348
Guidelines: OECD 305, EPA 165-4
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 F (Pyraclostrobin, Reg. No. 304428)
[Tolyl-U-^{14}C]-BAS 500 F; specific activity 4.62 MBq/mg,
[Chlorophenyl-U-^{14}C]-BAS 500 F; specific activity 4.32 MBq/mg,
Lot/Batch #: BAS 500 F: 00937-128
[Tolyl-U-^{14}C]-BAS 500 F: 566-2101
[Chlorophenyl-U-^{14}C]-BAS 500 F: 579-1201
Purity: BAS 500 F: 99.8% (Chemical purity)
[Tolyl-U-^{14}C]-BAS 500 F: > 98% (Radiochemical purity)
[Chlorophenyl-U-^{14}C]-BAS 500 F: > 98% (Radiochemical purity)
CAS#: 175013-18-0
Development code: not applicable
Stability of test compound: The test item was stable for up to 3 months.

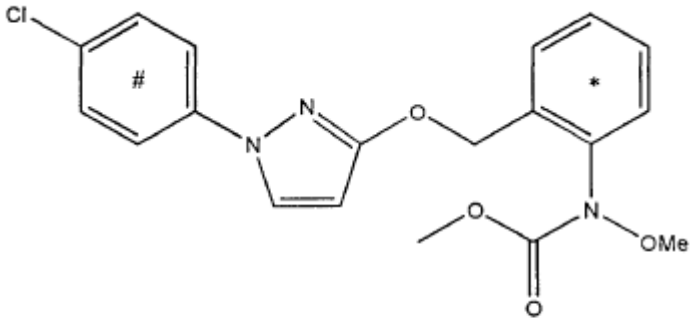
2. Test animals: Fish
Test species: Bluegill Sunfish (*Lepomis macrochirus*)
Body weight: 0.883 – 1.211 g/animal (wet, at the start of exposure, used for bioaccumulation and metabolism study)
Age: ca. 12 months (at the start of exposure)

B. STUDY DESIGN

1. Test procedure

Test fish were exposed to 300 ng as/L (nominal concentration) in a flow-through system. The test concentration was derived from acute toxicity data of pyraclostrobin for bluegill sunfish (ca. 1% of the 96 h LC_{50}). Since this concentration was already very low, a test with a second lower concentration could not be conducted, as results would have been below analytical limits of determination.
The active substance was dissolved in N,N-dimethyl formamide (DMF) and this stock solution was pumped into a mixing vessel supplied with a diluent stream of water. From there, the treated water flowed through the tanks at rates of 12.1 – 13.0 L/hour. Test fish (*ca*. 150 of *ca.* 1 g size for each label at day 0) were exposed for 37 days. Subsequently fish were exposed to a continuous flow of dilution water alone for either 14 days (chlorophenyl label) or 21 days (tolyl label). A control experiment with ca. 150 fish was conducted where fish were exposed to DMF and diluent water alone.

Figure 6.2.5-1: Structural formula of ^{14}C-BAS 500 F labelled at the chlorophenyl ring and the tolyl ring



= [^{14}C]-BAS 500F Chlorophenyl label
* = [^{14}C]-BAS 500F Tolyl label

2. Description of analytical procedures

Water and fish were sampled at suitable intervals. Fish were dissected into edible (muscle) and inedible (viscera) tissue fractions. Total radioactivity was determined in tissue and water samples.
Extracts of water, edibles and inedibles were analyzed by radio-HPLC. Unchanged parent compound and degradation products were identified by chromatographic comparison with reference compounds and metabolites previously identified in the course of a rat metabolism study.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

1. Test item concentration in the water

Chlorophenyl label
During the uptake phase the actual concentration of total radioactivity in water was in the range of 263 – 344 ng/L with a mean concentration of 305 ng/L. Water sampled at the first day of depuration contained 14% of the nominal concentration used in the uptake phase and thereafter the concentration dropped to levels below the limit of determination. The only radioactive component in water was unchanged pyraclostrobin.

Tolyl label
During the uptake phase the actual concentration of total radioactivity in water was in the range of 278 - 336 ng/L with a mean concentration of 300 ng/L. Water sampled at the first day of depuration contained 22% of the nominal concentration used in the uptake phase and thereafter the concentration dropped to levels below the limit of determination. The only radioactive component in water was unchanged pyraclostrobin.

2. Test item concentration in fish

Chlorophenyl label
Mean concentrations of radioactivity in the total fish reached an apparent steady state of 0.184 - 0.235 mg/kg after 4 days of exposure. Mean concentrations of radioactivity in the fillet increased from 0.047 mg/kg on day 1 of the exposure period to a plateau of 0.066 - 0.071 mg/kg. Mean concentrations of radioactivity in the inedible fraction (viscera) increased from 0.180 mg/kg on day 1 to a plateau of 0.314 - 0.404 mg/kg during days 4 - 35 of the exposure period.

Tolyl label
Mean concentrations of radioactivity in the total fish reached an apparent steady state of 0.192 - 0.243 mg/kg after 4 days of exposure. Mean concentrations of radioactivity in the fillet increased from 0.07 mg/kg on day 1 of the exposure period to a plateau of 0.074 - 0.084 mg/kg. Mean concentrations of radioactivity in the inedible fraction (viscera) increased from 0.197 mg/kg on day 1 to a plateau of 0.331 - 0.425 mg/kg during days 4 - 35 of the exposure period.

3. Bioconcentration factor

Chlorophenyl label
The bioconcentration factors and kinetic parameters based on total radioactivity concentrations were derived from Non-Linear-Regression Analysis using a 2-Compartment Model and are summarized in Table 6.2.5-1. The depuration half-life in whole fish was 0.9 day. Accordingly, the time to reach 90% depuration is 3.0 days. The bioconcentration factor calculated directly from the ratio of the ^{14}C-concentrations in water and tissue fractions (mean of days 4 – 35) was 673 for whole fish, 232 for edible tissues and 1169 for viscera (Table 6.2.5-2). The values were in good accordance with those obtained by kinetic modelling.

Tolyl label
The bioconcentration factors and kinetic parameters based on total radioactivity concentrations were derived from Non-Linear-Regression Analysis using a 2-Compartment Model and are summarized in Table 6.2.5-1. The depuration half-life in whole fish was 0.9 days. Accordingly, the time to reach 90% depuration is 2.8 days. The bioconcentration factor calculated directly from the ratio of the ^{14}C-concentrations in water and tissue fractions (mean of days 4 – 35) was 719 for whole fish, 262 for edible tissues and 1221 for viscera (Table 6.2.5-2). The values were in good accordance with those obtained by kinetic modelling.

Table 6.2.5-1: Kinetic parameters

Parameter	Tissue fraction					
	Chlorophenyl label			Tolyl label		
	Edibles	Viscera	Whole fish	Edibles	Viscera	Whole fish
Uptake rate constant k_1 [$mL^{-1} \cdot g^{-1} \cdot days^{-1}$]	236	833	514	243	994	598
Depuration rate constant k_2 [$days^{-1}$]	1.020	0.711	0.762	0.901	0.797	0.812
Depuration half-life [days]	0.7	1.0	0.9	0.8	0.9	0.9
Time to reach 90% depuration t_{90} [days]	2.3	3.2	3.0	2.6	2.9	2.8
Bioconcentration factor (BCF) k_1/k_2	232	1171	675	269	1246	736

Table 6.2.5-2: Bioconcentration factors calculated directly from the ratio of total radioactivity concentrations in water and tissues

Uptake phase Day No.	Tissue fraction					
	Chlorophenyl label			Tolyl label		
	Edibles	Viscera	Whole Fish	Edibles	Viscera	Whole Fish
1	177	677	421	248	699	443
2	212	956	566	199	1135	666
4	262	1066	623	233	1041	616
7	206	981	575	274	1065	625
14	223	1052	608	261	1252	732
21	217	1142	650	252	1123	661
28	259	1474	814	273	1417	810
31	230	1288	761	270	1288	782
35	228	1179	683	270	1358	805
Mean Days 4 – 35 (plateau)	232	1169	673	262	1221	719

The BCF values for unchanged BAS 500 F were 379 for whole fish, 191 for edibles and 574 for inedibles. The BCF values are shown in Table 6.2.5-3.

Table 6.2.5-3: Bioconcentration factors for unchanged parent compound calculated from the ratio of the BAS 500 F concentrations in tissues and water

Treatment	BCF		
	Edibles	Inedibles	Whole Fish
Chlorophenyl label	191	574	379
Tolyl label	178	853	507

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

1. Extraction of residues

Most of the radioactivity (>80.6% and >81.6% with the chlorophenyl label and tolyl label, respectively) was recovered in the methanol extracts. A further 2.3-5.3% (chlorophenyl label) and 2.4 - 4.8% (tolyl label) were extracted by water. The Residual Radioactive Residues (RRR) after solvent extraction were in the range of 3.9 to 9.9% (chlorophenyl label) and in the range of 5.5 to 11.2% (tolyl label) of the total radioactive residue. The residual radioactive residues from edible tissue (8.2 - 11.2%) were consistently higher than that from inedible tissue (3.9 - 7.6%). There were no obvious differences between the 2 sites of radiolabel.

Extractability data in fish tissue is shown in Table 6.2.5-4.

Table 6.2.5-4: Recovery during the extraction of fish tissue for chromatography

Treatment	Matrix type	Initial Sample [%]	Extract 1 [%]	Extract 2 [%]	RRR [%]	Total Recovery [%]
Uptake Day 21						
Chlorophenyl label	Edible	100.0	80.6	5.3	8.2	94.0
	Inedible	100.0	90.1	2.3	3.9	96.2
Tolyl label	Edible	100.0	88.4	4.2	11.2	103.8
	Inedible	100.0	101.1	2.4	5.5.	109.0
Uptake Day 28						
Chlorophenyl label	Edible	100.0	88.7	5.2	9.9	103.8
	Inedible	100.0	84.7	3.0	5.6	93.4
Tolyl label	Edible	100.0	81.9	4.8	11.2	98.0
	Inedible	100.0	81.6	4.5	7.6	93.7

Extract 1 = Methanol, Extract 2 = Water

In the experiment with the chlorophenyl label, between 82.1 and 99.4% of the total radioactivity in water was extracted by solid phase extraction leaving 1.2 - 7.5% in the aqueous residue. With the tolyl label, between 82.9 and 95.4% of the total radioactivity was extracted and 1.1 - 7.0% recovered in the aqueous residue. In both experiments, quantities of radioactivity recovered in the aqueous residue were lower in the pre-equilibration phase than after addition of the fish. This indicates the presence of unextracted polar components, possibly eliminated from the fish. Overall recoveries of radioactivity exceeded 85% and, considering the low levels of radioactivity in water from this study, were considered quantitative.

2. Identification, characterization and quantitation of extractable residues

In edible tissue, 62.8 - 73.7% (0.051 - 0.052 mg/kg) showed chromatography consistent with unchanged pyraclostrobin, 6.5 - 9.4% (0.005 - 0.007 mg/kg) with 500M07 and 2.5 - 3.6% (0.002 - 0.003 mg/kg) with 500M08. In the experiment conducted with the chlorophenyl label, 2.0% (0.001 mg/kg) of 500M04 was detected, whilst with the tolyl label, 4.1% (0.003 mg/kg) of 500M45 was detected. In the experiment with the tolyl label, 2 components of a highly polar nature, Unknowns 4 and 5, were detected at 2.0% (0.002 mg/kg) and 4.0% (0.003 mg/kg), respectively. Radioactivity in the water extract accounted for 4.8 - 5.2% (0.004 mg/kg) to leave residual radioactive residues of 9.9 - 11.2% (0.007 - 0.009 mg/kg), so these fractions were not characterized further.

Inedible tissue contained 38.6 - 57.4% (0.156 - 0.244 mg/kg) unchanged pyraclostrobin and 3.4 - 7.3% (0.014 - 0.029 mg/kg) 500M07. In the experiment with the chlorophenyl label, 4.5% (0.018 mg/kg) of 500M08 and 9.0% (0.036 mg/kg) of 500M04 were detected together with 5.3% (0.021 mg/kg) each of 2 unidentified components (Unknowns 1 and 2) and 14.7% (0.059 mg/kg) Unknown 3. In the experiment with the tolyl label, 16.1% (0.068 mg/kg) of Unknown 3 and 4.6% (0.012 mg/kg) of Unknown 5 were detected. Radioactivity in the water extract accounted for 3.0 - 4.5% (0.012 - 0.019 mg/kg), leaving residual radioactive residues of 5.6 - 7.6% (0.023 - 0.032 mg/kg), so these fractions were not characterized further.

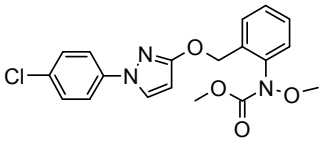
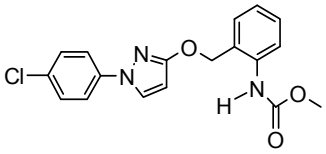
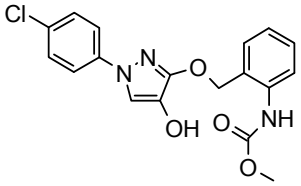
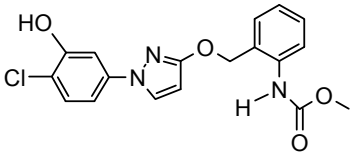
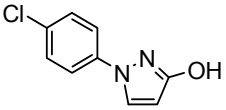
It appeared that pyraclostrobin was initially metabolized to form metabolite 500M07. Subsequent hydroxylation yielded metabolites 500M08 and 500M45. In addition, cleavage of the ether link between the pyrazole and tolyl rings lead to separation of the molecule into 500M04 and unidentified components (possibly the highly polar Unknowns 4 and 5). Up to 5 unidentified components were observed in fish tissues during the study. However, the major component in all tissue fractions was unchanged pyraclostrobin which comprised 39% - 74% of the Total Radioactive Residue (TRR).
A summary of identified metabolites in fish fractions from the steady-state period (28 days of exposure) is shown in Table 6.2.5-5. The structures of identified metabolites are presented in Table 6.2.5-6.

Table 6.2.5-5: Summary of Identified and Characterized Radioactivity Results in Fish Tissues - Day 28

Metabolite	Chlorophenyl label		Tolyl label	
	Edibles	Inedibles	Edibles	Inedibles
	mg/kg tissue (% TRR)	mg/kg tissue (% TRR)	mg/kg tissue (% TRR)	mg/kg tissue (% TRR)
BAS 500 F	0.052 (73.7)	0.156 (38.6)	0.051 (62.8)	0.244 (57.4)
500M07 (BF 500-3)	0.007 (9.4)	0.029 (7.3)	0.005 (6.5)	0.014 (3.4)
500M08	0.003 (3.6)	0.018 (4.5)	0.002 (2.5)	-
500M45	-	-	0.003 (4.1)	-
500M04 (BF 500-5)	0.001 (2.0)	0.036 (9.0)	-	-
Unknown 1	-	0.021 (5.3)	-	-
Unknown 2	-	0.021 (5.3)	-	-
Unknown 3	-	0.059 (14.7)	-	0.068 (16.1)
Unknown 4	-	-	0.002 (2.0)	-
Unknown 5	-	-	0.003 (4.0)	0.012 (4.6)
Water-soluble radioactivity	0.004 (5.2)	0.012 (3.0)	0.004 (4.8)	0.019 (4.5)
Unextracted Residue	0.007 (9.9)	0.023 (5.6)	0.009 (11.2)	0.032 (7.6)
Total	0.074 (103.8)	0.375 (93.3)	0.079 (97.9)	0.397 (93.6)

TRR = Total radioactive residues

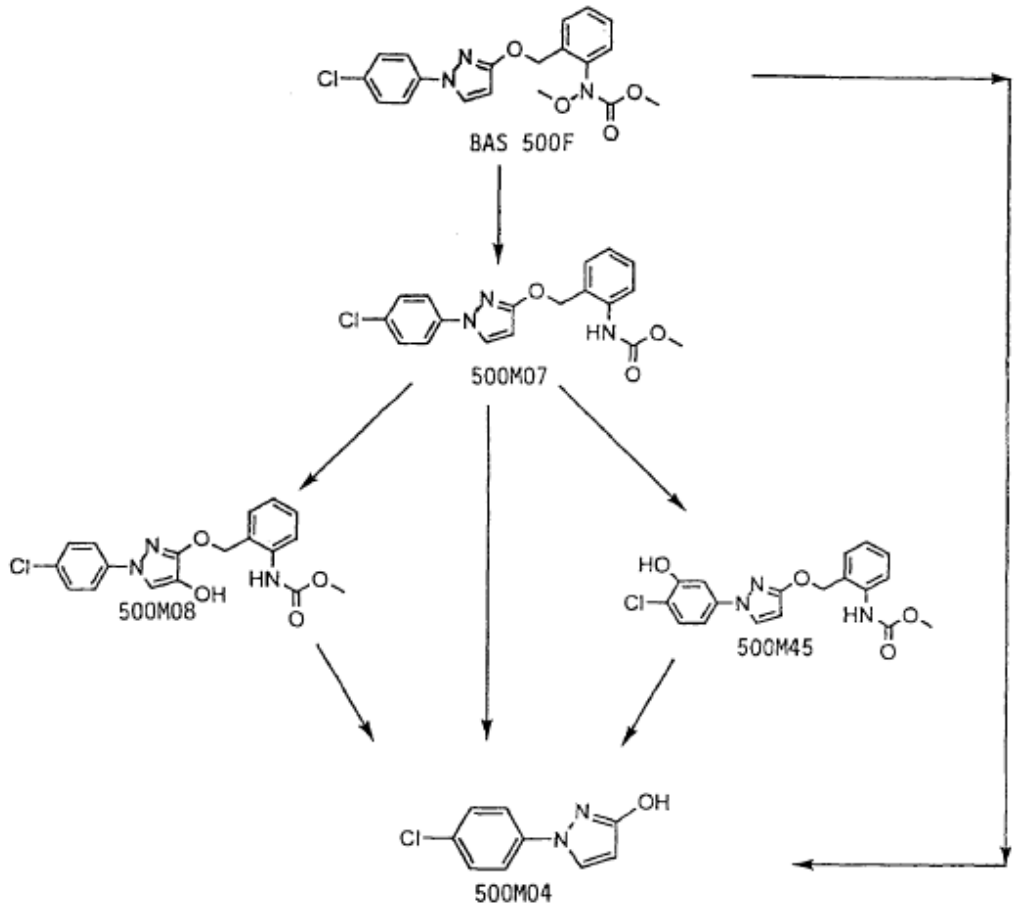
Table 6.2.5-6: Structures of identified metabolites

Metabolite	Structure
BAS 500 F	
500M07 (BF 500-3)	
500M08	
500M45	
500M04 (BF 500-5)	

3. Proposed metabolic pathway

The proposed pathway for the metabolism of pyraclostrobin in bluegill sunfish is presented in Figure 6.2.5-2.

Figure 6.2.5-2: Proposed pathway for the metabolism of pyraclostrobin in Bluegill Sunfish



6. Storage Stability

BAS 500 F and the radioactive components detected in fish are stable in fish tissue during storage at ca. -20°C for up to 3 months.

III. CONCLUSION

After exposure of fish to pyraclostrobin at a nominal exposure level of 300 ng/L, apparent steady state was reached after 2 - 4 days. After termination of the exposure, radioactivity levels in fish tissues decreased rapidly with a half-life of *ca.* 0.7 – 1.0 days. Bioconcentration factors based on total radioactivity were relatively low in edibles (232 - 262) and relatively high in inedibles (1169 - 1221). For unchanged parent compound, the BCF values were considerably lower in all tissues. This is an indication for an intensive metabolic clearance of pyraclostrobin. Only minor differences were observed between the two labelled forms of the test compound with regard to the kinetic parameters. In the edible tissues pyraclostrobin formed the major part of the residue, followed by 500M07 (BF 500-3). Cleavage of the ether bond resulting in the formation of the metabolite 500M04 (BF 500-5) occurred to a minor extent and mainly in the inedible portion of fish.

Report: CA 6.2.5/2
[redacted]., 2014a
The metabolism of 14C-BAS 500 F in rainbow trout (Oncorhynchus mykiss)
2014/1001601
Guidelines: EPA 860.1300: Nature of the Residue in Plants Livestock, OECD 305 (October 2012), SANCO/11187/2013 (31 January 2013)
GLP: yes
(certified by Ministerium fuer Arbeit, Integration und Soziales des Landes Nordrhein-Westfalen, Duesseldorf)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 F (Pyraclostrobin, Reg No. 304428)
Description: Chlorophenyl-U-^{14}C-pyraclostrobin, specific activity 6.15 MBq/mg
Tolyl-^{14}C-BAS 500 F, specific activity 6.6 MBq/mg
^{13}C-BAS 500 F
Lot/Batch #:[Chlorophenyl-U-^{14}C]-Label: 579-6301
[Tolyl-^{14}C]-Label: 566-5101
^{13}C-BAS 500 F: 1026-1018
Purity: [Chlorophenyl-U-^{14}C]-Label: 99.2% (CoA), radiochemical purity
[Tolyl-^{14}C]-Label: 566-5101: 99.9% (CoA), radiochemical purity
^{13}C-BAS 500 F: 99.8%
CAS#: 175013-18-0
Development code: not applicable
Stability of test compound: analysis of storage stability was not performed

2. Test animals: Fish
Test species: Rainbow Trout (Oncorhynchus mykiss)
Body weight: 330 – 465 g (at the start of exposure)
Body length: not applicable

B. STUDY DESIGN AND METHODS

1. Test procedure

Before starting the exposure phase, the fish were allowed to acclimatize to the laboratory environment for at least 14 days in de-chlorinated tap water. The experimental animals were fed a non-enriched diet for at least two weeks prior to the start of the experiment.
The experiment was carried out in two experimental tanks, each filled with approximately 1400 L of pre-conditioned tap-water under flow-through conditions with a flow rate of approximately 120 L/h. Each tank was stocked with 5 experimental animals. Water in the tank was constantly recirculated through two filter columns to avoid the accumulation of dissolved test item and metabolites. The water temperature was kept constant at around 15ºC (±2°C) throughout the experiment. Fish were housed under a 16 hour light/8 hour dark cycle throughout the experiment.

For the chlorphenyl label ^{14}C-labelled and ^{13}C-labelled test item were mixed in a ratio of 2:1 (specific activity: 3.72 MBq/mg). The tolyl label comprised a mixture of ^{14}C-labelled test item and non-labelled test items in a ratio of 5:3 (specific activity: 4.33 MBq/mg).

Figure 6.2.5-3: Structural formula of ^{14}C-BAS 500 F labelled at the chlorophenyl ring

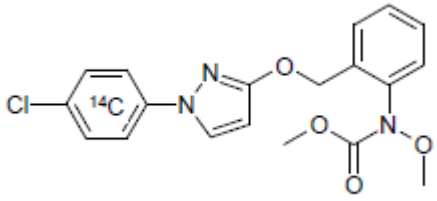


Figure 6.2.5-4: Structural formula of ^{14}C-BAS 500 F labelled at the tolyl ring

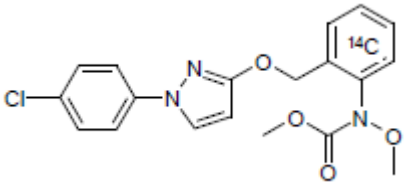


Figure 6.2.5-5: Structural formula of Carbon-13 labelled pyraclostrobin

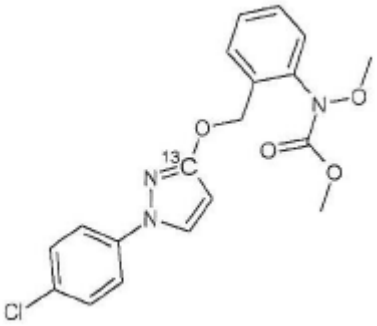
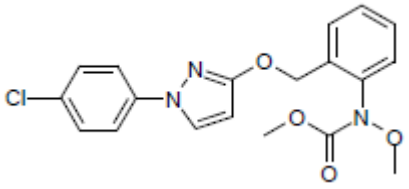


Figure 6.2.5-6: Structural formula unlabelled pyraclostrobin



During the study, the test item was dosed to the fish for 10 consecutive days for both labels at a nominal level of 10 mg/kg. The daily dose administered was 11.07 mg [Chlorophenyl-U-14C]-pyraclostrobin and 10.54 mg [Tolyl-14C]-pyraclostrobin respectively per kg food consumed. The daily ration was distributed on the surface of the water. Fish were fed at approximately 2.3% of wet body weight per day. The ration was adjusted weekly to account for the estimated weight gain of the experimental animals.

A pre-test showed that radioactivity in tissue reached a maximum plateau 10 days after the first dose. So the sacrifice time was set to the 10th day 6 h following the 10th dose. The fish were killed and edible tissues (liver, filet and filet skin) and pyloric caeca removed post mortem.
Samples not analyzed immediately were stored frozen (-20°C) until analysis. After analysis, samples were returned to storage at -20°C.

2. Description of analytical procedures

Extraction

Aliquots of the homogenized filet, the entire homogenized filet skins and unmodified livers were extracted with acetonitrile. The number of acetonitrile extractions depended on the sample weight. Liver samples were extracted with water after acetonitrile extraction.
Aliquots of pooled extracts containing significant amounts of radioactivity (A-extracts) were concentrated and were centrifuged; the supernatant was analyzed by HPLC, TLC and LC-MS. The radioactivity was determined by LSC.

Treatment of Residual Radioactive Residues (solubilization procedures)

Residual Radioactive Residues remaining in liver accounted for >10% TRR were further investigated following treatment of the post extraction tissue debris with digestive enzymes.
The samples were mixed with TRIS buffer to give a non-viscous suspension. Protease enzymes (from Streptomyces griseus) were added. Following incubation, solids were separated by centrifugation and decanting of the supernatant. The pellet was dried and an aliquot of remaining solids was determined by combustion/LSC. The supernatant was determined by LSC.
The residues after protease treatment were pooled and further digested with pepsin. Therefore, artificial gastric juice was added to the residue and the sample was incubated. After centrifugation, the supernatant was determined by LSC.
Artificial chyle (containing pancreatin) was added to the residue. The sample was incubated at a pH of 7.5. After centrifugation, the supernatant was determined by LSC.

The solid debris was dried and combusted. Radioactivity was determined via LSC.

Fat precipitation

The sample extracts were stored in a freezer (-20°C) directly after extraction. During storage, fat precipitation occurred in all samples. Fat was separated from extracts and dissolved in dichloromethane. The radioactive content of the fat was determined by LSC analysis.

Quantification and identification of residues

Pooled extracts of filet, filet skin and liver were analyzed with radio thin layer chromatography (radio TLC) on silica gel plates to identify radioactive residues. [Tolyl-14C]-pyraclostrobin and [Chlorophenyl-U-14C]-pyraclostrobin, as well as 10 known metabolites (certified reference items) were used to analyze the composition of the radioactive residues in the extracts.
As confirmatory method, an HPLC method with online radio detection was used to analyze the composition in pooled extracts. The identity of radiolabelled components was based on co-chromatography with certified reference items. HPLC was used to quantify the test item in the dose formulation as well as in homogeneity and stability tests. For HPLC separation, gradient elution on reversed-phase columns was applied.

In addition to HPLC analysis, the identity of components was confirmed by mass spectrometry in MRM mode. Two transitions were established for each component.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Edible Tissues
Radioactive residue data for the tissues are provided in Table 6.2.5-7. Residues in the filet, filet skin and liver were for [Chlorophenyl-U-14C]-pyraclostrobin 0.282, 0.229 and 0.556 mg/kg, and for [Tolyl-14C]-pyraclostrobin 0.239, 0.232 and 0.479 mg/kg, respectively.

Faeces
TRR data for faeces are provided in Table 6.2.5-7. Residues were analyzed for [Chlorophenyl-U-14C]-pyraclostrobin 7.33 mg/kg (6.42% TRR), and for [Tolyl-14C]-pyraclostrobin 4.87 mg /kg (5.28% TRR), respectively.

Table 6.2.5-7: Total radioactive residues (TRR) in tissues and faeces from rainbow trout following oral administration of ^{14}C-BAS 500 F

Total radioactive residues (TRR) [mg/kg]		
Label position	**Chlorophenyl label**	**Tolyl label**
Filet	0.282	0.239
Filet skin	0.229	0.232
Liver	0.556	0.479
Faeces	7.33	4.87

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

1. Extraction of residues

Filet
The homogenized filet was extracted with acetonitrile, resulting in extraction of 97.7% TRR (0.276 mg/kg) for chlorophenyl label and 97.2% TRR (0.232 mg/kg) for tolyl label. The solid debris was analyzed by combustion analysis and contained 2.2% TRR (0.006 mg/kg) for chlorophenyl label and 2.8% TRR (0.007 mg/kg) for tolyl label.

Filet Skin
The homogenized filet skin was extracted with acetonitrile, resulting in extraction of 97.8% TRR (0.225 mg/kg) for chlorophenyl label and 98.0% TRR (0.228 mg/kg) for tolyl label. The solid debris was analyzed by combustion analysis and contained 2.2% TRR (0.004 mg/kg) for chlorophenyl label and 2.0% TRR (0.005 mg/kg) for tolyl label.

Liver
The homogenized liver samples were extracted with acetonitrile and water, resulting in extraction of 84.5% TRR (0.470 mg/kg) for chlorophenyl label and 83.3% TRR (0.399 mg/kg) for tolyl label.

A summary of the extractability of residues of ^{14}C-pyraclostrobin in rainbow trout matrices is presented in Table 6.2.5-8.

Table 6.2.5-8: Extractability of residues of ^{14}C-BAS 500 F in rainbow trout matrices

Matrix	ERR[1]		PES[2]		TRR
	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Chlorophenyl label					
Filet	0.276	97.7	0.006	2.2	0.282
Filet skin	0.225	97.8	0.004	2.2	0.229
Liver	0.470	84.5	0.086	15.5	0.556
Tolyl label					
Filet	0.232	97.2	0.007	2.8	0.239
Filet skin	0.228	98.0	0.005	2.0	0.232
Liver	0.399	83.3	0.080	16.7	0.479

1 ERR = Extractable Radioactive Residue (acetonitrile extracts, fat precipitate, for liver: water extract)
[2] PES = Post-Extraction Solid

2. Identification, characterization and quantitation of extractable residues

Filet

For identification of labeled components, concentrated filet extract was analyzed with TLC directly without further purification. The analysis led to a pattern of 2 peaks, 1 main and 1 trace peak in both labels, which were identified as unchanged pyraclostrobin and 500M89 (Reg. No. 334089) by co-chromatography.

The residues were quantified by LSC analysis in combination with radio TLC. Unchanged pyraclostrobin accounted for 93.1% TRR (0.262 mg/kg) for chlorophenyl and 92.3% TRR (0.220 mg/kg) for tolyl label. Metabolite 500M89 (Reg. No. 334089) accounted for 2.6% TRR (0.007 mg/kg) for chlorophenyl and 3.0% TRR (0.007 mg/kg) for tolyl label. A small amount of radioactivity, representing the polar fraction, remained unidentified at the start point (0.005 mg/kg, 2.0% TRR for chlorophenyl and 0.004 mg/kg, 1.4% TRR for tolyl label).

The sum of identified residue from extractable radioactivity in filet samples was for chlorophenyl label 95.7% TRR (0.269 mg/kg) and for tolyl label 95.3% TRR (0.227 mg/kg). The sum of characterized residue in filet sample was for chlorophenyl label 2.0% TRR (0.005 mg/kg) and for tolyl label 1.8% TRR (0.005 mg/kg) from extractable radioactivity.
The sum of identified and characterized residue in extractable radioactivity in filet samples was for chlorophenyl label 97.7% TRR (0.274 mg/kg) and for tolyl label 97.1% TRR (0.232 mg/kg).

LC-MS analysis of the filet extracts confirmed the identification of only unchanged pyraclostrobin and 500M89 (Reg. No. 334089) in the samples 2 specific MRM transitions for each component were established and where these were detected in tissue extract, were used to confirm the assignments made by co-chromatography. MRM ion chromatograms for transitions A and B for compounds pyraclostrobin (Reg. No. 304428) and 500M89 (Reg. No. 334089) show peaks at 8.47 and 11.15 min, respectively, which confirms the presence of these compounds in this extracts.

Analysis of filet extract with HPLC method 2 led to only 1 peak of unchanged pyraclostrobin. Other residues were not detectable (below detection limit).

A summary of identified, characterized and final radioactive residues in fish filet sample is presented in Table 6.2.5-9.

Table 6.2.5-9: Summary of identified and characterized ^{14}C-residues extracted from filet (after 10 daily doses of ^{14}C-BAS 500 F at 10 mg/kg feed nominal)

Designation	Extract		Total	
	mg/kg	% TRR	mg/kg	% TRR
Chlorophenyl label Acetonitrile Extract 0.276 mg/kg, 97.7% TRR				
Identified[1]				
BAS 500 F (Reg. No. 304428)	0.262	93.1	0.262	93.1
500M89 (Reg. No. 334089)	0.007	2.6	0.007	2.6
Total identified in extractable radioactivity			**0.266**	**95.7**
Characterized				
Unknown at the start point of TLC			0.005	2.0
Fat Precipitate			0.0001	0.03
Total characterized in extractable radioactivity			**0.005**	**2.0**
Total identified and characterized in extractable radioactivity			**0.274**	**97.7**
Post-Extraction solid			**0.006**	**2.2**
Grand Total			**0.280**	**99.9**
Tolyl label Acetonitrile Extract 0.232 mg/kg, 97.2% TRR				
Identified[1]				
BAS 500 F (Reg. No. 304428)	0.220	92.3	0.220	92.3
500M89 (Reg. No. 334089)	0.007	3.0	0.007	3.0
Total identified in extractable radioactivity			**0.227**	**95.3**
Characterized				
Unknown at the start point of TLC			0.004	1.4
Fat Precipitate			0.001	0.4
Total characterized in extractable radioactivity			**0.005**	**1.8**
Total identified and characterized in extractable radioactivity			**0.232**	**97.1**
Post-Extraction solid			**0.007**	**2.8**
Grand Total			**0.239**	**99.9**

[1] Identified metabolites were resolved and quantified using LSC in combination with radio TLC and qualitative confirmation was performed by LC-MS

Filet Skin
For identification of labeled components, concentrated filet skin extract was analyzed with TLC directly without further purification. The analysis led to a pattern of 2 peaks, 1 main and 1 trace peak in both labels, which were identified as unchanged pyraclostrobin and 500M89 (Reg. No. 334089) by co-chromatography.

The residues were quantified by LSC analysis in combination with radio TLC. Unchanged pyraclostrobin accounted for 91.6% TRR (0.210 mg/kg) for chlorophenyl and 90.5% TRR (0.210 mg/kg) for tolyl label. Metabolite 500M89 (Reg. No. 334089) accounted for 3.7% TRR (0.008 mg/kg) for chlorophenyl and 4.3% TRR (0.010 mg/kg) for tolyl label. A small amount of radioactivity, representing the polar fraction, remained unidentified at the start point 2.2% TRR (0.006 mg/kg) for chlorophenyl and 2.6% TRR (0.006 mg/kg) for tolyl label.

The sum of identified residue from extractable radioactivity in filet skin samples was for chlorophenyl label 95.3% TRR (0.218 mg/kg) and for tolyl label 94.8% TRR (0.220 mg/kg). The sum of characterized residue in filet skin sample was for chlorophenyl label 2.6% TRR (0.007 mg/kg) and for tolyl label 3.0% TRR (0.007 mg/kg) from extractable radioactivity.
The sum of identified and characterized residue in extractable radioactivity in filet skin samples was for chlorophenyl label 97.9% TRR (0.225 mg/kg) and for tolyl label 97.8% TRR (0.227 mg/kg).

A summary of identified, characterized and final radioactive residues in fish filet skin sample is presented in Table 6.2.5-10.

Table 6.2.5-10: Summary of identified and characterized ^{14}C-residues extracted from filet skin (after 10 daily doses of ^{14}C-BAS 500 F at 10 mg/kg feed nominal)

Designation	Extract		Total	
	mg/kg	% TRR	mg/kg	% TRR
Chlorophenyl label Acetonitrile Extract 0.225 mg/kg, 97.8% TRR				
Identified[1]				
BAS 500 F (Reg. No. 304428)	0.210	91.6	0.210	91.6
500M89 (Reg. No. 334089)	0.008	3.7	0.008	3.7
Total identified in extractable radioactivity			**0.218**	**95.3**
Characterized				
Unknown at the start point of TLC			0.006	2.2
Fat Precipitate			0.001	0.4
Total characterized in extractable radioactivity			**0.007**	**2.6**
Total identified and characterized in extractable radioactivity			**0.225**	**97.9**
Post-Extraction solid			**0.004**	**2.2**
Grand Total			**0.229**	**100.1**
Tolyl label Acetonitrile Extract 0.228 mg/kg, 98.0% TRR				
Identified[1]				
BAS 500 F (Reg. No. 304428)	0.210	90.5	0.210	90.5
500M89 (Reg. No. 334089)	0.010	4.3	0.010	4.3
Total identified in extractable radioactivity			**0.220**	**94.8**
Characterized				
Unknown at the start point of TLC			0.006	2.6
Fat Precipitate			0.001	0.4
Total characterized in extractable radioactivity			**0.007**	**3.0**
Total identified and characterized in extractable radioactivity			**0.227**	**97.8**
Post-Extraction solid			**0.005**	**2.0**
Grand Total			**0.232**	**99.8**

[1] Identified metabolites were resolved and quantified using LSC in combination with radio TLC and qualitative confirmation was performed by LC-MS

LC-MS analysis of the filet skin extracts confirmed the identification of only unchanged pyraclostrobin and 500M89 (Reg. No. 334089) in the samples. 2 specific MRM transitions for each component were established and where these were detected in tissue extract, were used to confirm the assignments made by co-chromatography. MRM ion chromatograms for transitions A and B for compounds pyraclostrobin (Reg. No. 304428) and 500M89 (Reg. No. 334089) show peaks at 8.47 and 11.15 min, respectively, which confirms the presence of these compounds in these extracts.

Analysis of filet skin extract with HPLC method 2 led to only 1 peak of unchanged pyraclostrobin. Other residues were not detectable (below detection limit).

Liver
For identification of labeled components, concentrated liver extract was analyzed by TLC directly without further purification. The analysis led to a pattern of 2 peaks, 1 main and 1 trace peak in both labels, which were identified as unchanged pyraclostrobin and 500M89 (Reg. No. 334089) by co-chromatography.

The residues were quantified by LSC analysis in combination with radio TLC. Unchanged pyraclostrobin accounted for 73.6% TRR (0.409 mg/kg) for chlorophenyl and 70.4% TRR (0.337 mg/kg) for tolyl label. Metabolite 500M89 (Reg. No. 334089) accounted for 1.0% TRR (0.006 mg/kg) for chlorophenyl and 2.3% TRR (0.011 mg/kg) for tolyl label. A small amount of radioactivity, representing the polar fraction, remained unidentified at the start point (0.037 mg/kg, 6.7% TRR for chlorophenyl and 0.032 mg/kg, 6.9% TRR for tolyl label).

The sum of identified residue from extractable radioactivity in liver sample was for chlorophenyl label 74.6% TRR (0.415 mg/kg) and for tolyl label 72.7% TRR (0.348 mg/kg). The sum of characterized residue in liver sample was for chlorophenyl label 9.9% TRR (0.055 mg/kg) and for tolyl label 10.5% TRR (0.049 mg/kg) from extractable radioactivity, for chlorophenyl label 8.1% TRR (0.044 mg/kg) and for tolyl label 11.2% TRR (0.054 mg/kg) were released from post-extraction solids.
So the sum of identified and characterized residue in liver sample was for chlorophenyl label 92.6% TRR (0.514 mg/kg) and for tolyl label 94.4% TRR (0.451 mg/kg).
LC-MS analysis of the liver extracts confirmed the identification of only unchanged pyraclostrobin and 500M89 (Reg. No. 334089) in the samples.

A summary of identified, characterized and final radioactive residues in fish liver sample is presented in Table 6.2.5-11.

Table 6.2.5-11: Summary of identified and characterized ^{14}C-residues extracted from liver (after 10 daily doses of ^{14}C-BAS 500 F at 10 mg/kg feed nominal)

Designation	Extract		Total	
	mg/kg	% TRR	mg/kg	% TRR
Chlorophenyl label Acetonitrile Extract 0.470 mg/kg, 84.5% TRR				
Identified[1]				
BAS 500 F (Reg. No. 304428)	0.409	73.6	0.409	73.6
500M89 (Reg. No. 334089)	0.006	1.0	0.006	1.0
Total identified in extractable radioactivity			**0.415**	**74.6**
Characterized				
Unknown at the start point of TLC			0.037	6.7
Fat Precipitate			0.006	1.0
Water extract			0.012	2.2
Total characterized in extractable radioactivity			**0.055**	**9.9**
Total identified and characterized in extractable radioactivity			**0.470**	**84.5**
Post-Extraction solid			**0.086**	**15.5**
Grand Total			**0.556**	**100.0**
Tolyl label Acetonitrile Extract 0.399 mg/kg, 83.3% TRR				
Identified[1]				
BAS 500 F (Reg. No. 304428)	0.337	70.4	0.337	70.4
500M89 (Reg. No. 334089)	0.011	2.3	0.011	2.3
Total identified in extractable radioactivity			**0.348**	**72.7**
Characterized				
Unknown at the start point of TLC			0.032	6.9
Fat Precipitate			0.005	1.1
Water extract			0.012	2.5
Total characterized in extractable radioactivity			**0.049**	**10.5**
Total identified and characterized in extractable radioactivity			**0.397**	**83.2**
Post-Extraction solid			**0.080**	**16.7**
Grand Total			**0.477**	**99.9**

[1] Identified metabolites were resolved and quantified using LSC in combination with radio TLC and qualitative confirmation was performed by LC-MS

The nature of residual radioactive residues in the liver was investigated by treatment with digestive enzyme (protease). The process resulted in a further 6.1% TRR (0.034 mg/kg) for chlorophenyl and 10.0% TRR (0.048 mg/kg TRR) for tolyl being liberated. The digestion with pepsin enzyme resulted in liberation of 0.4% TRR (0.002 mg/kg) for chlorophenyl and 0.4% TRR (0.002 mg/kg TRR) for tolyl.
The digestion with pancreatin enzyme resulted in liberation of 1.4% TRR (0.008 mg/kg) for chlorophenyl and 0.8% TRR (0.004 mg/kg TRR) for tolyl.

A summary of characterized residues released from residual radioactive residues of fish liver is presented in Table 6.2.5-12.

Table 6.2.5-12: Summary characterized ^{14}C-residues released from residual radioactive residues of liver (after 10 daily doses of ^{14}C-BAS 500 F at 10 mg/kg feed nominal)

Designation	Post Extraction Solid (PES) Fractions			
	PES of liver		Sum of fractions	
	mg/kg	% TRR	mg/kg	% TRR
Chlorophenyl label PES of liver 0.086 mg/kg, 15.5% TRR				
Characterized				
Extractable following treatment with protease enzyme	0.034	6.1	0.034	6.1
Extractable following treatment with pepsin enzyme	0.002	0.4	0.002	0.4
Extractable following treatment with pancreatin enzyme	0.008	1.4	0.008	1.4
Total characterized in released radioactivity			**0.044**	**8.1**
Total identified and/or characterized radioactivity released from post-extraction solids			**0.044**	**8.1**
Tolyl label PES of liver 0.080 mg/kg, 16.7% TRR				
Characterized				
Extractable following treatment with protease enzyme	0.048	10.0	0.048	10.0
Extractable following treatment with pepsin enzyme	0.002	0.4	0.002	0.4
Extractable following treatment with pancreatin enzyme	0.004	0.8	0.004	0.8
Total characterized in released radioactivity			**0.054**	**11.2**
Total identified and/or characterized radioactivity released from post-extraction solids			**0.054**	**11.2**

Note: No meaningful chromatogram was obtained for the liver extract following treatment of post extraction solids with protease enzyme. The radioactive residues in the extract were characterized as extractable following treatment with protease, pepsin or pancreatin enzyme.

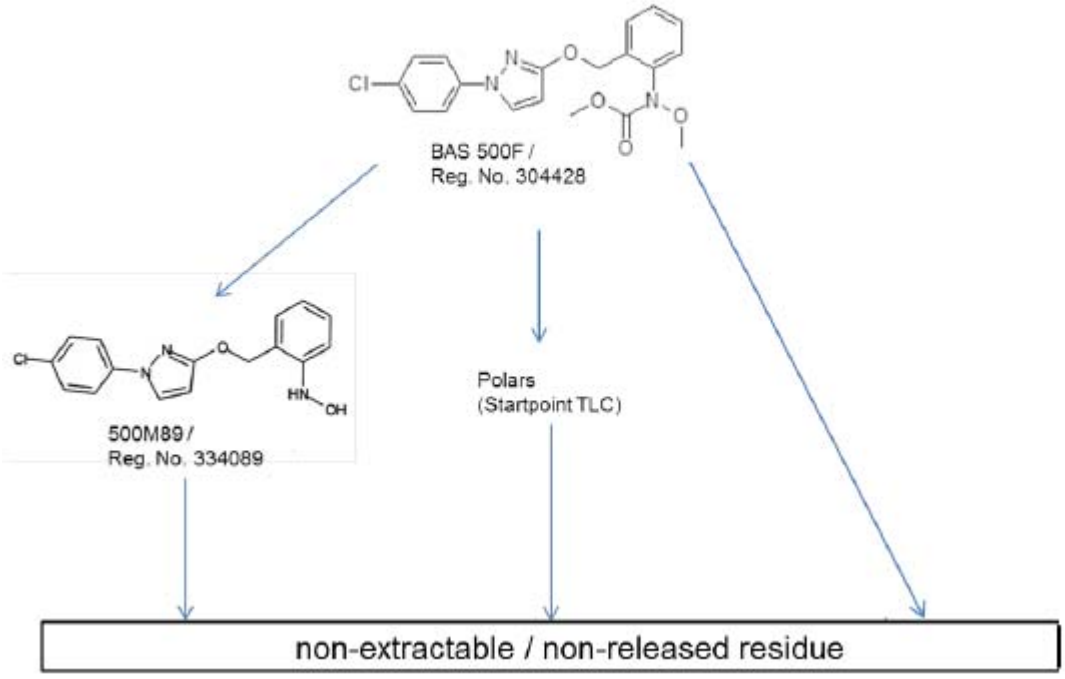
Two specific MRM transitions for each component were established and where these were detected in tissue extract, were used to confirm the assignments made by co-chromatography. MRM ion chromatograms for transitions A and B for compounds pyraclostrobin (Reg. No. 304428) and 500M89 (Reg. No. 334089) show peaks at 8.47 and 11.15 min, respectively, which confirms the presence of these compounds in this extracts.

Analysis of liver extract with HPLC method 2 led to only 1 peak of unchanged pyraclostrobin. Other residues were not detectable (below detection limit).

3. Proposed metabolic pathway

In all matrices (liver, filet skin, filet) and in both labels (chlorophenyl and tolyl) most of the extracted residues consisted only of unchanged parent (70.4-93.1% TRR). The metabolite 500M89 (Reg. No. 334089) occurred in minor amounts (1-4% TRR). An amount of 1.6 to 2.6% TRR in filet and filet skin were characterized as polar transformation products by TLC analysis. In liver samples 6.7 and 6.9% TRR can be assigned to this polar not identified fraction.
Thus, one metabolic transformation could be identified: cleavage of the amide yielding the metabolite 500M89 (Reg. No. 334089). Reactions leading to a cleavage of the molecule did not occur. Not identifiable residues in a range of 1-7% TRR were characterized by TLC as very polar compounds. In liver ca. 8-11% TRR were incorporated in the tissue and just releasable with digestive enzymes.

Figure 6.2.5-7: Pathway for the metabolism of pyraclostrobin in rainbow trout



4. Storage stability

All extraction, fractionation and HPLC analysis was completed within 6 months of sacrifice, therefore storage stability analysis was not performed.

III. CONCLUSION

The daily dose administered was 11.07 mg [Chlorophenyl-U-14C]-pyraclostrobin and 10.54 mg [Tolyl-14C]-pyraclostrobin respectively per kg food consumed.

The radioactive residues in filet accounted for 0.282 mg/kg (chlorophenyl label) and 0.239 mg/kg (tolyl label). In filet skin 0.229 mg/kg (skin chlorophenyl label) and 0.232 mg/kg (skin tolyl label) were found. Radioactive residues in liver accounted for 0.556 mg/kg (liver chlorophenyl label) and 0.479 mg/kg (liver tolyl label).

Approximately 99.40% of the [Chlorophenyl-U-14C]-pyraclostrobin dose and 85.79% of the [Tolyl-14C]-pyraclostrobin were recovered as a total mass balance, the majority of which was present in the active coal (ca 55%, respectively 46%).

The recovery of radioactivity in filet was relatively high compared to filet skin and liver. In the filet samples 4.21% for chlorophenyl label and 3.84% for tolyl label were recovered. In filet skin 0.20% for chlorophenyl label and 0.31% for tolyl label, in liver 0.39% for chlorophenyl label and 0.37% for tolyl label were recovered.

The extractability of the filet and filet skin was high, for filet 97.7% TRR (0.276 mg/kg; chlorophenyl) and 97.2% TRR (0.232 mg/kg; tolyl label) as well as for filet skin 97.8% TRR (0.225 mg/kg; chlorophenyl) and 98.0% TRR (0.228 mg/kg; tolyl), respectively. The extractability of liver accounted for 84.5% TRR (0.470 mg/kg; chlorophenyl) and 83.3% TRR (0.399 mg/kg; tolyl). The residue after extraction of liver was 15.5% (0.086 mg/kg) TRR for chlorophenyl and 16.7% TRR (0.080 mg/kg) for tolyl and was further hydrolyzed with digestive enzymes, whereby an additional 8.1% TRR (0.044 mg/kg) for chlorophenyl and 11.2% TRR (0.054 mg/kg) for tolyl was released.

The identification of 1 metabolite was based on co-chromatography in a radio TLC system and confirmation by LC-MS/MS in MRM mode.

In all matrices (filet, filet skin, liver) and in both labels (chlorophenyl and tolyl) most of the extracted residues consisted mainly of unchanged parent (70.4-93.1% TRR; 0.210-0.409 mg/kg). Only minor amounts of the metabolite 500M89 (Reg. No. 334089) were detected in extracted residue (1-4% TRR; 0.006-0.011 mg/kg). Taking the actual feed burden and the overdosing factor (8 x) into account, no significant residues of 500M89 will occur when fish are fed with commodities being treated with pyraclostrobin.

Only one metabolic transformation could be identified: Cleavage of the amide residue yielding the metabolite 500M89 (Reg. No. 334089). Reactions leading to a cleavage of the molecule between the ring systems did not occur. Therefore, both labels are comparable with respect to the overall result regarding metabolite formation of both studies. A degradation to polar residues occurred in a range of 1-7% TRR (0.005-0.037 mg/kg), as characterized by TLC. In liver ca. 8-11% TRR (0.044-0.054 mg/kg) were incorporated in the tissue and just releasable with digestive enzymes.

The results achieved in analysis of the chlorophenyl and tolyl label were very well comparable. The amounts of residues in the same tissues of both labels were very similar and in both labels the main identified residue consisted of unchanged pyraclostrobin. The results are in accordance with those obtained during the BCF study.

Although not requested by guideline, it was intended to conduct an in vitro metabolism study in rainbow trout cryopreserved hepatocytes and microsomes as supplementary information in comparison to the full metabolism study. However, due to so far insufficient metabolic activity in the cells reliable results are currently not available. The study will be continued to find out if this approach can principally be used in this context.
Due to the situation described above, the study is not part of the supplemental dossier, although it was included in the study list of the application for renewal of approval as data point KCA 6.2.5/3 (BASF DocID 2014/1001602).

CA 6.3 Magnitude of residues trials in plants

Pyraclostrobin is registered in multiple crops belonging to different EU crop groups. Within this dossier residue data are only provided for the representative uses supporting the renewal of approval. The solo EC formulation BAS 500 06 F and the mixed formulation BAS 516 07 F (containing pyraclostrobin and boscalid) have been selected as representative formulations.

Consequently, in this dossier section the relevant data for the following crops are summarized:

- Potatoes (BAS 516 07 F)
- Cereals (BAS 500 06 F)
- Maize including sweet corn (BAS 500 06 F)

In order to increase the readability of the dossier, the data for mixing partners are not shown in this dossier. The studies provided below for potatoes and cereals have been submitted before in context of national or zonal product registration dossiers. They have not been evaluated within the MRL re-evaluation process according to Reg. 396/2005, Art. 12. Consequently, they are not considered as peer-reviewed.

In case of maize/sweet corn and driven by the high importance of maize silage as feed item, parts of the studies had been provided to EFSA in context of the MRL evaluation process. The studies which had been assessed before are indicated accordingly by a remark above the study header so that a differentiation between “old” and new data should be possible without problems.

In M-CA 6.7 the residue levels found in samples destined for human food or for animal feed are compared with the data that have already been evaluated by EFSA. The relevant residue levels for parent are underlined. For the desmethoxy metabolite the codes 500M07 and BF 500-3 are used synonymously.

CA 6.3.1 Potatoes

The formulation BAS 516 07 F is registered in the Northern part of the EU. Due to the favorable residue behavior in potatoes with residues consistently below the LOQ of 0.02 mg/kg at all sampling intervals, only a reduced number of residue data is required.

In order to allow a more comprehensive evaluation in potatoes, the residue trials with the formulation BAS 536 01 F are additionally summarized. As shown in the table below, the mixed formulation containing pyraclostrobin and dimethomorph is registered at higher rates (100 g pyraclostrobin/ha compared to 17 g pyraclostrobin/ha), but is also registered in additional countries. The results obtained confirm the favorable residue behavior since even at the higher rates the residues found in tuber were well below the LOQ of 0.02 mg/kg.

Table 6.3.1-1: Summary of the critical GAP for the proposed use in potatoes for BAS 516 07 F

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)
					Rate* (kg as/ha)	Water (L/ha)	
Potatoes (N-EU)	O	41 – 89	4	5 -10	0.017	150-1000	0-3

* amount of pyraclostrobin per ha and application

Table 6.3.1-2: Summary of the critical GAP for the proposed use in potatoes for BAS 536 01 F (supplemental information)

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)
					Rate* (kg as/ha)	Water (L/ha)	
Potatoes (N-EU)	O	10 – 89	3	5	0.100	100-400	7
Potatoes (S-EU)	O	10 - 89	3	5	0.100	100-1000	3

* amount of pyraclostrobin per ha and application

Table 6.3.1-3: Number of residue trials conducted per geographical region and vegetation period

Crop	Vegetation period	Number of trials					Reference
		EU North	Country	EU South	Country	Total	
Potatoes	2003	5	DE,NL,DK,FR,UK	1	FR	6	6.3.1/1
	2005	5	DE,FR (2x), BE,DK	5	GR,ES,IT,FR (2x)	10	6.3.1/2
	2015	0	n.a.	4	GR,ES,IT,FR	4	6.3.1/3
Total number of trials per region		**10**		**10**	**Total number of trials**	**20**	

Report:	CA 6.3.1/1 Schulz H., 2004a Study on the residue behaviour of BAS 510 F and BAS 500 F in potatoes after application of BAS 516 00 F under field conditions in The Netherlands, Germany, United Kingdom, Denmark, France North and South, 2003 2004/1015948
Guidelines:	EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	BAS 516 00 F
	Description:	BAS 516 00 F: 6.7% of BAS 500 F (pyraclostrobin), 26.7% of BAS 510 F (boscalid), WG formulation
	Lot/Batch #:	2002-1
	Purity:	Not relevant
	CAS#:	Pyraclostrobin: 175013-18-0 Boscalid: 188425-85-6
	Development code:	not applicable
	Spiking levels:	Pyraclostrobin: 0.02 and 0.2 mg/kg
2.	**Test Commodity:**	Root and tuber vegetables
	Crop:	Potato tuber
	Type:	not applicable
	Variety:	Astate, Gloria, Sava, Europa, Agatha, Victoria
	Botanical name:	*Solanum tuberosum* L.
	Crop parts(s) or processed Commodity:	tuber
	Sample size:	min. 2.0 kg

B. STUDY DESIGN AND METHODS

1. Test procedure

In 2003, a total of 6 residue trials were conducted in potatoes in The Netherlands, Germany, United Kingdom, Denmark and France (North and South). The formulation BAS 516 00 F was applied 4 times at a rate of 0.02 kg as/ha of pyraclostrobin. The applications took place at 33 (±1), 23 (±1), 13 (±1) and 3 (±1) days before harvest with a spray rate of 150 L/ha. Samples were taken immediately after application and 3 ±1, 7 ±1 and 13 ±1 days thereafter. The samples were stored until analysis. The maximum storage interval from harvest until extraction was 162 days.

2. Description of analytical procedures

The specimens were analysed for residues of pyraclostrobin and its metabolite BF 500-3 using BASF method No. 445/0 which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.02 mg/kg for pyraclostrobin and its metabolite BF 500-3 in all of the specimen material. The mean recovery results for pyraclostrobin and its metabolite BF 500-3 were 87.7 ± 27.0% and 83.0 ± 14.5%, respectively, at fortification levels of 0.02 and 0.20 mg/kg.
Pyraclostrobin and BF 500-3 residues were extracted from the homogenized specimens with methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination of pyraclostrobin and BF 500-3 was performed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

In none of the samples analysed, any pyraclostrobin or BF 500-3 residues were found above the limit of quantitation of 0.02 mg/kg.

A summary of residues is presented in Table 6.3.1-4. Details are shown in Table 6.3.1-5.

Table 6.3.1-4: Summary of pyraclostrobin and BF 500-3 residues in potatoes after 4 applications of BAS 516 00 F

Matrix	Year	DALA[1]	Range of residues (mg/kg)		
			BAS 500 F	BF 500-3	BAS 500 F, total [2, 3]
Potato tuber	2003	0	<0.02	<0.02	<0.04
		3-4	<0.02	<0.02	<0.04
		6-8	<0.02	<0.02	<0.04
		12-14	<0.02	<0.02	<0.04

1 days after last application
2 as pyraclostrobin equivalent (conversion factor is 1.08)
3 for calculation purposes, "< 0.02" is set 0.02

III. CONCLUSION

No residues of pyraclostrobin or its metabolite BF 500-3 (500M07) above the limit of quantitation of 0.02 mg/kg were found in any of the analysed specimens.

Table 6.3.1-5: Residues of pyraclostrobin and BF 500-3 in potatoes after 4 applications of BAS 516 00 F

Trial details	Crop	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total[1, 2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 160753	Potatoes	Germany	BAS 516 00 F, Pyraclostrobin: 4x 0.020 kg as/ha	BBCH 48	0	tuber	<0.02	<0.02	<0.04	**BAS 500 F Method No.:** 445/0				
Doc ID: 2004/1015948					3	tuber	<0.02	<0.02	<0.04	fruit	0.02 / 0.20	4	87.7	27.0
Trial No. ACK/07/03					7	tuber	<0.02	<0.02	<0.04					
GLP: yes					13	tuber	<0.02	<0.02	<0.04	**BF 500-3 Method No.:** 445/0				
Year: 2003										fruit	0.02 / 0.20	4	83.0	14.5
Study code: 160753	Potatoes	The Nether-lands	BAS 516 00 F, Pyraclostrobin: 4x 0.020 kg as/ha	BBCH 48	0	tuber	<0.02	<0.02	<0.04					
Doc ID: 2004/1015948					4	tuber	<0.02	<0.02	<0.04					
Trial No. AGR/04/03					8	tuber	<0.02	<0.02	<0.04					
GLP: yes					14	tuber	<0.02	<0.02	<0.04					
Year: 2003														
Study code: 160753	Potatoes	Denmark	BAS 516 00 F, Pyraclostrobin: 4x 0.020 kg as/ha	BBCH 97	0	tuber	<0.02	<0.02	<0.04					
Doc ID: 2004/1015948					4	tuber	<0.02	<0.02	<0.04					
Trial No. ALB/09/03					7	tuber	<0.02	<0.02	<0.04					
GLP: yes					14	tuber	<0.02	<0.02	<0.04					
Year: 2003														
Study code: 160753	Potatoes	France (N)	BAS 516 00 F, Pyraclostrobin: 4x 0.020 kg as/ha	BBCH 47	0	tuber	<0.02	<0.02	<0.04					
Doc ID: 2004/1015948					3	tuber	<0.02	<0.02	<0.04					
Trial No. FAN/01/03					6	tuber	<0.02	<0.02	<0.04					
GLP: yes					12	tuber	<0.02	<0.02	<0.04					
Year: 2003														
Study code: 160753	Potatoes	France (S)	BAS 516 00 F, Pyraclostrobin: 4x 0.020 kg as/ha	BBCH 49	0	tuber	<0.02	<0.02	<0.04					
Doc ID: 2004/1015948					3	tuber	<0.02	<0.02	<0.04					
Trial No. FTL/08/03					7	tuber	<0.02	<0.02	<0.04					
GLP: yes					14	tuber	<0.02	<0.02	<0.04					
Year: 2003														

Table 6.3.1-5: Residues of pyraclostrobin and BF 500-3 in potatoes after 4 applications of BAS 516 00 F

Trial details	Crop	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total[1, 2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 160753	Potatoes	United Kingdom	BAS 516 00 F, Pyraclostrobin: 4x 0.020 kg as/ha	BBCH 81	0	tuber	<0.02	<0.02	<0.04					
Doc ID: 2004/1015948					3	tuber	<0.02	<0.02	<0.04					
Trial No. OAT/13/03					7	tuber	<0.02	<0.02	<0.04					
GLP: yes					14	tuber	<0.02	<0.02	<0.04					
Year: 2003														

[1] as BAS 500 F equivalent (conversion factor is 1.08)
[2] for calculation purposes, "< 0.02" is set 0.02

Report: CA 6.3.1/2
Schulz H., 2006a
Study on the residue behaviour of Dimethomorph and Pyraclostrobin in potatoes after treatment with BAS 536 01 F under field conditions in France (N & S), Denmark, Germany, Belgium, Italy, Spain and Greece, 2005
2006/1000581

Guidelines: EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 96/46, SANCO/825/00 rev. 7 (17 March 2004)

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 536 01 F

Description:	BAS 536 01 F: 40 g/L BAS 500 F (pyraclostrobin), 72 g/L BAS 550 F (dimethomorph), EC formulation
Lot/Batch #:	5041
Purity:	Not relevant
CAS#:	Pyraclostrobin: 175013-18-0 Dimethomorph: 110488-70-5
Development code:	not applicable
Spiking levels:	Pyraclostrobin: 0.02 and 0.02 mg/kg

2. Test Commodity: Root and tuber vegetables

Crop:	Potato tuber
Type:	Not relevant
Variety:	Dailfa, Gliceta, Primura, Kuras, Agata, Cesar, Vitesse, Cherie, Bintje, Hamlet
Botanical name:	*Solanum tuberosum* L.
Crop parts(s) or processed Commodity:	tuber
Sample size:	min. 2 kg

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2005 growing season, a total of 10 field trials were conducted in potatoes in order to determine the magnitude of the residues of active ingredient(s) in or on Raw Agricultural Commodities (RAC). BAS 536 01 F, an EC formulation of pyraclostrobin (40 g/L) and dimethomorph (72 g/L) was foliar applied 3 times at a rate of 2.5 L/ha of formulated product (0.1 kg as/ha of pyraclostrobin) to potatoes beginning about 21 days before expected harvest with a spray interval of 7 days. The spray volume used was 300 L/ha.
Potato tuber specimens were collected directly after the last application (DALA) from each plot at all locations as well as 3, 7 and 14 days thereafter.
The samples were stored frozen until analysis. The maximum storage interval from harvest until extraction was 234 days.

2. Description of analytical procedures

All samples were analysed with BASF method no. 445/0, which quantifies the residues of pyraclostrobin and its metabolite BF 500-3 with a limit of quantitation of 0.02 mg/kg each in all sample materials. Procedural recoveries obtained with the analytical series averaged at 87.3% for pyraclostrobin and at 85.6% for BF 500-3 at fortification levels of 0.02 and 0.20 mg/kg.
Pyraclostrobin and BF 500-3 residues were extracted from the homogenized specimens with methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination of pyraclostrobin and BF 500-3 was performed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

The analysis for pyraclostrobin and its metabolite BF 500-3 showed that in none of the potato tuber samples analysed any residues above limit of quantitation could be determined.

A summary of residues is presented in Table 6.3.1-6. Details are shown in Table 6.3.1-7.

Table 6.3.1-6: Summary of pyraclostrobin and BF 500-3 residues in potatoes after 3 applications of BAS 536 01 F

Matrix	Year	DALA[1]	Range of residues (mg/kg)		
			BAS 500 F	BF 500-3	BAS 500 F, total [2, 3]
Tuber	2005	0-15	<0.02	<0.02	<0.04

[1] days after last application
[2] as BAS 500 F equivalent (conversion factor is 1.084)
[3] for calculation purposes, "< 0.02" is set 0.02

III. CONCLUSION

No residues of pyraclostrobin or its metabolite BF 500-3 (500M07) above the limit of quantitation were found in any of the analysed specimens.

Table 6.3.1-7: Residues of pyraclostrobin and BF 500-3 in potatoes after 3 applications of BAS 536 01 F

Trial details	Crop	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total [1, 2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 210253	Potatoes	Greece	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	BBCH 47	0	tuber	<0.02	<0.02	<0.04	**BAS 500 F Method No.:** 445/0				
Doc ID: 2006/1000581					3	tuber	**<u><0.02</u>**	<0.02	<0.04	fruit	0.02 / 0.20	8	87.3	11.3
Trial No. 05RF048					7	tuber	<0.02	<0.02	<0.04					
GLP: yes					14	tuber	<0.02	<0.02	<0.04	**BF 500-3 Method No.:** 445/0				
Year: 2005										fruit	0.02 / 0.20	8	85.6	6.7
Study code: 210253	Potatoes	Spain	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	BBCH 47	0	tuber	<0.02	<0.02	<0.04					
Doc ID: 2006/1000581					4	tuber	**<u><0.02</u>**	<0.02	<0.04					
Trial No. 05ES083R					7	tuber	<0.02	<0.02	<0.04					
GLP: yes					14	tuber	<0.02	<0.02	<0.04					
Year: 2005														
Study code: 210253	Potatoes	Italy	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	BBCH 85-87	0	tuber	<0.02	<0.02	<0.04					
Doc ID: 2006/1000581					4	tuber	**<u><0.02</u>**	<0.02	<0.04					
Trial No. 0538R					7	tuber	<0.02	<0.02	<0.04					
GLP: yes					14	tuber	<0.02	<0.02	<0.04					
Year: 2005														
Study code: 210253	Potatoes	Germany	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	BBCH 71	0	tuber	<0.02	<0.02	<0.04					
Doc ID: 2006/1000581					3	tuber	<0.02	<0.02	<0.04					
Trial No. AT-05/006-1					7	tuber	**<u><0.02</u>**	<0.02	<0.04					
GLP: yes					15	tuber	<0.02	<0.02	<0.04					
Year: 2005														
Study code: 210253	Potatoes	France (N)	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	BBCH 45	0	tuber	<0.02	<0.02	<0.04					
Doc ID: 2006/1000581					3	tuber	<0.02	<0.02	<0.04					
Trial No. 05 F PT FR P21					7	tuber	**<u><0.02</u>**	<0.02	<0.04					
GLP: yes					14	tuber	<0.02	<0.02	<0.04					
Year: 2005														

Table 6.3.1-7: Residues of pyraclostrobin and BF 500-3 in potatoes after 3 applications of BAS 536 01 F

Trial details	Crop	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total [1, 2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 210253 **Doc ID:** 2006/1000581 **Trial No.** 05 F PT FR P22 **GLP:** yes **Year:** 2005	Potatoes	France (N)	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	BBCH 47	0	tuber	<0.02	<0.02	<0.04					
					2	tuber	<0.02	<0.02	<0.04					
					7	tuber	**<u><0.02</u>**	<0.02	<0.04					
					15	tuber	<0.02	<0.02	<0.04					
Study code: 210253 **Doc ID:** 2006/1000581 **Trial No.** 05 F PT FR P23 **GLP:** yes **Year:** 2005	Potatoes	France (S)	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	BBCH 47	0	tuber	<0.02	<0.02	<0.04					
					3	tuber	**<u><0.02</u>**	<0.02	<0.04					
					7	tuber	<0.02	<0.02	<0.04					
					14	tuber	<0.02	<0.02	<0.04					
Study code: 210253 **Doc ID:** 2006/1000581 **Trial No.** 05 F PT FR P24 **GLP:** yes **Year:** 2005	Potatoes	France (S)	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	BBCH 47	1	tuber	<0.02	<0.02	<0.04					
					3	tuber	**<u><0.02</u>**	<0.02	<0.04					
					7	tuber	<0.02	<0.02	<0.04					
					14	tuber	<0.02	<0.02	<0.04					
Study code: 210253 **Doc ID:** 2006/1000581 **Trial No.** G018-05 F-B **GLP:** yes **Year:** 2005	Potatoes	Belgium	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	BBCH 47-48	0	tuber	<0.02	<0.02	<0.04					
					2	tuber	<0.02	<0.02	<0.04					
					7	tuber	**<u><0.02</u>**	<0.02	<0.04					
					14	tuber	<0.02	<0.02	<0.04					
Study code: 210253 **Doc ID:** 2006/1000581 **Trial No.** ALB/190506-01 **GLP:** yes **Year:** 2005	Potatoes	Denmark	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	BBCH 89	0	tuber	<0.02	<0.02	<0.04					
					3	tuber	<0.02	<0.02	<0.04					
					7	tuber	**<u><0.02</u>**	<0.02	<0.04					
					14	tuber	<0.02	<0.02	<0.04					

<u>underlined values</u> are used for MRL calculation
[1] as BAS 500 F equivalent (conversion factor is 1.084)
[2] for calculation purposes, "< 0.02" is set 0.02

Report:	CA 6.3.1/3 Galvez O., Moreno S., 2016 a Study on the residue behaviour of Boscalid (BAS 510 F) and Pyraclostrobin (BAS 500 F) on potato after treatment with BAS 516 07 F under field conditions in South Europe, season 2015 2016/1000547
Guidelines:	2004/10/EC of 11 February 2004, Real Decreto 1369/2000, International guidelines for distribution and pesticides application AEPLA FAO 1985, EC 1107/2009 of the European Parliament, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997), OECD 509 Crop Field Trial (2009), ENV/MC/CHEM(98)17, OECD-ENV/JM/MONO(99)22, OECD-ENV/JM/MONO(2002)/9, EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	BAS 516 07 F
	Description:	BAS 516 07 F: 6.7% of BAS 500 F (pyraclostrobin), 26.7% of BAS 510 F (boscalid), WG formulation
	Lot/Batch #:	FRE-001182
	Purity:	Not relevant
	CAS#:	Pyraclostrobin: 175013-18-0 Boscalid: 188425-85-6
	Development code:	not applicable
	Spiking levels:	Pyraclostrobin: 0.01 and 0.1 mg/kg

2.	**Test Commodity:**	Root and tuber vegetables
	Crop:	Potato
	Type:	not applicable
	Variety:	Actrice, Jearla, Spunta, El Mundo
	Botanical name:	*Solanum tuberosum* L.
	Crop parts(s) or processed Commodity:	tuber
	Sample size:	1.0 - 5.0 kg/24 tubers

B. STUDY DESIGN AND METHODS

1. Test procedure

During 2015, a total of 4 residue trials were conducted in potatoes in representative potato growing areas in South Europe to determine the residue level of pyraclostrobin (BAS 500 F) and boscalid (BAS 510 F) in/on potato tubers after application of BAS 516 07 F. Each field consisted of one untreated (plot 1) and one treated plot (plot2).

In this summary only the results of residues of pyraclostrobin were reported. Results and conclusions of the other active substance (boscalid) are not considered as relevant in this summary.

The test item was foliar applied four times at 18 (±1), 12 (±1), 8 (±1) and 3 (±1) days before harvest at a rate of 16.75 g as/ha of pyraclostrobin and 66.75 g as/ha boscalid with a water volume of 300 L/ha. Plot 1 remained untreated.

Untreated and treated specimen of potato (tuber) were sampled at 0, 3, 7, and 13 - 14 DALA (days after last application). Control (untreated) specimens were collected prior to the treated specimens to avoid any contamination. All samples were double bagged.

The maximum storage interval from sampling to extraction for pyraclostrobin (BAS 500 F) and its metabolite 500M07 and boscalid (BAS 510 F) was 234 days. Storage stabilities of boscalid and pyraclostrobin and its metabolite were shown for 24 months.

2. Description of analytical procedures

Specimen from all trials were analyzed for pyraclostrobin (BAS 500 F) and its metabolite 500M07 (BF 500- 3) and boscalid (BAS 510 F) according to BASF analytical method No. 535/1 (L0076/01), which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg for all analytes.

The overall mean recovery for pyraclostrobin was at 92.6% (n=6) and for its metabolite 500M07 at 99.6% (n=6), respectively, at fortification levels of 0.01 and 0.10 mg/kg.

Pyraclostrobin and 500M07 residues were extracted from the homogenized specimens with methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination of pyraclostrobin and 500M07 was performed by LC-MS/MS.

The results of procedural recovery experiments are summarised in Table 6.3.1-8.

Table 6.3.1-8 Summary of recoveries of pyraclostrobin and its metabolite in potato tuber specimen in Southern Europe

Matrix	Fortification level (mg/kg)	Summary recoveries							
		n	Mean (%)	SD (+/-)	RSD (%)	n	Mean (%)	SD (+/-)	RSD (%)
Method No. 535/1		Pyraclostrobin				500M07			
Potato tuber	0.01	3	96.5	8.7	9.0	3	102	9.2	9.0
	0.1	3	88.7	5.8	6.6	3	97.6	8.4	8.7
overall		**6**	**92.6**	**7.9**	**8.5**	**6**	**99.6**	**8.2**	**8.2**

II. RESULTS AND DISCUSSION

In none of the analysed samples any pyraclostrobin or 500M07 residues were found above the limit of quantitation of 0.01 mg/kg.

A summary of residues is presented in Table 6.3.1-4. Details are shown in Table 6.3.1-5.

Table 6.3.1-9: Summary of pyraclostrobin and 500M07 residues in potatoes after four applications of BAS 516 07 F

Matrix	Year	DALA[1]	Range of residues (mg/kg)		
			BAS 500 F	500M07	BAS 500 F, total [2, 3]
Potato tuber	2015	0	<0.01	<0.011	<0.021
		3	<0.01	<0.011	<0.021
		7	<0.01	<0.011	<0.021
		13-14	<0.01	<0.011	<0.021

[1] days after last application
[2] as pyraclostrobin equivalent (conversion factor is 1.08)
[3] for calculation purposes, "< 0.01" is set 0.01

III. CONCLUSION

No residues of pyraclostrobin or its metabolite 500M07 above the limit of quantitation of 0.01 mg/kg were found in any of the analysed specimens.

Table 6.3.1-10: Residues of pyraclostrobin and 500M07 in potatoes after 4 applications of BAS 516 07 F

Trial details	Crop	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	500M07 [1]	Total [2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 773801 **Doc ID:** 2016/1000547 **Trial No.** L150224 **GLP:** yes **Year:** 2015	Potatoes	France	BAS 516 07 F, Pyraclostrobin: 4x 0.01675 kg as/ha	BBCH 48	0	tuber	<0.01	<0.011	<0.021	**BAS 500 F Method No.:** 535/1				
					3	tuber	<0.01	<0.011	<0.021	tuber	0.01 / 0.10	6	92.6	8.5
					7	tuber	<0.01	<0.011	<0.021					
					14	tuber	<0.01	<0.011	<0.021	**500M07 Method No.:** 535/1				
										tuber	0.01 / 0.10	6	99.6	8.2
Study code: 773801 **Doc ID:** 2016/1000547 **Trial No.** L150225 **GLP:** yes **Year:** 2015	Potatoes	Greece	BAS 516 07 F, Pyraclostrobin: 4x 0.01675 kg as/ha	BBCH 48	0	tuber	<0.01	<0.011	<0.021					
					3	tuber	<0.01	<0.011	<0.021					
					7	tuber	<0.01	<0.011	<0.021					
					14	tuber	<0.01	<0.011	<0.021					
Study code: 773801 **Doc ID:** 2016/1000547 **Trial No.** L150226 **GLP:** yes **Year:** 2015	Potatoes	Italy	BAS 516 07 F, Pyraclostrobin: 4x 0.01675 kg as/ha	BBCH 48	0	tuber	<0.01	<0.011	<0.021					
					3	tuber	<0.01	<0.011	<0.021					
					7	tuber	<0.01	<0.011	<0.021					
					13	tuber	<0.01	<0.011	<0.021					
Study code: 773801 **Doc ID:** 2016/1000547 **Trial No.** L150227 **GLP:** yes **Year:** 2015	Potatoes	Spain	BAS 516 07 F, Pyraclostrobin: 4x 0.01675 kg as/ha	BBCH 48	0	tuber	<0.01	<0.011	<0.021					
					3	tuber	<0.01	<0.011	<0.021					
					7	tuber	<0.01	<0.011	<0.021					
					13	tuber	<0.01	<0.011	<0.021					

[1] expressed as parent equivalent, conversion factor for calculation of 500M07 to parent is 1.08
[2] for residues < 0.01 mg/kg (for 500M07 < 0.011 mg/kg), value was set to 0.01 mg/kg (for 500M07 to 0.011 mg/kg) for calculation of sum

CA 6.3.2 Cereals (wheat, barley)

Multiple residue studies have been performed in cereals for the active substance pyraclostrobin in solo and mixed formulations in cereal crops. The residue data were evaluated during the previous Annex I inclusion process, various national submissions, but also in context of the EU MRL re-evaluation according to Reg. 396/055 Art. 12. They were considered as suitable for establishing an EU MRL for wheat, rye, barley and oats. Between 1998 and 2005 more than 70 trials were conducted in representative growing areas of the Northern and Southern part of Europe. In the trials, two applications were performed at 250 g as/ha.

Despite the broad coverage, new residue trials in cereals have been performed between 2009 and 2012. The purpose of these trials was to provide a consistent residue data package for the formulation BAS 500 06 F, but also to ensure that the residue levels in grain are covered by the established EU MRLs. The trials were performed in accordance with the most critical registered GAP (see Table 6.3.2-1). The distribution of these trials is shown in Table 6.3.2-2.

Table 6.3.2-1: Summary of the critical GAP for the proposed use in cereals for BAS 500 06 F

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)**
					Rate* (kg as/ha)	Water (L/ha)	
Barley	O	25 -69	2	21	0.25	100 – 400	35
Oat	O	25 -69	2	21	0.25	100 – 400	35
Wheat	O	25 -69	2	21	0.25	100 – 400	35
Triticale	O	25 -69	2	21	0.25	100 – 400	35
Rye	O	25 -69	2	21	0.25	100 – 400	35

* amount of pyraclostrobin per ha and application
** for cereal crops the actual preharvest interval is defined by the latest growth stage of application (which is BBCH 69)

Table 6.3.2-2: Number of residue trials conducted per geographical region and vegetation period

Crop	Vegetation period	Number of trials					Reference
		EU North	Country	EU South	Country	Total	
Barley	2011	4	DE (2x),NL,FR	4	FR,GR,IT,ES	8	6.3.2/1
	2010	4	DE,UK,DK,FR	4	FR,GR,IT,ES	8	6.3.2/2
	2009[1]	2	DE,FR	2	IT,ES	4	6.3.2/3
	2012[1]	4	DE (2x),UK,FR	4	FR,GR,IT,ES	8	6.3.2/4, 6.3.2/5
Wheat	2010	4	DE,UK,DK,FR	4	FR,GR,IT,ES	8	6.3.2/6
	2011	4	DE (2x),NL,UK	4	FR,GR,IT,ES	8	6.3.2/7
	2011[1]	2	DE,UK	2	FR,IT	4	6.3.2/8, 6.3.2/9
	2012[1]	2	DE,UK	2	FR,ES	4	6.3.2/10
Total number of trials per region		**14+12**		**14+12**	**Total number of trials**	**52**	

[1] BAS 500 06 F was included for comparison purposes in bridging trials.

Report:	CA 6.3.2/1 Plier S., 2013a Determination of residues of BAS 500 F in barley after two applications of BAS 500 06 F in Germany, Netherlands, France (North), France (South), Greece, Italy and Spain, 2011 2012/1067587
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, OECD 509 Crop Field Trial (2009), Working document of the Commission of the European Communities Directorate General for Agriculture VI B II-1 Appendix B, EEC 7029/VI/95 rev. 5
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F

Description:	BAS 500 06 F: pyraclostrobin (BAS 500 F), 200.0 g/L nominal content, EC
Lot/Batch #:	0003223026
Purity:	99.9%
CAS#:	Pyraclostrobin: 175013-18-0 BF 500-3: 512165-96-7
Development code:	not applicable
Spiking levels:	0.01-10 mg/kg

2. Test Commodity: Cereals

Crop:	Barley
Type:	not applicable
Variety:	Marthe, Laverda, Lomerit, Cervoise, Franzi, Moutso, Mattina, Caballar
Botanical name:	*Hordeum vulgare L.*
Crop parts(s) or processed Commodity:	Whole plant w/o roots, ears, rest of plant w/o roots, grain, straw
Sample size:	Whole plant w/o roots: ≥0.5 mg/kg, ears: ≥1.0 mg/kg, rest of plant w/o roots ears: ≥0.7 mg/kg, grain: ≥1.0 mg/kg, straw: ≥0.5 mg/kg

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2011 growing season, 8 field trials were conducted in Northern and Southern Europe in order to determine the magnitude of residues of pyraclostrobin in barley after two applications of BAS 500 06 F. Each field trial consisted of a control and a treated plot. BAS 500 06 F (200 g/L pyraclostrobin), was applied twice with an application rate of 0.25 kg as/ha and a spray volume of 200 L/ha at growth stages BBCH 49 and 69. Whole plant no roots specimens were taken directly after the last application. Depending on the maturity ears and "rest of plant without roots" or grain and straw specimens were taken at 28 ± 1, 35 ± 1 and 42 ± 1 days after last application. If crop stage BBCH 89 was not reached at 42 ± 1 DALA, an additional sampling of grain and straw was made.
The samples were stored deep frozen until analysis. The maximum storage interval from harvest until analysis for plant samples was 385 days.

Table 6.3.2-3: Application and sampling details for trials conducted in 2011

Region	No. of trials	No. of appl.	F, P, or G	Test item	Active substance	Application		Target timing	
						Rate as (kg/ha)	Water vol. (L/ha)	Application	Sampling (DALA)[1]
Northern and Southern Europe	8	2	F	BAS 500 06 F (EC)	BAS 500 F	0.25	200	BBCH 49 BBCH 69	0 28 +/- 1 35 +/- 1 42 +/- 1 BBCH 89

[1] days after last application

2. Description of analytical procedures

All specimens were analysed for pyraclostrobin and its metabolite BF 500-3 using BASF method 535/1 (synonym: L0076/01).
The analytes were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method for pyraclostrobin and its metabolite BF 500-3 was 0.01 mg/kg.
The results of procedural recovery experiments were 88% for pyraclostrobin and 85% for BF 500-3 at fortification levels between 0.01 and 10 mg/kg.

II. RESULTS AND DISCUSSION

At pre harvest interval (PHI) of 34-35 days residues of pyraclostrobin ranged between 0.06 and 0.24 mg/kg in ears, 1.02 and 3.08 mg/kg in rest of plant without roots, 0.02 and 0.22 mg/kg in grain and 0.50 and 7.68 mg/kg in straw.
The residues of the metabolite BF 500-3 ranged between 0.05 and 0.21 mg/kg in ears, 0.14 and 0.92 mg/kg in rest of plant without roots, 0.01 and 0.09 mg/kg in grain and 0.26 and 2.89 mg/kg in straw. In the control samples no residues at or above the limit of quantitation were found.

A summary of residues is presented in Table 6.3.2-4. Details are shown in Table 6.3.2-5.

Table 6.3.2-4: Summary of pyraclostrobin and BF 500-3 residues in the treated barley specimens after application of BAS 500 06 F

Crop	Year	Appl.	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)			
					Matrix	BAS 500 F	BF 500-3[3]	Total[4]
Barley	2011	BAS 500 06 F (EC)	0	69	whole plant[2]	4.64 – 8.16	0.08 – 0.36	4.76 – 8.48
			27-28	72-87	ears	0.05 – 0.86	0.04 – 0.49	0.09 – 1.35
					rest of plant[2]	0.37 – 6.10	0.15 – 2.16	0.53 – 8.26
			34-35	73-87	ears	0.06 – 0.24	0.05 – 0.21	0.11 – 0.45
			34-35	85-89	rest of plant[2]	1.02 – 3.08	0.14 – 0.92	1.16 – 4.00
					grain	0.02 – 0.22	0.01 – 0.09	0.03 – 0.31
					straw	0.50 – 7.68	0.26 – 2.89	0.76 – 10.57
			41	77	ears	0.06	0.05	0.11
					rest of plant[2]	0.87	0.14	1.01
			41-42	85-89	grain	0.01 – 0.22	0.01 – 0.11	0.02 – 0.31
					straw	0.42 – 8.73	0.26 – 3.93	0.68 – 12.66
			48-56	89	grain	<0.01 – 0.08	<0.01 – 0.03	<0.02 – 0.11
					straw	0.29 – 2.23	0.19 – 0.80	0.48 – 3.03

[1] days after last application
[2] no roots
[3] as BAS 500 F equivalent (conversion factor is 1.084)
[4] for calculation purposes, "< 0.01" is set 0.01

III. CONCLUSION

At pre harvest interval (PHI) of 34-35 days residues of pyraclostrobin ranged between 0.06 and 0.24 mg/kg in ears, 1.02 and 3.08 mg/kg in rest of plant without roots, 0.02 and 0.22 mg/kg in grain and 0.50 and 7.68 mg/kg in straw.
The residues of the metabolite BF 500-3 (500M07) ranged at pre harvest interval (PHI) of 34-35 days between 0.05 and 0.21 mg/kg in ears, 0.14 and 0.92 mg/kg in rest of plant without roots, 0.01 and 0.09 mg/kg in grain and 0.26 and 2.89 mg/kg in straw.

Table 6.3.2-5: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371238 **Doc ID:** 2012/1067587 **Trial No.** L110150 **GLP:** yes **Year:** 2011	Barley / Marthe	Germany	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	4.92	0.17	5.09	**BAS 500 F Method No.:535/1 (L0076/01)**				
					27	ears	0.05	0.04	0.09	all sample matrices	0.01 - 10	27	88.2	8.7
					27	rest of plant[1]	0.37	0.16	0.53					
					35	grain	**0.02**	0.01	0.03	**BF 500-3 Method No.: 535/1 (L0076/01)**				
					35	straw	**0.50**	0.26	0.76	all sample matrices	0.01 - 10	27	84.5	11.3
					42	grain	0.01	0.01	0.02					
					42	straw	0.42	0.26	0.68					
					52	grain	<0.01	<0.01	<0.02					
					52	straw	0.29	0.19	0.48					
Study code: 371238 **Doc ID:** 2012/1067587 **Trial No.** L110151 **GLP:** yes **Year:** 2011	Barley / Laverda	Germany	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	8.16	0.32	8.48					
					28	ears	0.86	0.49	1.35					
					28	rest of plant[1]	6.10	2.16	8.26					
					34	grain	**0.22**	0.09	0.31					
					34	straw	7.68	2.89	10.57					
					42	grain	0.22	0.09	0.31					
					42	straw	**8.73**	3.93	12.66					
Study code: 371238 **Doc ID:** 2012/1067587 **Trial No.** L110152 **GLP:** yes **Year:** 2011	Barley / Lomerit	The Nether lands	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	5.76	0.15	5.91					
					28	ears	0.20	0.11	0.31					
					28	rest of plant[1]	2.72	0.59	3.31					
					34	ears	0.15	0.09	0.24					
					34	rest of plant[1]	2.24	0.62	2.86					
					41	grain	**0.09**	0.03	0.12					
					41	straw	**2.61**	0.73	3.34					
					48	grain	0.08	0.03	0.11					
					48	straw	2.23	0.80	3.03					

Table 6.3.2-5: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371238 **Doc ID:** 2012/1067587 **Trial No.** L110153 **GLP:** yes **Year:** 2011	Barley / Cervoise	France (N)	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	4.64	0.12	4.76					
					27	ears	0.22	0.19	0.41					
					27	rest of plant[1]	3.02	0.81	3.83					
					35	ears	0.21	0.20	0.41					
					35	rest of plant[1]	3.04	0.88	3.92					
					41	grain	**0.07**	0.05	0.12					
					41	straw	**3.98**	1.15	5.13					
Study code: 371238 **Doc ID:** 2012/1067587 **Trial No.** L110154 **GLP:** yes **Year:** 2011	Barley / Franzi	France (S)	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	6.40	0.36	6.76					
					28	ears	0.24	0.18	0.42					
					28	rest of plant[1]	2.68	0.73	3.41					
					35	ears	0.24	0.21	0.45					
					35	rest of plant[1]	3.08	0.92	4.00					
					42	grain	**0.03**	0.02	0.05					
					42	straw	**3.36**	1.08	4.44					
Study code: 371238 **Doc ID:** 2012/1067587 **Trial No.** L110155 **GLP:** yes **Year:** 2011	Barley / Moutso	Greece	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	7.04	0.32	7.36					
					28	ears	0.10	0.09	0.19					
					28	rest of plant[1]	1.29	0.15	1.44					
					35	ears	0.06	0.05	0.11					
					35	rest of plant[1]	1.02	0.14	1.16					
					41	ears	0.06	0.05	0.11					
					41	rest of plant[1]	0.87	0.14	1.01					
					56	grain	**<0.01**	<0.01	<0.02					
					56	straw	**1.04**	0.23	1.27					

Table 6.3.2-5: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371238 **Doc ID:** 2012/1067587 **Trial No.** L110156 **GLP:** yes **Year:** 2011	Barley / Mattina	Italy	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	5.82	0.28	6.10					
					27	ears	0.57	0.31	0.88					
					27	rest of plant[1]	2.04	0.52	2.56					
					34	grain	0.13	0.09	0.22					
					34	straw	3.33	0.80	4.13					
					41	grain	**0.14**	0.11	0.25					
					41	straw	**3.53**	1.08	4.61					
Study code: 371238 **Doc ID:** 2012/1067587 **Trial No.** L110157 **GLP:** yes **Year:** 2011	Barley / Caballar	Spain	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	5.64	0.08	5.72					
					27	ears	0.70	0.35	1.05					
					27	rest of plant[1]	3.28	0.86	4.14					
					34	grain	**0.09**	0.05	0.14					
					34	straw	2.77	0.82	3.59					
					41	grain	0.07	0.04	0.11					
					41	straw	**3.05**	1.06	4.11					

underlined values are used for MRL calculation
[1] w/o roots
[2] as BAS 500 F equivalent (conversion factor is 1.084)
[3] for calculation purposes, "< 0.01" is set 0.01

Report: CA 6.3.2/2
Plier S., 2011a
Determination of residues of BAS 500 F in barley after two applications of BAS 500 06 F in Germany, United Kingdom, Denmark, France (North), France (South), Greece, Italy and Spain, 2010
2011/1135916

Guidelines: EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, OECD 509 Crop Field Trial (2009)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F

Description:	BAS 500 06 F: pyraclostrobin (BAS 500 F), 200.0 g/L nominal content, EC
Lot/Batch #:	0003223026
Purity:	99.9%
CAS#:	Pyraclostrobin: 175013-18-0 BF 500-3: 512165-96-7
Development code:	not applicable
Spiking levels:	0.01 - 1.0 mg/kg

2. Test Commodity: Cereals

Crop:	Barley
Type:	not applicable
Variety:	Zoom, Saffron, Quench, Abondance, Yoole, Moutso, Mattina, Henley
Botanical name:	*Hordeum vulgare L.*
Crop parts(s) or processed Commodity:	Whole plant w/o roots, ears, rest of plant w/o roots, grain, straw
Sample size:	Whole plant w/o roots: ≥0.5 mg/kg, ears: ≥1.0 mg/kg, rest of plant w/o roots ears: ≥1.0 mg/kg, grain: ≥1.0 mg/kg, straw: ≥0.5 mg/kg

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2010 growing season, a total of 8 field trials were conducted in Northern and Southern Europe in order to determine the magnitude of residues of pyraclostrobin in barley after two applications of BAS 500 06 F. Each field trial consisted of a control and a treated plot. BAS 500 06 F (200 g/L pyraclostrobin), was foliar applied twice with an application rate of 0.25 kg as/ha and a spray volume of 200 L/ha at growth stages BBCH 49 and 69. "Whole plant without roots" specimens were taken directly after the last application. Depending on the maturity ears and "rest of plant without roots" or grain and straw specimens were taken at 28 ± 1, 35 ± 1 and 42 ± 1 days after last application. If crop stage BBCH 89 was not reached at 42 ± 1 DALA, an additional sampling was made.
The samples were stored deep frozen until analysis. The maximum storage interval from harvest until analysis for plant samples was 406 days.

Table 6.3.2-6: Application and sampling details for trials conducted in 2010

Region	No. of trials	No. of appl.	F, P, or G	Test item	Active substance	Application		Target timing	
						Rate as (kg/ha)	Water vol. (L/ha)	Application	Sampling (DALA)[1]
Northern and Southern Europe	8	2	F	BAS 500 06 F (EC)	BAS 500 F	0.25	200	BBCH 49 BBCH 69	0 28 +/- 1 35 +/- 1 42 +/- 1 BBCH 89

[1] days after last application

2. Description of analytical procedures

All specimens were analysed for pyraclostrobin and its metabolite BF 500-3 using BASF method 535/1 (synonym: L0076/01).
The analytes were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method for pyraclostrobin and its metabolite BF 500-3 was 0.01 mg/kg.
The results of procedural recovery experiments were 89.1% for pyraclostrobin and 87.3% for BF 500-3 at fortification levels between 0.01 and 1.0 mg/kg.

II. RESULTS AND DISCUSSION

At pre harvest interval (PHI) of 34-36 days residues of pyraclostrobin ranged between 0.10 and 2.66 mg/kg in ears, 0.59 and 7.13 mg/kg in rest of plant without roots, 0.13 and 0.54 mg/kg in grain and 3.79 and 4.24 mg/kg in straw. The residues of the metabolite BF 500-3 ranged between 0.02 and 0.98 mg/kg in ears, 0.10 and 1.69 mg/kg in rest of plant without roots, 0.09 and 0.35 mg/kg in grain and 1.09 and 1.59 mg/kg in straw.
In untreated specimens residues were always below 0.01 mg/kg with two exceptions. One grain specimen contained 0.02 mg/kg of pyraclostrobin and 0.01 mg/kg of the metabolite BF 500-3. One straw specimen contained 0.11 mg/kg of pyraclostrobin and 0.04 mg/kg of the metabolite BF 500-3. The residues in the control sample are most likely caused by an unknown contamination.

A summary of residues is presented in Table 6.3.2-7. Details are shown in Table 6.3.2-8.

Table 6.3.2-7: Summary of pyraclostrobin and BF 500-3 residues in the treated barley specimens after application of BAS 500 06 F

Crop	Year	Appl.	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)			
					Matrix	BAS 500 F	BF 500-3[3]	Total[4]
Barley	2010	BAS 500 06 F (EC)	0	69	whole plant[2]	3.60 – 7.46	0.08 – 0.35	3.76 – 7.77
			28-29	73/75-85	ears	0.14 – 3.93	0.03 – 1.22	0.17 – 5.15
					rest of plant[2]	0.70 – 7.92	0.09 – 1.70	0.79 – 9.62
			27	89	grain	0.13, 0.65	0.7, 0.41	0.20, 1.06
					straw	2.45, 3.33	0.79, 0.91	3.24, 4.24
			35-36	77-89	ears	0.10 – 2.66	0.02 – 0.98	0.12 – 3.64
					rest of plant[2]	0.59 – 7.13	0.10 – 1.69	0.69 – 8.82
			34-36	89	grain	0.13, 0.54	0.09, 0.35	0.22, 0.89
					straw	3.79, 4.24	1.09, 1.59	4.88, 5.83
			41-44	85-89	ears	0.20, 0.26	0.14, 0.26	0.34, 0.52
					rest of plant[2]	0.71, 1.99	0.13, 0.54	0.84, 2.53
			41-44	87-89	grain	0.08 – 0.82	0.02 – 0.31	0.13 – 0.95
					straw	0.77 – 6.30	0.14 – 1.83	0.91 – 8.13
			49-52	89	grain	0.06 – 0.09	0.02 – 0.04	0.10 – 0.12
					straw	0.75 – 1.28	0.15 – 0.42	0.90 – 1.70

[1] days after last application
[2] no roots
[3] as BAS 500 F equivalent (conversion factor is 1.084)
[4] for calculation purposes, "< 0.01" is set 0.01

III. CONCLUSION

At pre harvest interval (PHI) of 34-36 days, residues of pyraclostrobin ranged between 0.10 and 2.66 mg/kg in ears, 0.59 and 7.13 mg/kg in rest of plant without roots, 0.13 and 0.54 mg/kg in grain and 3.79 and 4.24 mg/kg in straw.
The residues of the metabolite BF 500-3 (500M07) ranged in specimens, sampled at the PHI of 34-36 days, between 0.02 and 0.98 mg/kg in ears, 0.10 and 1.69 mg/kg in rest of plant without roots, 0.09 and 0.35 mg/kg in grain and 1.09 and 1.59 mg/kg in straw.

Table 6.3.2-8: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371234 **Doc ID:** 2011/1135916 **Trial No.** L100093 **GLP:** yes **Year:** 2010	Barley / Zoom	Germany	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	5.80	0.10	5.90	**BAS 500 F Method No.:535/1 (L0076/01)**				
					28	ears	0.26	0.16	0.42	all sample matrices	0.01, 0.1, 1.0	23	89.1	7.5
					28	rest of plant[1]	1.67	0.40	2.07					
					35	ears	0.44	0.25	0.69	**BF 500-3 Method No.: 535/1 (L0076/01)**				
					35	rest of plant[1]	2.17	0.54	2.71	all sample matrices	0.01, 0.1, 1.0	23	87.3	8.0
					41	grain	**0.10**	0.06	0.16					
					41	straw	**2.75**	0.71	3.46					
Study code: 371234 **Doc ID:** 2011/1135916 **Trial No.** L100094 **GLP:** yes **Year:** 2010	Barley / Saffron	United Kingdom	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	3.60	0.16	3.76					
					28	ears	0.16	0.08	0.24					
					28	rest of plant[1]	2.26	0.48	2.74					
					35	ears	0.27	0.18	0.45					
					35	rest of plant[1]	1.06	0.24	1.30					
					42	ears	0.20	0.14	0.34					
					42	rest of plant[1]	1.99	0.54	2.53					
					49	grain	**0.08**	0.04	0.12					
					49	straw	**1.28**	0.42	1.70					
Study code: 371234 **Doc ID:** 2011/1135916 **Trial No.** L100095 **GLP:** yes **Year:** 2010	Barley / Quench	Denmark (N)	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	7.34	0.31	7.65					
					29	ears	3.93	1.22	5.15					
					29	rest of plant[1]	7.92	1.70	9.62					
					36	ears	2.66	0.98	3.64					
					36	rest of plant[1]	7.13	1.69	8.82					
					42	grain	**0.82**	0.13	0.95					
					42	straw	**6.30**	1.83	8.13					

Table 6.3.2-8: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371234 **Doc ID:** 2011/1135916 **Trial No.** L100096 **GLP:** yes **Year:** 2010	Barley / Abon-dance	France (N)	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	5.44	0.34	5.78					
					28	ears	0.13	0.08	0.21					
					28	rest of plant[1]	2.35	0.66	3.01					
					35	ears	0.22	0.15	0.37					
					35	rest of plant[1]	2.32	0.74	3.06					
					42	grain	**0.11**	0.06	0.17					
					42	straw	**2.42**	0.83	3.25					
Study code: 371234 **Doc ID:** 2011/1135916 **Trial No.** L100097 **GLP:** yes **Year:** 2010	Barley / Yoole	France (S)	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	4.32	0.11	4.43					
					28	ears	0.14	0.03	0.17					
					28	rest of plant[1]	0.70	0.09	0.79					
					36	ears	0.10	0.02	0.12					
					36	rest of plant[1]	0.59	0.10	0.69					
					44	grain	**0.11**	0.02	0.13					
					44	straw	**0.77**	0.14	0.91					
					51	grain	0.09	0.02	0.11					
					51	straw	0.75	0.15	0.90					
Study code: 371234 **Doc ID:** 2011/1135916 **Trial No.** L100098 **GLP:** yes **Year:** 2010	Barley / Moutso	Greece	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	6.54	0.35	6.89					
					28	ears	0.32	0.22	0.54					
					28	rest of plant[1]	1.25	0.19	1.44					
					35	ears	0.18	0.17	0.35					
					35	rest of plant[1]	0.76	0.13	0.89					
					42	ears	0.26	0.26	0.52					
					42	rest of plant[1]	0.71	0.13	0.84					
					52	grain	**0.06**	0.04	0.10					
					52	straw	**0.98**	0.18	1.16					

Table 6.3.2-8: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371234 **Doc ID:** 2011/1135916 **Trial No.** L100099 **GLP:** yes **Year:** 2010	Barley / Mattina	Italy	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	5.14	0.08	5.22					
					27	grain	0.65	0.41	1.06					
					27	straw	3.33	0.91	4.24					
					36	grain	**0.54**	0.35	0.89					
					36	straw	**3.79**	1.09	4.88					
					41	grain	0.44	0.31	0.75					
					41	straw	3.53	0.88	4.41					
Study code: 371234 **Doc ID:** 2011/1135916 **Trial No.** L100100 **GLP:** yes **Year:** 2010	Barley / Henley	Spain	BAS 500 06 F, Pyraclostrobin: 2 x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	7.46	0.31	7.77					
					27	grain	0.13	0.07	0.20					
					27	straw	2.45	0.79	3.24					
					34	grain	**0.13**	0.09	0.22					
					34	straw	**4.24**	1.59	5.83					
					41	grain	0.08	0.06	0.14					
					41	straw	2.30	0.80	3.10					

underlined values are used for MRL calculation
[1] w/o roots
[2] as BAS 500 F equivalent (conversion factor is 1.084)
[3] for calculation purposes, "< 0.01" is set 0.01

Report: CA 6.3.2/3
Erdmann H.-P., 2010a
Study on the residue behaviour of BAS 700 F, Epoxiconazole and Pyraclostrobin in barley after application of BAS 702 01 F, BAS 700 00 F, BAS 500 06 F and BAS 480 38 F under field condition in France, Spain, Italy and Germany, 2009
2010/1006342

Guidelines: EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7

GLP: yes
(certified by Land Brandenburg Ministerium fuer Laendliche Entwicklung, Umwelt und Verbraucherschutz, Potsdam, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 702 01 F, BAS 700 00 F, BAS 500 06 F, BAS 480 38 F

Description:	BAS 702 01 F (BAS 700 F, BAS 500 F, BAS 480 F), BAS 700 00 F (BAS 700 F), BAS 500 06 F (BAS 500 F), BAS 480 38 F (BAS 480 F)	
Lot/Batch #:	205005, BAS 700 F: 42 g/L, BAS 500 F: 67 g/L, BAS 480 F: 42 g/L (BAS 702 01 F) 201149, BAS 700 F: 62.5 g/L (BAS 700 00 F) 8265, BAS 500 F: 200 g/L (BAS 500 06 F) 15146, BAS 480 F: 83 g/L (BAS 480 38 F)	
Purity:	not relevant	
CAS#:	Pyraclostrobin (BAS 500 F): 175013-18-0 Fluxapyroxad (BAS 700 F): 907204-31-3 Epoxiconazole (BAS 480 F): 133855-98-8	
Development code:	not applicable	
Spiking levels:	Pyraclostrobin:	0.01 – 10.0 mg/kg
	Fluxapyroxad:	0.01 – 10.0 mg/kg
	Epoxiconazole:	0.01 – 10.0 mg/kg

2. Test Commodity: Cereals

Crop:	Barley
Type:	not relevant
Variety:	Marthe, Prestige, Cecilia, Otis
Botanical name:	*Hordeum vulgare* L.
Crop parts(s) or processed Commodity:	whole plant (no roots), rest of plant (no roots), ears, grain, straw
Sample size:	min. 1.0 kg (straw min. 0.5 kg)

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2009 growing season, 4 trials in barley were conducted in different representative growing areas in France, Spain, Italy and Germany to determine the residue level of fluxapyroxad, pyraclostrobin and epoxiconazole in or on raw agricultural commodities (RAC). Therefore, the new fungicidal formulation BAS 702 01 F (fluxapyroxad, pyraclostrobin and epoxiconazole) was compared to the solo formulations BAS 700 00 F (fluxapyroxad), BAS 500 06 F (pyraclostrobin) and BAS 480 38 F (epoxiconazole).
All trials consisted of five plots: plot 1 (control), plot 2 (treated with BAS 702 01 F), plot 3 (treated with BAS 700 00 F), plot 4 (treated with BAS 500 06 F) and plot 5 (treated with BAS 480 38 F).
BAS 702 01 F (42 g/L fluxapyroxad + 67 g/L pyraclostrobin + 42 g/L epoxiconazole, EC) was applied twice at a single rate equivalent to 0.126 kg as/ha of epoxiconazole, 0.201 kg as/ha of pyraclostrobin and 0.126 kg as/ha of fluxapyroxad on plot 2.
BAS 700 00 F (62.5 g/L fluxapyroxad, EC) was applied twice at a single rate equivalent to 0.125 kg as/ha of fluxapyroxad on plot 3.
BAS 500 06 F (200 g/L pyraclostrobin, EC) was applied twice at a single rate equivalent to 0.250 kg as/ha of pyraclostrobin on plot 4.
BAS 480 38 F (83 g/L epoxiconazole, EC) was applied twice at a single rate equivalent to 0.125 kg as/ha of epoxiconazole on plot 5.
In all trials the applications were made at crop stages BBCH 39-49 and 69 (exception: in trial L090027: the 1st application was carried out at BBCH 71). The spray volume used was 200 L/ha.
Barley "whole plant without roots" specimens were collected directly after the last application.
After 28 (±1), 35 (±1) and 42 (±1) days, either ears and rest of plant or grain and straw were taken depending on the crop maturity (exception: trial L090027 4th sampling was not carried out).
Commercial harvest (BBCH 89) was reached in two trials at 35 DALA (L090027, L090028), and in two trials at 41 DALA.
The samples were stored frozen until analysis. The maximum storage interval from harvest until analysis was 406 days.

2. Description of analytical procedures

All specimens of plot 2 and 4 were analysed for pyraclostrobin and its metabolite BF 500-3 using BASF method 535/1 (synonym: L0076/01), which has a limit of quantitation of 0.01 mg/kg. The results of procedural recovery experiments at fortification levels between 0.01 and 10 mg/kg averaged at about 100% for pyraclostrobin and 95% for BF 500-3.
Pyraclostrobin and its metabolite BF 500-3 were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

Although the application rate of pyraclostrobin was 24% higher in the application of the solo formulation on plot 4 (2 x 0.250 kg as/ha) compared to the mixed formulation on plot 2 (2 x 0.201 kg as/ha), the residues levels found in both variants were comparable at all times.
Directly after the last application, the pyraclostrobin residues in barley "whole plant no roots" specimens ranged between 3.44 and 6.40 mg/kg in plot 2 (mixed formulation) and between 3.54 and 6.60 mg/kg in plot 4 (solo formulation).
In barley grain collected at 29 DALA residues decreased and were at 0.06 mg/kg in plot 2 and 0.08 mg/kg in plot 4. At about 35 DALA residues ranged from 0.02 - 0.21 mg/kg (plot 2) and from 0.01 - 0.09 mg/kg (plot 4). After longer PHI residues up to 0.07 mg/kg were found.
Residues found in ears were higher than the results for grain. At 27 - 29 DALA the residues in ears in plot 2 reached 0.21 - 0.99 mg/kg and in plot 4 0.17 - 1.01 mg/kg.
The residues in straw are dependent on the ripening stage / water content of the matrix and at 29 DALA residues of 2.02 mg/kg for plot 2 and 2.23 mg/kg for plot 4 were found. After longer PHI's the maximum residues, found in straw, were comparable for the mix and the solo formulation (about 35 DALA: 1.63 to 2.42 mg/kg in plot 2 and 0.79 - 2.50 mg/kg in plot 4; 41 - 42 DALA: 1.19 to 2.09 mg/kg in plot 2 and 0.46 to 2.87 mg/kg plot 4).
At 34-42 DALA, residues of metabolite BF 500-3 in barley grain ranged between 0.02 - 0.09 mg/kg after application of the mixed formulation and 0.02 – 0.07 mg/kg after application of the solo formulation. At 34-42 DALA, residues of metabolite BF 500-3 in barley straw ranged between 0.26 - 0.81 mg/kg after application of BAS 702 01 F and 0.22 – 1.08 mg/kg after application of BAS 500 06 F.

None of the analyzed untreated specimens did contain any residues of pyraclostrobin or BF 500-3 above LOQ.

A summary of residues is presented in
Table 6.3.2-9. Details are shown in Table 6.3.2-10.

Table 6.3.2-9: Summary of pyraclostrobin and BF 500-3 residues in barley after 2 applications of BAS 702 01 F and BAS 500 06 F

Matrix	Year	DALA[1]	Range of residues (mg/kg)		
			BAS 500 F	BF 500-3	BAS 500 F, total [2, 3]
BAS 702 01 F					
plant without roots	2009	0	3.44 – 6.40	0.10 – 0.21	3.59 – 6.61
ears		27-29	0.21 – 0.99	0.09 – 0.29	0.30 – 1.28
rest of plant without roots		27-29	1.26 – 2.84	0.10 – 0.73	1.36 – 3.57
grain		29	0.06	0.05	0.11
straw		29	2.02	0.59	2.61
grain		34-35	0.02 – 0.21	0.02 – 0.09	0.04 – 0.30
straw		34-35	1.63 – 2.42	0.26 – 0.70	2.05 – 3.12
grain		41-41	0.02 – 0.07	0.02 – 0.05	0.04 – 0.12
straw		41-42	1.19 – 2.09	0.53 – 0.81	1.72 – 2-90
BAS 500 06 F					
plant without roots	2008	0	3.54 – 6.60	0.12 – 0.36	3.66 – 6.96
ears		27-29	0.17 – 1.01	0.10 – 0.38	0.27 – 1.39
rest of plant without roots		27-29	0.88 – 4.17	0.20 – 1.34	1.08 – 5.51
grain		29	0.08	0.08	0.16
straw		29	2.23	0.89	3.12
grain		34-35	0.01 – 0.09	0.02 – 0.07	0.03 – 0.14
straw		34-35	0.79 – 2.50	0.22 – 0.99	1.07 – 3.49
grain		41-41	0.02 – 0.06	0.02 – 0.07	0.04 – 0.13
straw		41-42	0.46 – 2.87	0.27 – 1.08	0.73 – 3.95

[1] days after last application
[2] as BAS 500 F equivalent (conversion factor is 1.08)
[3] for calculation purposes, "< 0.01" is set 0.01

III. CONCLUSION

The results of the study show that the residue behaviour of pyraclostrobin in/on barley specimens after application of mixed formulation BAS 702 01 F is in correspondence to the behaviour found after use of the respective solo formulation BAS 500 06 F.
At harvest maturity, the pyraclostrobin residues in barley grain (34-42 DALA: BAS 702 01 F → 0.02 – 0.21 mg/kg; BAS 500 06 F → 0.01 – 0.09 mg/kg) and in barley straw are at a comparable level (34-42 DALA: BAS 702 01 F → 1.19 – 2.42 mg/kg; BAS 500 06 F → 0.46 – 2.50 mg/kg).
At 34-42 DALA, residues of metabolite BF 500-3 in barley grain ranged between 0.02 and 0.09 mg/kg after application of the mixed formulation and between 0.02 and 0.07 mg/kg after application of the solo formulation. At 34-42 DALA, residues of metabolite BF 500-3 in barley straw ranged between 0.26 and 0.81 mg/kg after application of BAS 702 01 F and between 0.22 and 1.08 mg/kg after application of BAS 500 06 F.

Table 6.3.2-10: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 702 01 F and BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 316160	Barley / Marthe	Germany	BAS 702 01 F, Pyraclostrobin: 2x 0.201 kg as/ha	BBCH 69	0	plant without roots	3.89	0.10	3.99	**BAS 500 F Method No.: 535/1 (L0076/01)**				
Doc ID: 2010/1006342					29	grain	0.06	0.05	0.11	whole plant[1]	0.01/10.0	6	107.1	12.5
Trial No. L090026					29	straw	2.02	0.59	2.61	ears	0.01/1.0	3	95.6	8.8
GLP: yes					34	grain	0.05	0.04	0.09	rest of plant[1]	0.01/10.0	3	95.9	13.4
Year: 2009					34	straw	1.66	0.60	2.26	grain	0.01/1.0	6	98.3	2.9
					41	grain	**0.07**	0.05	0.12	straw	0.01/10.0	12	99.4	10.5
					41	straw	**2.09**	0.81	2.90	**BF 500-3 Method No.: 535/1 (L0076/01)**				
			BAS 500 06 F, Pyraclostrobin: 2x 0.250 kg as/ha		0	plant without roots	4.59	0.20	4.79	whole plant[1]	0.01/10.0	6	100.8	7.0
					29	grain	0.08	0.08	0.16	ears	0.01/1.0	3	99.0	5.3
					29	straw	2.23	0.89	3.12	rest of plant[1]	0.01/10.0	3	88.0	12.4
					34	grain	0.05	0.07	0.12	grain	0.01/1.0	6	96.7	7.1
					34	straw	**2.50**	0.99	3.49	straw	0.01/10.0	13	93.0	12.8
					41	grain	**0.06**	0.07	0.13					
					41	straw	2.05	1.04	3.09					

Table 6.3.2-10: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 702 01 F and BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 316160 **Doc ID:** 2010/1006342 **Trial No.** L090027 **GLP:** yes **Year:** 2009	Barley / Prestige	France (N)	BAS 702 01 F, Pyraclostrobin: 2x 0.201 kg as/ha	BBCH 71	0	plant without roots	4.34	0.10	4.44					
					27	ears	0.99	0.29	1.28					
					27	rest of plant w/o roots	1.26	0.10	1.36					
					35	grain	**0.21**	0.09	0.30					
					35	straw	**2.10**	0.26	2.36					
			BAS 500 06 F, Pyraclostrobin: 2x 0.250 kg as/ha		0	plant without roots	3.54	0.12	3.66					
					27	ears	1.01	0.38	1.39					
					27	rest of plant w/o roots	0.88	0.20	1.08					
					35	grain	**0.09**	0.05	0.14					
					35	straw	**1.17**	0.22	1.39					
Study code: 316160 **Doc ID:** 2010/1006342 **Trial No.** L090028 **GLP:** yes **Year:** 2009	Barley	Spain	BAS 702 01 F, Pyraclostrobin: 2x 0.201 kg as/ha	BBCH 69	0	plant without roots	3.44	0.15	3.59					
					29	ears	0.21	0.09	0.30					
					29	rest of plant w/o	2.63	0.48	3.11					
					35	grain	**0.02**	0.02	0.04					
					35	straw	1.63	0.42	2.05					
					42	grain	0.02	0.02	0.04					
					42	straw	**1.94**	0.59	2.53					
			BAS 500 06 F, Pyraclostrobin: 2x 0.250 kg as/ha		0	plant without roots	6.60	0.36	6.96					
					29	ears	0.59	0.32	0.91					
					29	rest of plant w/o	4.17	1.34	5.51					
					35	grain	**0.03**	0.03	0.06					
					35	straw	1.77	0.70	2.47					
					42	grain	0.03	0.03	0.06					
					42	straw	**2.87**	1.08	3.95					

Table 6.3.2-10: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 702 01 F and BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 316160 **Doc ID:** 2010/1006342 **Trial No.** L090029 **GLP:** yes **Year:** 2009	Barley	Italy	BAS 702 01 F, Pyraclostrobin: 2x 0.201 kg as/ha	BBCH 69	0	plant without roots	6.40	0.21	6.61					
					27	ears	0.26	0.12	0.38					
					27	rest of plant w/o	2.84	0.73	3.57					
					34	grain	**0.03**	0.03	0.06					
					34	straw	**2.42**	0.70	3.12					
					41	grain	0.03	0.02	0.05					
					41	straw	1.19	0.53	1.72					
			BAS 500 06 F, Pyraclostrobin: 2x 0.250 kg as/ha		0	plant without roots	6.60	0.15	6.75					
					27	ears	0.17	0.10	0.27					
					27	rest of plant w/o	2.46	0.79	3.25					
					34	grain	0.01	0.02	0.03					
					34	straw	**0.79**	0.28	1.07					
					41	grain	**0.02**	0.02	0.04					
					41	straw	0.46	0.27	0.73					

underlined values are used for MRL calculation
[1] w/o roots
[2] as BAS 500 F equivalent (conversion factor is 1.084)
[3] for calculation purposes, "< 0.01" is set 0.01

Report: CA 6.3.2/4
Tandy R., 2012a
Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in winter barley after treatment with either BAS 556 03 F, BAS 500 06 F or BAS 555 00 F in Northern and Southern Europe during 2011
2012/1194990

Guidelines: EEC 91/414 (1607/VI/97), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 556 03 F, BAS 500 06 F or BAS 555 00 F

Description:	BAS 556 03 F: BAS 500 F (pyraclostrobin, 130 g/L), BAS 555 F (metconazole, 80 g /L), EC BAS 500 06 F: BAS 500 F (pyraclostrobin, 200 g/L), EC BAS 555 00 F: BAS 555 F (metconazole, 60 g/L), EC
Lot/Batch #:	BAS 556 03 F: 380009, BAS 500 06 F: 0003223026, BAS 555 00 F: 0003255328
Purity:	not applicable
CAS#:	Pyraclostrobin: 175013-18-0, metconazole: 125116-23-6
Development code:	not applicable
Spiking levels:	whole plant w/o roots: 0.01 and 25 mg/kg, ears: 0.01 and 1.0 mg/kg, grain: 0.01 and 1.0 mg/kg, straw: 0.01, 1.0 and 10 mg/kg

2. Test Commodity: Cereals

Crop:	Barley
Type:	not relevant
Variety:	Souleyka, Cavia, Azurel, Amorosa
Botanical name:	*Hordeum vulgare* L.
Crop parts(s) or processed	
Commodity:	whole plant (no roots), rest of plant (no roots), ears, grain, straw
Sample size:	whole plant (no roots): ≥0.5 kg, rest of plant (no roots) ≥1.0 kg, ears ≥1.0 kg, grain, straw ≥0.5 kg

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2011 growing season, four field trials were conducted at representative winter barley growing areas in Germany, UK, Southern France and Italy to determine the residue level of pyraclostrobin and metconazole (BAS 555 F) in or on Raw Agricultural Commodities (RAC).
Plot 2 was treated with **BAS 556 03 F**, an EC formulation of metconazole (80 g/L) and pyraclostrobin (130 g/L) and was foliar applied twice at a rate of 0.143 kg as/ha pyraclostrobin and 0.088 kg as/ha metconazole to barley at BBCH 49 and BBCH 69-73. The nominal spray volume used was 200 L/ha.
Plot 3 was treated with **BAS 500 06 F**, an EC formulation of pyraclostrobin (200 g/L) and was foliar applied twice at a rate of 0.25 kg as/ha pyraclostrobin to barley at BBCH 49 and BBCH 69-73. The nominal spray volume used was 200 L/ha.
Plot 4 was treated with BAS 555 00 F, an EC formulation of metconazole (60 g/L) and was foliar applied twice at a rate of 0.09 kg as/ha metconazole to barley at BBCH 49 and BBCH 69-73. The nominal spray volume used was 200 L/ha.
Whole plant specimens were collected directly before (plot 1) and after (plot 2 to 4) the last application. At 28-29 days after last application (DALA), 34-36 DALA and 41-42 DALA either, ears and "rest of plant without roots", or grain and straw specimens were taken depending on the maturity.
The samples were stored frozen until analysis. The maximum storage interval from harvest until analysis was 406 days.

Table 6.3.2-11: Application and sampling details for trials conducted in 2011

Region	No. of trials	No. of appl.	F, P, or G	Test item	Active substance	Application		Target timing	
						Rate as (kg/ha)	Water vol. (L/ha)	Appl.	Sampling (DALA)[1]
Northern and Southern Europe	4	2	F	BAS 556 03 F	metconazole	0.088	200	BBCH 49 BBCH 69	0 28-29 34-36 41-42 BBCH 89
					pyraclostrobin	0.143			
				BAS 500 06 F	pyraclostrobin	0.25			
				BAS 555 00 F	metconazole	0.09			

[1] days after last application

2. Description of analytical procedures

All specimens were analysed for pyraclostrobin and its metabolite BF 500-3 using BASF method No. 535/1 (synonym: L0076/01):
Pyraclostrobin and its metabolite BF 500-3 were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS. The limit of quantification of the method was 0.01 mg/kg.
The results of procedural recovery experiments were 81-94% for pyraclostrobin and 80-96% for BF 500-3 at fortification levels between 0.01 and 25 mg/kg.

II. RESULTS AND DISCUSSION

Residues of pyraclostrobin parent analysed in whole plant specimens, taken 0 days after last application, ranged between 2.3 - 5.1 mg/kg in plot 2 and 1.9 - 9.2 mg/kg in plot 3. At 28-29 DALA residues in ear specimens ranged between 0.049 - 0.26 mg/kg in plot 2; 0.066 - 0.39 mg/kg in plot 3, while residues in "rest of plant w/o roots" ranged between 0.25 - 2.0 mg/kg in plot 2; 0.39 - 3.5 mg/kg in plot 3. At BBCH 83-89, residues in grain ranged between 0.019 - 0.059 mg/kg in plot 2; 0.029 - 0.065 mg/kg in plot 3, and residues in straw specimens ranged between 0.42 - 3.2 mg/kg in plot 2; 0.46 - 4.1 mg/kg in plot 3.
Residues of metabolite BF 500-3 analyzed in barley grain were found in maximum up to 0.042 mg/kg. In rest of plant and straw samples maximum residues of 1.9 mg/kg in plot 2 and of 2.4 mg/kg in plot 4 were found.

No residues of pyraclostrobin and its metabolite BF 500-3 above the limit of quantitation (0.01 mg/kg) were detected in the untreated specimens of this study.
All specimens, apart from the S1 whole plant, S2 rest of plant and S3 ear specimens from trial S11-00713-02 (L110193) and the S3 rest of plant specimen from trial S11-00713-04 (L110195), showed that the new formulation BAS 556 03 F does not lead to higher pyraclostrobin residues than the solo formulation BAS 500 06 F.

A summary of residues is presented in Table 6.3.2-12 and
Table 6.3.2-13. Details are shown in Table 6.3.2-14.

Table 6.3.2-12: Summary of pyraclostrobin and BF 500-3 residues in the treated barley specimens after 2 applications of BAS 556 03 F (Plot 2)

Crop	Year	Appl.	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)			
					Matrix	BAS 500 F	BF 500-3[3]	Total[4]
Barley	2011	BAS 556 03 F	0	69-73	whole plant[2]	2.3 - 5.1	0.086 -0.37	2.4 – 5.4
			28-29	77-85	ears	0.049 – 0.26	0.062 – 0.14	0.13 – 0.39
					rest of plant[2]	0.25 – 2.0	0.13 – 0.92	0.39 – 2.9
			34-36	81-89	ears	0.042 – 0.23	0.060 – 0.18	0.11 – 0.42
					rest of plant[2]	0.27 – 1.7	0.15 – 0.82	0.42 – 2.5
			35	87-89	grain	0.038	0.020	0.058
					straw	1.5	0.48	1.9
			41-42	83-89	grain	0.019 - 0.059	0.020 – 0.036	0.038 – 0.095
					straw	0.55 – 3.2	0.30 – 1.9	0.85 – 5.1
			54	89	grain	0.030	0.022	0.052
					straw	0.42	0.25	0.67

[1] days after last application
[2] w/o roots
[3] as BAS 500 F equivalent (conversion factor is 1.084)
[4] for calculation purposes, "< 0.01" is set 0.01

Table 6.3.2-13: Summary of pyraclostrobin and BF 500-3 in the treated barley specimens after 2 applications of BAS 500 06 F (Plot 3)

Crop	Year	Appl.	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)			
					Matrix	BAS 500 F	BF 500-3[3]	Total[4]
Barley	2011	BAS 500 06 F	0	69-73	whole plant[2]	1.9 – 9.2	0.078 – 0.56	2.0 – 9.8
			28-29	77-85	ears	0.066 – 0.39	0.048 – 0.25	0.13 – 0.64
					rest of plant[2]	0.39 – 3.5	0.17 – 1.3	0.56 – 4.9
			34-36	81-89	ears	0.049 – 0.34	0.040 – 0.23	0.089 – 0.57
					rest of plant[2]	0.48 – 3.2	0.19 – 1.5	0.67 – 4.7
			35	87-89	grain	0.049	0.033	0.081
					straw	2.1	0.69	2.8
			41-42	83-89	grain	0.029 – 0.065	0.025 – 0.042	0.054 – 0.11
					straw	0.93 – 4.1	0.47 – 2.4	1.4 – 6.5
			54	89	grain	0.030	0.026	0.056
					straw	0.046	0.25	0.71

[1] days after last application
[2] w/o roots
[3] as BAS 500 F equivalent (conversion factor is 1.084)
[4] for calculation purposes, "< 0.01" is set 0.01

III. CONCLUSION

Residues of pyraclostrobin parent analysed in whole plant specimens, taken 0 days after last application, ranged between 2.3 and 5.1 mg/kg in plot 2 (treated with the mixed formulation BAS 556 03 F) and between 1.9 and 9.2 mg/kg in plot 3 (treated with BAS 500 06 F). At BBCH 83-89, residues in grain ranged between 0.019 and 0.059 mg/kg in plot 2 and between 0.029 and 0.065 mg/kg in plot 3; residues in straw specimens ranged between 0.42 and 3.2 mg/kg in plot 2 and between 0.46 and 4.1 mg/kg in plot 3.
Residues of metabolite BF 500-3 analysed in barley grain were found in maximum up to 0.042 mg/kg. In rest of plant and straw samples maximum residues of 1.9 mg/kg in plot 2 and of 2.4 mg/kg in plot 3 were found.
In general, it was proven with only a few exceptions that a double treatment with formulation BAS 556 03 F at of 0.143 kg as/ha leads to lower pyraclostrobin residues compared to the corresponding treatment with solo formulation BAS 500 06 F at 0.25 kg as/ha.

Table 6.3.2-14: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 556 03 F and BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 401831_1	Barley / Souleyka	Germany	BAS 556 03 F, Pyraclostrobin: 2x 0.143 kg as/ha	BBCH 69-73	0	whole plant[1]	5.1	0.37	5.4	**BAS 500 F Method No.:** 535/1				
Doc ID: 2012/1194990					28	ears	0.26	0.14	0.39	whole plant[1]	0.01 / 25	8	81	6
Trial No. L110192					28	rest of plant[1]	2.0	0.92	2.9	ears	0.01 / 1.0	8	92	6
GLP: yes					36	ears	0.23	0.18	0.42	grain	0.01 / 1.0	8	85	11
Year: 2011					36	rest of plant[1]	1.7	0.82	2.5	straw	0.01 / 1.0 / 10	9	94	8
					42	grain	0.059	0.036	0.095					
					42	straw	3.2	1.9	5.1	**BF 500-3 Method No.:** 535/1				
			BAS 500 06 F, Pyraclostrobin: 2x 0.250 kg as/ha		0	whole plant[1]	9.2	0.56	9.8	whole plant[1]	0.01 / 25	8	80	6
					28	ears	0.39	0.25	0.64	ears	0.01 / 1.0	8	87	11
					28	rest of plant[1]	3.5	1.3	4.9	grain	0.01 / 1.0	8	80	14
					36	ears	0.34	0.23	0.57	straw	0.01 / 1.0 / 10	9	96	10
					36	rest of plant[1]	3.2	1.5	4.7					
					42	grain	**0.065**	0.042	0.11					
					42	straw	**4.1**	2.4	6.5					
Study code: 401831_1	Barley / Cavia	United Kingdom	BAS 556 03 F, Pyraclostrobin: 2x 0.143 kg as/ha	BBCH 69	0	whole plant[1]	2.3	0.086	2.4					
Doc ID: 2012/1194990					28	ears	0.082	0.062	0.14					
Trial No. L110193					28	rest of plant[1]	0.25	0.13	0.39					
GLP: yes					34	ears	0.078	0.060	0.14					
Year: 2011					34	rest of plant[1]	0.27	0.15	0.42					
					41	grain	0.035	0.022	0.057					
					41	straw	0.65	0.39	1.0					
					54	grain	0.030	0.022	0.052					
					54	straw	0.42	0.25	0.67					

Table 6.3.2-14: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 556 03 F and BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 401831_1 **Doc ID:** 2012/1194990 **Trial No.** L110193 **GLP:** yes **Year:** 2011	Barley / Cavia	United Kingdom	BAS 500 06 F, Pyraclostrobin: 2x 0.250 kg as/ha	BBCH 69	0	whole plant[1]	1.9	0.078	2.0					
					28	ears	0.082	0.048	0.13					
					28	rest of plant[1]	0.39	0.17	0.56					
					34	ears	0.049	0.040	0.089					
					34	rest of plant[1]	0.48	0.19	0.67					
					41	grain	**0.039**	0.027	0.066					
					41	straw	**0.93**	0.47	1.4					
					54	grain	0.030	0.026	0.056					
					54	straw	0.46	0.25	0.71					
Study code: 401831_1 **Doc ID:** 2012/1194990 **Trial No.** L110194 **GLP:** yes **Year:** 2011	Barley / Azurel	France (S)	BAS 556 03 F, Pyraclostrobin: 2x 0.143 kg as/ha	BBCH 69	0	whole plant[1]	3.7	0.21	3.9					
					28	ears	0.049	0.082	0.13					
					28	rest of plant[1]	0.60	0.23	0.83					
					35	ears	0.042	0.072	0.11					
					35	rest of plant[1]	0.41	0.24	0.66					
					42	grain	0.019	0.020	0.038					
					42	straw	0.55	0.30	0.85					
			BAS 500 06 F, Pyraclostrobin: 2x 0.250 kg as/ha		0	whole plant[1]	6.3	0.21	6.5					
					28	ears	0.066	0.064	0.13					
					28	rest of plant[1]	1.2	0.35	1.5					
					35	ears	0.11	0.16	0.27					
					35	rest of plant[1]	0.63	0.30	0.93					
					42	grain	**0.029**	0.025	0.054					
					42	straw	**1.3**	0.52	1.8					

Table 6.3.2-14: Residues of pyraclostrobin and BF 500-3 in barley after 2 applications of BAS 556 03 F and BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 401831_1 **Doc ID:** 2012/1194990 **Trial No.** L110195 **GLP:** yes **Year:** 2011	Barley / Amorosa	Italy	BAS 556 03 F, Pyraclostrobin: 2x 0.143 kg as/ha	BBCH 69-71	0	whole plant[1]	4.2	0.18	4.3					
					29	ears	0.11	0.071	0.18					
					29	rest of plant[1]	1.5	0.43	1.9					
					35	ears	0.11	0.11	0.22					
					35	rest of plant[1]	1.3	0.42	1.7					
					35	grain	0.038	0.020	0.058					
					35	straw	1.5	0.48	1.9					
			BAS 500 06 F, Pyraclostrobin: 2x 0.250 kg as/ha		0	whole plant[1]	7.3	0.33	7.7					
					29	ears	0.18	0.16	0.35					
					29	rest of plant[1]	1.9	0.70	2.6					
					35	ears	0.21	0.23	0.44					
					35	rest of plant[1]	1.2	0.46	1.6					
					35	grain	**0.049**	0.033	0.081					
					35	straw	**2.1**	0.69	2.8					

underlined values are used for MRL calculation
[1] w/o roots
[2] as BAS 500 F equivalent (conversion factor is 1.084)
[3] for calculation purposes, "< 0.01" is set 0.01

Report: CA 6.3.2/5
Meyer M., 2013a
Study on the residue behaviour of Fluxapyroxad (BAS 700 F) and Pyraclostrobin (BAS 500 F) in barley after treatment with either BAS 703 04 F or BAS 700 00 F or BAS 500 06 F under field conditions in Germany, N-France, Spain and Greece, 2012
2013/1282605

Guidelines: EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 9 (March 2011)

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 703 04 F, BAS 700 00 F, BAS 500 06 F

Description: BAS 703 04 F: 150 g/L of BAS 500 F (pyraclostrobin) and 75 g/L of BAS 700 F (fluxapyroxad), EC
BAS 500 06 F: 200 g/L of BAS 500 F (pyraclostrobin), EC
BAS 700 00 F: 62.5 g/L of BAS 700 F (fluxapyroxad), EC

Lot/Batch #: BAS 703 04 F: 208088
BAS 700 00 F: 201454
BAS 500 06 F: 0004863761

Purity: 99.9%

CAS#: BAS 500 F: 175013-18-0
BAS 700 F: 907204-31-3

Development code: not applicable

Spiking levels: 0.01-100 mg/kg

2. Test Commodity: Cereals

Crop: Barley

Type: not applicable

Variety: Propino, Cervoise, Pewter, Arta

Botanical name: *Hordeum vulgare L.*

Crop parts(s) or processed

Commodity: Whole plant without roots, ears, grain, rest of plant without roots, straw

Sample size: 0.5-1.0 kg

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2012 growing season, 4 trials in barley were conducted in different representative growing areas in Northern France, Spain, Greece and Germany to determine the residue level of fluxapyroxad and pyraclostrobin in or on raw agricultural commodities (RAC).
Therefore, the fungicidal formulation BAS 703 04 F (fluxapyroxad and pyraclostrobin) was compared to the solo formulations BAS 700 00 F (fluxapyroxad) and BAS 500 06 F (pyraclostrobin). All trials consisted of four plots: plot 1 (control), plot 2 (treated with BAS 703 04 F), plot 3 (treated with BAS 700 00 F) and plot 4 (treated with BAS 500 06 F).

BAS 703 04 F (75 g/L fluxapyroxad and 150 g/L pyraclostrobin, EC) was applied twice at a single rate equivalent to 0.1125 kg as/ha of fluxapyroxad and 0.225 kg as/ha of pyraclostrobin on plot 2.
BAS 700 00 F (62.5 g/L fluxapyroxad, EC) was applied twice at a single rate equivalent to 0.125 kg as/ha of fluxapyroxad on plot 3. **BAS 500 06 F** (200 g/L pyraclostrobin, EC) was applied twice at a single rate equivalent to 0.250 kg as/ha of pyraclostrobin on plot 4.

In all trials the applications were made at crop stages BBCH 49 and 69. The spray volume used was 200 L/ha. "Whole plant without roots" specimens were collected directly after the last application. After 28±1, 35±1 and 42±1 days, either ears or "rest of plant" or grain and straw were taken depending on the crop maturity. Commercial harvest (BBCH 89) was reached at 36 to 47 days after the last application (DALA).

The specimens were shipped under deep-frozen conditions from the field stations to the specimen management side, homogenized using dry ice and stored deep frozen until analysis. Maximum storage interval from harvest until analysis was about 12 months.

Table 6.3.2-15: Application and sampling details for trials conducted in 2012

Region	No. of trials	No. of appl.	F, P, or G	Test item	Active substance	Application		Target timing	
						Rate as (kg/ha)	Water vol. (L/ha)	Appl.	Sampling (DALA)[1]
Northern and Southern Europe	4	2	F	BAS 703 04 F (EC)	BAS 700 F	0.1125	200	BBCH 49 BBCH 69	0 28±1 35±1 42±1 BBCH 89
					BAS 500 F	0.225			
				BAS 700 00 F (EC)	BAS 700 F	0.125			
				BAS 500 06 F (EC)	BAS 500 F	0.250			

[1] days after last application

2. Description of analytical procedures

All specimens were analysed for pyraclostrobin and its metabolite BF 500-3 using BASF method No. 535/1 (synonym: L0076/01):
Pyraclostrobin and its metabolite BF 500-3 were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS. The limit of quantification of the method was 0.01 mg/kg.
The results of procedural recovery experiments were 93.6% for pyraclostrobin and 95.2% for BF 500-3 at fortification levels between 0.01 and 100 mg/kg.

II. RESULTS AND DISCUSSION

Although the application rate of pyraclostrobin was higher in the application of the BAS 500 06 F formulation (2 x 250 g as/ha) compared to the mixed formulation BAS 703 04 F (2 x 225 g as/ha), the residue levels found in both variants were very comparable in barley matrices.

Directly after the last application, the residues of pyraclostrobin in barley "whole plant without roots" specimens ranged between 3.6 and 5.1 mg/kg (mixed formulation) and between 4.4 and 6.0 mg/kg (solo formulation).

In barley grain collected at 34 to 36 DALA residues of pyraclostrobin ranged between 0.04 and 0.25 mg/kg (mixed formulation) and between 0.056 and 0.33 mg/kg (solo formulation). Residues of metabolite BF 500-3 ranged from 0.020 to 0.089 mg/kg (mixed formulation) and 0.03 to 0.16 mg/kg (solo formulation). At 41-47 DALA parent pyraclostrobin residues in barley grain ranged between <0.01 - 0.27 mg/kg (mixed formulation) and between <0.01 - 0.35 mg/kg (solo formulation), residues of the metabolite ranged between <0.01 - 0.082 mg/kg (mixed formulation) and for the solo formulation between <0.01 - 0.15 mg/kg.

The residues of pyraclostrobin in barley straw collected at 34 to 36 DALA ranged between 0.80 and 1.9 mg/kg (mixed formulation) and between 1.2 and 2.8 mg/kg (solo formulation), while residues of metabolite BF 500-3 ranged from 0.20 to 0.46 mg/kg (mixed formulation) and from 0.44 to 0.99 mg/kg (solo formulation). At 41 - 47 DALA parent pyraclostrobin residues in barley straw ranged between <0.01 - 1.7 mg/kg (mixed formulation) and between 0.56 - 2.9 mg/kg (solo formulation), residues of the metabolite ranged between <0.01 - 0.50 mg/kg (mixed formulation) and for the solo formulation between 0.22 - 0.85 mg/kg.

No residues equal or above the limit of quantitation (0.01 mg/kg) of pyraclostrobin and its metabolite BF 500-3 were present in control specimens.

The summarized results are given in
Table 6.3.2-16. The detailed results are given in Table 6.3.2-17.

Table 6.3.2-16: Summary of pyraclostrobin and BF 500-3 residues in the treated barley specimens after application of BAS 703 04 F and BAS 500 06 F

Crop	Year	Application	DALA[1]	BBCH[2]	Residues found (mg/kg)			
					Matrix	BAS 500 F	BF 500-3[3]	Total residue[4]
Barley	2012	BAS 703 04 F (EC)	0	49-69	whole plant[5]	3.6-5.1	0.028-0.16	3.7-5.1
			27-29	75-85	ears	0.011-0.78	0.010-0.36	0.021-1.1
			35	81	ears	0.27	0.25	0.51
			27-29	75-85	rest of plant[5]	0.36-1.4	0.038-0.33	0.40-1.7
			35	81	rest of plant[5]	0.55	0.12	0.67
			34-36	85-89	grain	0.04-0.25	0.020-0.089	0.060-0.34
			41-47	89-92	grain	<0.01-0.27	<0.01-0.082	<0.02-0.35
			34-36	85-89	straw	0.80-1.9	0.20-0.46	1.0-2.3
			41-47	89-92	straw	<0.01-1.7	<0.01-0.50	<0.02-1.7
		BAS 500 06 F (EC)	0	49-69	whole plant[5]	4.4-6.0	0.04-0.32	4.7-6.0
			27-29	75-85	ears	0.020-0.51	0.019-0.26	0.039-0.77
			35	81	ears	<0.01	<0.01	<0.02
			27-29	75-85	rest of plant[5]	0.58-1.9	0.079-0.37	0.76-2.2
			35	81	rest of plant[5]	0.60	0.074	0.67
			34-36	85-89	grain	0.056-0.33	0.030-0.16	0.086-0.49
			41-47	89-92	grain	<0.01-0.35	<0.01-0.15	<0.02-0.49
			34-36	85-89	straw	1.2-2.8	0.44-0.99	1.6-3.8
			41-47	89-92	straw	0.56-2.9	0.22-0.85	0.78-3.6

[1] days after last application
[2] BBCH stage at respective sampling
[3] conversion factor for calculation of BF 500-3 (500M07) to parent equivalent is 1.08
[4] for residues <0.01 mg/kg, value was set to 0.01 mg/kg for calculation of sum
[5] without roots

The values of trial L120231 (L1202310028 and L1202310029, DALA 42, GS 89) are not considered for any further evaluation, due to an obvious mix up of sample material most likely during the threshing process.
There are no further consequences on the trial L120231, previous samplings showed residues equal to the other trial.

III. CONCLUSION

In barley grain collected at 34 to 36 DALA, residues of pyraclostrobin ranged between 0.04 and 0.25 mg/kg (mixed formulation) and between 0.056 and 0.33 mg/kg (solo formulation). Residues of metabolite BF 500-3 ranged from 0.020 to 0.089 mg/kg (mixed formulation) and from <0.01 to 0.16 mg/kg (solo formulation).
At 41-47 DALA, parent pyraclostrobin residues in barley grain ranged between <0.01 - 0.27 mg/kg (mixed formulation) and between <0.01 - 0.35 mg/kg (solo formulation), residues of the metabolite BF 500-3 ranged between <0.01 - 0.082 mg/kg (mixed formulation) and for the solo formulation between <0.01 and 0.15 mg/kg.

The residues of pyraclostrobin in barley straw collected at 34 to 36 DALA ranged between 0.80 and 1.9 mg/kg (mixed formulation) and between 1.2 and 2.8 mg/kg (solo formulation), while residues of metabolite BF 500-3 ranged from 0.20 to 0.46 mg/kg (mixed formulation) and from 0.44 to 0.99 mg/kg (solo formulation).
At 41 - 47 DALA, parent pyraclostrobin residues in barley straw ranged between <0.01 and 1.7 mg/kg (mixed formulation) and between 0.56 and 2.9 mg/kg (solo formulation), residues of the metabolite BF 500-3 ranged between <0.01 and 0.50 mg/kg (mixed formulation) and for the solo formulation between 0.22 and 0.85 mg/kg.

Table 6.3.2-17: Residues of pyraclostrobin and BF 500-3 in barley after applications of BAS 703 04 F or BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[1]	Total[2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 419891 **Doc ID:** 2013/1282605 **Trial No.** L120228 **GLP:** yes **Year:** 2012	Barley / Propino	Germany	BAS 703 04 F 2 x 0.225	BBCH 69	0	whole plant[3]	4.6	0.13	4.8	**BAS 500 F Method No.:** 535/1				
					27	ears	0.11	0.060	0.17	whole plant[3]	0.01, 0.1, 1.0, 10 and 100	18	93.6	7.9
					27	rest of plant[3]	1.4	0.29	1.7	rest of plant[3]				
					34	grain	0.040	0.020	0.060	ears				
					34	straw	**1.9**	0.46	2.3	grain				
					41	grain	**0.043**	0.023	0.066	straw				
					41	straw	1.7	0.50	1.2	**BF 500-3 Method No.:** 535/1				
			BAS 500 06 F 2 x 0.250		0	whole plant[3]	5.2	0.13	5.3	whole plant[3]	0.01, 0.1, 1.0, 10 and 100	17	95.2	10.3
					27	ears	0.11	0.068	0.17	rest of plant[3]				
					27	rest of plant[3]	1.9	0.37	2.2	ears				
					34	grain	**0.056**	0.030	0.086	grain				
					34	straw	2.4	0.59	3.0	straw				
					41	grain	0.052	0.031	0.083					
					41	straw	**2.9**	0.78	3.6					
Study code: 419891 **Doc ID:** 2013/1282605 **Trial No.** L120229 **GLP:** yes **Year:** 2012	Barley / Cervoise	France (N)	BAS 703 04 F 2 x 0.225	BBCH 69	0	whole plant[3]	3.6	0.16	3.7					
					28	ears	0.78	0.36	1.1					
					28	rest of plant[3]	1.4	0.33	1.7					
					36	grain	**0.25**	0.089	0.34					
					36	straw	1.2	0.30	1.5					
					47	grain	0.22	0.072	0.29					
					47	straw	**1.4**	0.39	1.7					

Table 6.3.2-17: Residues of pyraclostrobin and BF 500-3 in barley after applications of BAS 703 04 F or BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[1]	Total[2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 419891 **Doc ID:** 2013/1282605 **Trial No.** L120229 **GLP:** yes **Year:** 2012	Barley / Cervoise	France (N)	BAS 500 06 F 2 x 0.250	BBCH 69	0	whole plant[3]	4.4	0.32	4.7					
					28	ears	0.34	0.22	0.56					
					28	rest of plant[3]	0.67	0.21	0.88					
					36	grain	0.33	0.16	0.49					
					36	straw	**2.8**	0.99	3.8					
					47	grain	**0.35**	0.15	0.49					
					47	straw	2.3	0.85	3.2					
Study code: 419891 **Doc ID:** 2013/1282605 **Trial No.** L120230 **GLP:** yes **Year:** 2012	Barley / Pewter	Spain	BAS 703 04 F 2 x 0.225	BBCH 69	0	whole plant[3]	5.1	0.028	5.1					
					28	ears	0.69	0.25	0.94					
					28	rest of plant[3]	0.52	0.14	0.66					
					36	grain	0.21	0.066	0.28					
					36	straw	0.80	0.20	1.0					
					42	grain	**0.27**	0.082	0.35					
					42	straw	**0.81**	0.23	1.0					
			BAS 500 06 F 2 x 0.250		0	whole plant[3]	6.0	0.040	6.0					
					28	ears	0.51	0.26	0.77					
					28	rest of plant[3]	0.58	0.20	0.78					
					36	grain	**0.15**	0.085	0.23					
					36	straw	**1.2**	0.44	1.6					
					42	grain	0.12	0.069	0.19					
					42	straw	1.2	0.39	1.6					

Table 6.3.2-17: Residues of pyraclostrobin and BF 500-3 in barley after applications of BAS 703 04 F or BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[1]	Total[2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 419891 **Doc ID:** 2013/1282605 **Trial No.** L120231 **GLP:** yes **Year:** 2012	Barley / Arta	Greece	BAS 703 04 F 2 x 0.225	BBCH 69	0	whole plant[3]	4.4	0.11	4.5					
					29	ears	0.011	0.010	0.021					
					29	rest of plant[3]	0.36	0.038	0.40					
					35	ears	0.27	0.25	0.51					
					35	rest of plant[3]	0.55	0.12	0.67					
					42	grain	**<u><0.01</u>**	<0.01	<0.02					
					42	straw	**<u><0.01</u>**[4]	<0.01[4]	<0.02[4]					
			BAS 500 06 F 2 x 0.250		0	whole plant[3]	4.5	0.16	4.7					
					29	ears	0.020	0.019	0.039					
					29	rest of plant[3]	0.68	0.079	0.76					
					35	ears	<0.01	<0.01	<0.02					
					35	rest of plant[3]	0.60	0.074	0.67					
					42	grain	**<u><0.01</u>**	<0.01	<0.02					
					42	straw	**<u>0.56</u>**	0.22	0.78					

[1] conversion factor for calculation of BF 500-3 to parent BAS 500 F is 1.084
[2] for residues <0.01 mg/kg, value was set to 0.01 mg/kg for calculation of sum
[3] without roots
[4] mean of three extractions/injections, values are both <0.01 mg/kg
<u>underlined values</u> are used for MRL calculation

Report:	CA 6.3.2/6 Plier S., 2011b Determination of residues of BAS 500 F in wheat after two applications of BAS 500 06 F in Germany, United Kingdom, France (North), Denmark, France (South), Greece, Italy and Spain, 2010 2011/1135915
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, OECD 509 Crop Field Trial (2009), Working document of the Commission of the European Communities Directorate General for Agriculture VI B II-1 Appendix B, IVA Guideline IA-II (1992), BBA IV 3-3, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F

Description:	BAS 500 06 F (BAS 500 F, 200 g/L pyraclostrobin), EC formulation
Lot/Batch #:	BAS 500 06 F: 0003223036
Purity:	99.9%
CAS#:	BAS 500 F: 175013-18-0 BF 500-3: 512165-96-7
Development code:	not applicable
Spiking levels:	0.01 - 1.0 mg/kg

2. Test Commodity: Cereals

Crop:	Wheat
Type:	not applicable
Variety:	Cubus, Solstice, Mercato, Frument, Euclide, Svevo, Aubusson, Prospero
Botanical name:	*Triticum L.*
Crop parts(s) or processed	
Commodity:	Whole plant without roots, ears, grain, rest of plant without roots, straw
Sample size:	whole plant (no roots): ≥0.5 kg, rest of plant (no roots) ≥1.0 kg, ears ≥1.0 kg, grain, straw ≥0.5 kg

B. STUDY DESIGN AND METHODS

During the 2010 growing season, a total of 8 field trials were conducted in Northern and Southern Europe in order to determine the magnitude of residues of pyraclostrobin after two applications of BAS 500 06 F. Each trial consisted of a control and a treated plot. BAS 500 06 F (200 g/L pyraclostrobin, EC), was applied twice with an application rate of 0.250 kg as/ha and a spray volume of 200 L/ha at growth stages BBCH 49 and 69. "Whole plant no roots" specimens were taken directly after the last application. Depending on the maturity ears and "rest of plant without roots" or grain and straw specimens were taken at 28±1, 35±1 and 42±1 DALA.
The samples were stored deep frozen until analysis. The maximum storage interval from harvest until analysis for plant samples was 334 days.

Table 6.3.2-18: Application and sampling details for trials conducted in 2010

Region	No. of trials	No. of appl.	F, P, or G	Test item	Active substance	Application		Target timing	
						Rate as (kg/ha)	Water vol. (L/ha)	Appl.	Sampling (DALA)[1]
Northern and Southern Europe	8	2	F	BAS 500 06 F	BAS 500 F	0.250	200	BBCH 49 BBCH 69	0 28±1 35±1 42±1

[1] days after last application

2. Description of analytical procedures

The specimens were analysed for residues of pyraclostrobin and its metabolite BF 500-3 using BASF method 535/1 (synonym: L0076/01), which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg for pyraclostrobin and its metabolite BF 500-3 in all of the specimen material. The results of procedural recovery experiments were 89% for pyraclostrobin and 86% for BF 500-3 at fortification levels between 0.01 and 1.0 mg/kg.
Pyraclostrobin and BF 500-3 residues were extracted from the homogenized specimens with methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination of pyraclostrobin and BF 500-3 was performed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

At pre harvest interval (PHI) of 34-36 days residues of pyraclostrobin ranged between 0.18 and 0.49 mg/kg in ears, 1.89 and 5.59 mg/kg in rest of plant without roots, <0.01 and 0.02 mg/kg in grain and 4.08 and 5.76 mg/kg in straw.

The residues of the metabolite BF 500-3 ranged between 0.06 and 0.37 mg/kg in ears, 0.63 and 1.90 mg/kg in rest of plant without roots, <0.01 mg/kg in grain and 1.72 and 1.93 mg/kg in straw.

No residues of either pyraclostrobin or its metabolite BF 500-3 above the respective limits of quantitation were found in any of the analysed untreated specimens.

A summary of residues is presented in
Table 6.3.2-19. Details are shown in Table 6.3.2-20.

Table 6.3.2-19: Summary of pyraclostrobin and BF 500-3 residues in the treated wheat specimens after application of BAS 500 06 F

Crop	Year	Appl.	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)			
					Matrix	BAS 500 F	BF 500-3[3]	Total[4]
Wheat	2010	BAS 500 06 F (EC)	0	69	whole plant[2]	2.41 – 6.10	0.17 – 0.73	2.64 – 6.83
			28-29	83-87	ears	0.11 – 1.06	0.04 – 0.44	0.15 – 1.43
					rest of plant[2]	1.46 – 6.18	0.49 – 1.91	2.19 – 8.09
			34-36	85-87	ears	0.18 – 0.49	0.06 – 0.37	0.24 – 0.76
			35	87-89	rest of plant[2]	1.89 – 5.59	0.63 – 1.90	2.87 – 7.49
					grain	<0.01 – 0.02	<0.01	<0.02 – 0.03
					straw	4.08 – 5.76	1.72 – 1.93	5.89 – 7.48
			41-43	89	grain	<0.01 – 0.02	<0.01	<0.02 – 0.03
					straw	1.55 – 5.43	0.76 – 2.22	2.50 – 7.65

[1] days after last application
[2] no roots
[3] as BAS 500 F equivalent (conversion factor is 1.084)
[4] for calculation purposes, "< 0.01" is set 0.01

III. CONCLUSION

At PHI of 34-36 days, residues of pyraclostrobin ranged between 0.18 and 0.49 mg/kg in ears, between 1.89 and 5.59 mg/kg in rest of plant without roots, between <0.01 and 0.02 mg/kg in grain and between 4.08 and 5.76 mg/kg in straw.

The residues of the metabolite BF 500-3 (500M07) ranged in specimens sampled at 34 to 36 DALA between 0.06 and 0.37 mg/kg in ears, between 0.63 and 1.90 mg/kg in rest of plant without roots, between <0.01 mg/kg in grain and between 1.72 and 1.93 mg/kg in straw.

Table 6.3.2-20: Residues of pyraclostrobin and BF 500-3 in wheat after 2 applications of BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total[2, 3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371232 **Doc ID:** 2011/1135915 **Trial No.** L100085 **GLP:** yes **Year:** 2010	Wheat / Cubus	Germany	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	5.55	0.37	5.92	**BAS 500 F Method No.: 535/1 (L0076/01)**				
					28	ears	0.22	0.07	0.29	all sample matrices	0.01 – 1.0	18	89.3	8.6
					28	rest of plant[1]	1.98	0.49	2.47					
					35	ears	0.38	0.11	0.49	**BF 500-3 Method No.: 535/1 (L0076/01)**				
					35	rest of plant[1]	2.66	0.63	3.29	all sample matrices	0.01 – 1.0	17	85.8	14.4
					43	grain	**<u><0.01</u>**	<0.01	<0.02					
					43	straw	**<u>1.74</u>**	0.76	2.50					
Study code: 371232 **Doc ID:** 2011/1135915 **Trial No.** L100086 **GLP:** yes **Year:** 2010	Wheat / Solstice	United Kingdom	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	2.41	0.23	2.64					
					28	ears	0.63	0.15	0.78					
					28	rest of plant[1]	4.31	1.20	5.51					
					35	grain	**<u>0.02</u>**	<0.01	0.03					
					35	straw	**<u>5.76</u>**	1.72	7.48					
					42	grain	0.02	<0.01	0.03					
					42	straw	4.02	1.37	5.39					
Study code: 371232 **Doc ID:** 2011/1135915 **Trial No.** L100087 **GLP:** yes **Year:** 2010	Wheat / Mercato	France (N)	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	2.65	0.26	2.91					
					29	ears	0.34	0.08	0.42					
					29	rest of plant[1]	3.88	0.80	4.68					
					36	ears	0.36	0.10	0.46					
					36	rest of plant[1]	3.27	0.83	4.10					
					42	grain	**<u>0.02</u>**	<0.01	0.03					
					42	straw	**<u>3.01</u>**	0.94	3.95					

Table 6.3.2-20: Residues of pyraclostrobin and BF 500-3 in wheat after 2 applications of BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total[2, 3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371232 **Doc ID:** 2011/1135915 **Trial No.** L100088 **GLP:** yes **Year:** 2010	Wheat / Frument	Denmark	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	2.97	0.24	3.21					
					29	ears	1.06	0.37	1.43					
					29	rest of plant[1]	6.18	1.91	8.09					
					36	ears	0.49	0.13	0.62					
					36	rest of plant[1]	5.59	1.90	7.49					
					43	grain	**0.02**	<0.01	0.03					
					43	straw	**5.43**	2.22	7.65					
Study code: 371232 **Doc ID:** 2011/1135915 **Trial No.** L100089 **GLP:** yes **Year:** 2010	Wheat / Euclide	France (S)	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	2.74	0.18	2.92					
					28	ears	0.11	0.04	0.15					
					28	rest of plant[1]	1.71	0.48	2.19					
					35	ears	0.18	0.06	0.24					
					35	rest of plant[1]	2.39	0.72	3.11					
					41	grain	**<0.01**	<0.01	<0.02					
					41	straw	**2.26**	1.11	3.37					
Study code: 371232 **Doc ID:** 2011/1135915 **Trial No.** L100090 **GLP:** yes **Year:** 2010	Wheat / Svevo	Greece	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	4.99	0.51	5.50					
					28	ears	0.34	0.36	0.70					
					28	rest of plant[1]	1.46	0.77	2.23					
					34	ears	0.39	0.37	0.76					
					34	rest of plant[1]	1.89	0.98	2.87					
					41	grain	**<0.01**	<0.01	<0.02					
					41	straw	**1.55**	1.00	2.55					

Table 6.3.2-20: Residues of pyraclostrobin and BF 500-3 in wheat after 2 applications of BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total[2, 3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371232 **Doc ID:** 2011/1135915 **Trial No.** L100091 **GLP:** yes **Year:** 2010	Wheat / Aubusson	Italy	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	4.98	0.17	5.15					
					28	ears	0.93	0.44	1.37					
					28	rest of plant[1]	3.48	1.17	4.65					
					35	grain	**0.01**	<0.01	0.02					
					35	straw	**4.17**	1.93	6.10					
					42	grain	0.01	<0.01	0.02					
					42	straw	2.83	1.44	4.27					
Study code: 371232 **Doc ID:** 2011/1135915 **Trial No.** L100092 **GLP:** yes **Year:** 2010	Wheat / Prospero	Spain	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	6.10	0.73	6.83					
					28	ears	0.46	0.21	0.67					
					28	rest of plant[1]	2.91	1.00	3.91					
					35	grain	**<0.01**	<0.01	<0.02					
					35	straw	**4.08**	1.81	5.89					
					42	grain	<0.01	<0.01	<0.02					
					42	straw	2.27	1.32	3.59					

underlined values are used for MRL calculation
[1] w/o roots
[2] as BAS 500 F equivalent (conversion factor is 1.084)
[3] for calculation purposes, "< 0.01" is set 0.01

Report:	CA 6.3.2/7 Plier S., 2013b Determination of residues of BAS 500 F in wheat after two applications of BAS 500 06 F in Germany, United Kingdom, Netherlands, France (South), Greece, Italy and Spain, 2011 2012/1067588
Guidelines:	EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, OECD 509 Crop Field Trial (2009), Working document of the Commission of the European Communities Directorate General for Agriculture VI B II-1 Appendix B, EEC 7029/VI/95 rev. 5
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F

Description:	BAS 500 06 F (BAS 500 F, 200 g/L pyraclostrobin), EC formulation
Lot/Batch #:	BAS 500 06 F: 0003223036
Purity:	99.9%
CAS#:	BAS 500 F: 175013-18-0 BF 500-3: 512165-96-7
Development code:	not applicable
Spiking levels:	0.01 - 10 mg/kg

2. Test Commodity: Cereals

Crop:	Wheat
Type:	not applicable
Variety:	Asano, Solstice, Tabasco, Quality, Svevo, Aubusson, Califa Sur
Botanical name:	*Triticum L.*
Crop parts(s) or processed	
Commodity:	Whole plant without roots, ears, grain, rest of plant without roots, straw
Sample size:	whole plant (no roots): ≥0.5 kg, rest of plant (no roots) ≥0.65 kg, ears ≥1.0 kg, grain, straw ≥0.5 kg

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2011 growing season, a total of 8 field trials were conducted in Northern and Southern Europe in order to determine the magnitude of residues of pyraclostrobin after two applications of BAS 500 06 F. Each trial consisted of a control and a treated plot. The fungicidal formulation BAS 500 06 F (200 g/L pyraclostrobin), was applied twice with an application rate of 0.25 kg as/ha and a spray volume of 200 L/ha at growth stages BBCH 49 and 69. "Whole plant no roots" specimens were taken directly after the last application. Depending on the maturity ears and "rest of plant without roots" or grain and straw specimens were taken at 28 ± 1, 35 ± 1, 42 ± 1 DALA. If crop stage BBCH 89 was not reached, an additional sampling was made.

The samples were stored deep frozen until analysis. The maximum storage interval from harvest until analysis for plant samples was 408 days.

Table 6.3.2-21: Application and sampling details for trials conducted in 2011

Region	No. of trials	No. of appl.	F, P, or G	Test item	Active substance	Application		Target timing	
						Rate as (kg/ha)	Water vol. (L/ha)	Appl.	Sampling (DALA)[1]
Northern and Southern Europe	8	2	F	BAS 500 06 F	BAS 500 F	0.25	200	BBCH 49 BBCH 69	0 28±1 35±1 42±1 BBCH 89

[1] days after last application

2. Description of analytical procedures

The specimens were analysed for residues of pyraclostrobin and its metabolite BF 500-3 using BASF method 535/1 (synonym: L0076/01), which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg for pyraclostrobin and its metabolite BF 500-3 in all of the specimen material. The results of procedural recovery experiments were 87% for pyraclostrobin and 83% for BF 500-3 at fortification levels between 0.01 and 10 mg/kg.

Pyraclostrobin and BF 500-3 residues were extracted from the homogenized specimens with methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination of pyraclostrobin and BF 500-3 was performed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

At PHI of 34-36 days residues of pyraclostrobin ranged between 0.05 and 0.54 mg/kg in ears, 0.23 and 5.32 mg/kg in rest of plant without roots, <0.01 and 0.02 mg/kg in grain and 4.18 and 4.40 mg/kg in straw.
The residues of the metabolite BF 500-3 ranged between 0.02 and 0.29 mg/kg in ears, 0.11 and 1.66 mg/kg in rest of plant without roots, <0.01 mg/kg in grain and 1.53 and 1.79 mg/kg in straw.
In two untreated 'rest of plant without root' samples residues of 0.01 mg/kg and 0.06 mg/kg of pyraclostrobin and 0.02 mg/kg of BF 500-3 were found. In the respective untreated straw samples residues of 0.01 and 0.03 mg/kg of pyraclostrobin and 0.01 mg/kg of BF 500-3 were found. The detected residues in the control samples are caused by unknown contamination. In all other than the above mentioned control samples no residues at or above the limit of quantitation were found.

A summary of residues is presented in Table 6.3.2-22. Details are shown in Table 6.3.2-23.

Table 6.3.2-22: Summary of pyraclostrobin and BF 500-3 residues in the treated wheat specimens after application of BAS 500 06 F

Crop	Year	Appl.	DALA[1]	Growth stage (BBCH)	Residues found (mg/kg)			
					Matrix	BAS 500 F	BF 500-3[3]	Total[4]
Wheat	2011	BAS 500 06 F (EC)	0	69	whole plant[2]	2.68 – 8.20	0.15 – 0.51	2.83 – 8.56
			27-29	75-87	ears	0.04 – 0.32	0.02 – 0.22	0.06 – 0.49
					rest of plant[2]	0.16 – 4.66	0.07 – 1.25	0.22 – 5.91
			28	87	grain	0.02	<0.01	0.03
					straw	4.18	1.63	5.81
			34-36	77-89	ears	0.05 – 0.54	0.02 – 0.29	0.07 – 0.83
					rest of plant[2]	0.23 – 5.32	0.11 – 1.66	0.34 – 6.98
			35-36	87-89	grain	<0.01, 0.02	<0.01	<0.02, 0.03
					straw	4.18, 4.40	1.53, 1.79	5.71, 6.19
			41-42	87	ears	0.09, 0.29	0.05, 0.21	0.14, 0.50
					rest of plant[2]	0.45, 0.48	0.20, 0.19	0.65, 0.67
			41-43	87-89	grain	<0.01 – 0.03	<0.01 – 0.01	<0.02 – 0.04
					straw	0.23 – 6.96	0.13 – 2.38	0.36 – 9.34
			48-56	89	grain	<0.01	<0.01	<0.02
					straw	0.30 – 1.30	0.20 – 0.66	0.50 – 1.96

[1] days after last application
[2] no roots
[3] as BAS 500 F equivalent (conversion factor is 1.084)
[4] for calculation purposes, "< 0.01" is set 0.01

III. CONCLUSION

At PHI 34-36 days, residues of pyraclostrobin ranged between 0.05 and 0.54 mg/kg in ears, between 0.23 and 5.32 mg/kg in rest of plant without roots, between <0.01 and 0.02 mg/kg in grain and between 4.18 and 4.40 mg/kg in straw.
The residues of the metabolite BF 500-3 (500M07) ranged between 0.02 and 0.29 mg/kg in ears, between 0.11 and 1.66 mg/kg in rest of plant without roots, between 1.53 and 1.79 mg/kg in straw and were at < 0.01 mg/kg in grain.

Table 6.3.2-23: Residues of pyraclostrobin and BF 500-3 in wheat after 2 applications of BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total[2, 3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371236 **Doc ID:** 2012/1067588 **Trial No.** L110158 **GLP:** yes **Year:** 2011	Wheat / Asano	Germany	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	4.90	0.19	5.09	**BAS 500 F Method No.: 535/1 (L0076/01)**				
					29	ears	0.04	0.02	0.06	all sample matrices	0.01 – 10	31	86.5	9.2
					29	rest of plant[1]	0.15	0.07	0.22					
					36	ears	0.05	0.02	0.07	**BF 500-3 Method No.: 535/1 (L0076/01)**				
					36	rest of plant[1]	0.23	0.11	0.34	all sample matrices	0.01 – 10	31	83.3	12.6
					42	grain	**<0.01**	<0.01	<0.02					
					42	straw	0.23	0.13	0.36					
					56	grain	<0.01	<0.01	<0.02					
					56	straw	**0.30**	0.20	0.50					
Study code: 371236 **Doc ID:** 2012/1067588 **Trial No.** L110159 **GLP:** yes **Year:** 2011	Wheat / Asano	Germany	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	5.86	0.20	6.06					
					27	ears	0.12	0.04	0.16					
					27	rest of plant[1]	1.57	0.55	2.12					
					35	grain	**0.14**	0.05	0.19					
					35	straw	**1.98**	0.82	2.80					
					42	grain	<0.01	<0.01	<0.02					
					42	straw	1.96	0.86	2.82					
Study code: 371236 **Doc ID:** 2012/1067588 **Trial No.** L110160 **GLP:** yes **Year:** 2011	Wheat / Lomerit	United Kingdom	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	2.68	0.15	2.83					
					27	ears	0.07	0.03	0.10					
					27	rest of plant[1]	0.53	0.17	0.70					
					34	ears	0.07	0.03	0.10					
					34	rest of plant[1]	0.73	0.26	0.99					
					41	ears	0.09	0.05	0.14					
					41	rest of plant[1]	0.45	0.20	0.65					
					48	grain	**<0.01**	<0.01	<0.02					
					48	straw	**0.51**	0.29	0.80					

Table 6.3.2-23: Residues of pyraclostrobin and BF 500-3 in wheat after 2 applications of BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total[2, 3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371236 **Doc ID:** 2012/1067588 **Trial No.** L110161 **GLP:** yes **Year:** 2011	Wheat / Tabasco	The Nether lands	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	5.32	0.15	5.47					
					27	ears	0.21	0.08	0.29					
					27	rest of plant[1]	0.73	0.28	1.01					
					35	ears	0.15	0.05	0.20					
					35	rest of plant[1]	0.85	0.28	1.31					
					41	grain	**0.03**	0.01	0.04					
					41	straw	0.81	0.38	1.19					
					54	grain	<0.01	<0.01	<0.02					
					54	straw	**1.30**	0.66	1.96					
Study code: 371236 **Doc ID:** 2012/1067588 **Trial No.** L110162 **GLP:** yes **Year:** 2011	Wheat / Quality	France (S)	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	6.98	0.33	7.31					
					28	ears	0.32	0.17	0.49					
					28	rest of plant[1]	4.66	1.25	5.91					
					36	ears	0.54	0.29	0.83					
					36	rest of plant[1]	5.32	1.66	6.98					
					43	grain	**<0.01**	<0.01	<0.02					
					43	straw	**6.96**	2.38	9.34					
Study code: 371236 **Doc ID:** 2012/1067588 **Trial No.** L110163 **GLP:** yes **Year:** 2011	Wheat / Svevo	Greece	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	7.02	0.24	7.26					
					28	ears	0.27	0.22	0.49					
					28	rest of plant[1]	0.60	0.22	0.82					
					35	ears	0.28	0.22	0.50					
					35	rest of plant[1]	0.87	0.40	1.27					
					42	ears	0.29	0.21	0.50					
					42	rest of plant[1]	0.48	0.19	0.67					
					49	grain	**<0.01**	<0.01	<0.02					
					49	straw	**0.85**	0.36	1.21					

Table 6.3.2-23: Residues of pyraclostrobin and BF 500-3 in wheat after 2 applications of BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total[2, 3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 371236 **Doc ID:** 2012/1067588 **Trial No.** L110164 **GLP:** yes **Year:** 2011	Wheat / Aubusson	Italy	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	7.50	0.51	8.01					
					27	ears	0.19	0.07	0.26					
					27	rest of plant[1]	2.84	0.93	3.77					
					35	grain	0.01	<0.01	<0.02					
					35	straw	**4.18**	1.53	5.71					
					43	grain	**0.02**	<0.01	0.03					
					43	straw	3.66	1.35	5.01					
Study code: 371236 **Doc ID:** 2012/1067588 **Trial No.** L110165 **GLP:** yes **Year:** 2011	Wheat / Califa Sur	Spain	BAS 500 06 F, Pyraclostrobin: 2x 0.25 kg as/ha	BBCH 69	0	whole plant[1]	8.20	0.36	8.56					
					28	grain	0.02	<0.01	0.03					
					28	straw	4.18	1.63	5.81					
					36	grain	**0.02**	<0.01	0.03					
					36	straw	**4.40**	1.79	6.19					
					43	grain	0.02	<0.01	0.03					
					43	straw	3.78	1.61	5.39					

underlined values are used for MRL calculation
[1] w/o roots
[2] as BAS 500 F equivalent (conversion factor is 1.084)
[3] for calculation purposes, "< 0.01" is set 0.01

Report:	CA 6.3.2/8 Tandy R., 2012b Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in winter wheat after treatment with either BAS 556 03 F, BAS 500 06 F or BAS 555 00 F in Northern and Southern Europe during 2011 2012/1194991
Guidelines:	EEC 91/414 (1607/VI/97), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 6.3.2/9 Tandy R., 2014a Final report amendment No. 1: Study on the residue behaviour of Pyraclostrobin (BAS 500 F) and Metconazole (BAS 555 F) in winter wheat after treatment with either BAS 556 03 F, BAS 500 06 F or BAS 555 00 F in Northern and Southern Europe during 2011 2014/1090810
Guidelines:	EEC 91/414 (1607/VI/97), SANCO/3029/99 rev. 4 (11 July 2000), EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	BAS 556 03 F, BAS 500 06 F, BAS 555 00 F
	Description:	BAS 556 03 F: BAS 500 F (pyraclostrobin, 130 g/L) and BAS 555 F (metconazole, 80 g/L), EC BAS 500 06 F: BAS 500 F (pyraclostrobin, 200 g/L), EC BAS 555 00 F: BAS 555 F (metconazole, 60 g/L), EC
	Lot/Batch #:	BAS 556 03 F: 380009, BAS 500 06 F: 0003223026, BAS 555 00 F: 0003255328
	Purity:	not relevant
	CAS#:	Pyraclostrobin: 175013-18-0 Metconazole: 125116-23-6
	Development code:	not applicable
	Spiking levels:	whole plant (no roots): 0.1 and 20 mg/kg, ears: 0.01 and 2.0 mg/kg, grain: 0.01 and 1.0 mg/kg, straw: 0.01 and 8.0 mg/kg

2. Test Commodity: Cereals

Crop:	Wheat
Type:	not relevant
Variety:	Julius, Oakley, PR22 R58, Bologna
Botanical name:	*Triticum L.*
Crop parts(s) or processed	
Commodity:	whole plant (no roots), rest of plant (no roots), ears, grain, straw
Sample size:	whole plant (no roots): ≥0.5 kg, rest of plant (no roots) ≥1.0 kg, ears ≥1.0 kg, grain, straw ≥0.5 kg

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2011 growing season, four field trials were conducted at representative winter wheat growing areas in Germany, the UK, Southern France and Italy to determine the residue level of pyraclostrobin (BAS 500 F) and metconazole (BAS 555 F) in or on Raw Agricultural Commodities (RAC).

Plot 2 was treated with **BAS 556 03 F**, an EC formulation of metconazole (80 g/L) and pyraclostrobin (130 g/L) and was foliar applied twice at a rate of 0.143 kg as/ha pyraclostrobin and 0.088 kg as/ha metconazole to wheat at BBCH 49-51 and BBCH 69. The nominal spray volume used was 200 L/ha.

Plot 3 was treated with **BAS 500 06 F**, an EC formulation of pyraclostrobin (200 g/L) and was foliar applied twice at a rate of 0.250 kg as/ha pyraclostrobin to wheat at BBCH 49-51 and BBCH 69. The nominal spray volume used was 200 L/ha.

Plot 4 was treated with BAS 555 00 F, an EC formulation of metconazole (60 g/L) and was foliar applied twice at a rate of 0.090 kg as/ha metconazole to wheat at BBCH 49-51 and BBCH 69. The nominal spray volume used was 200 L/ha.

Whole plant specimens were collected directly before (plot 1) and after (plot 2 to 4) the last application. At 27-29 days after last application (DALA), 34-36 DALA and 39-43 DALA either ears and "rest of plant without roots", or grain and straw specimens were taken depending on the maturity. At trials S11-00711-01 and 02 (L110188 and L110189) where BBCH 89 was not reached at 42 (±) DALA, grain and straw specimens were taken 57 DALA when BBCH 89 was reached. The samples were stored frozen until analysis. The maximum storage interval from harvest until extraction was 343 days.

2. Description of analytical procedures

All specimens of plot 2 and 3 were analysed for pyraclostrobin and its metabolite BF 500-3 using BASF method 535/1 (synonym: L0076/01), which has a limit of quantitation of 0.01 mg/kg. The results of procedural recovery experiments at fortification levels between 0.01 and 20 mg/kg were between 85 - 98% for pyraclostrobin and 75 - 91% for BF 500-3.

Pyraclostrobin and its metabolite BF 500-3 were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

Residues of parent pyraclostrobin analysed in whole plant specimens, taken 0 days after last application, ranged between 2.0 - 3.8 mg/kg in plot 2; 3.9 – 10 mg/kg in plot 3. At 27-29 DALA residues in ear specimens ranged between 0.11 - 0.31 mg/kg in plot 2; 0.19 - 0.36 mg/kg in plot 3, while residues in "rest of plant w/o roots" ranged between 0.57 - 3.0 mg/kg in plot 2; 1.3 - 6.1 mg/kg in plot 3. At BBCH 85-89, residues in grain ranged between <0.01 - 0.020 mg/kg in plot 2; <0.01 - 0.039 mg/kg in plot 3, and residues in straw specimens ranged between 0.49 - 1.9 mg/kg in plot 2; 0.95 - 5.5 mg/kg in plot 3.

Residues of metabolite BF 500-3 analysed in whole plant specimens, taken 0 days after last application, ranged between 0.069 – 0.24 mg/kg in plot 2; 0.14 – 0.46 mg/kg in plot 3. At 27-29 DALA residues in ear specimens ranged between 0.044 - 0.12 mg/kg in plot 2; 0.078 - 0.17 mg/kg in plot 3, while residues in "rest of plant w/o roots" ranged between 0.22 - 1.1 mg/kg in plot 2; 0.38 – 1.6 mg/kg in plot 3. At BBCH 85-89, residues in grain were <0.01 mg/kg in plot 2; <0.01 - 0.023 mg/kg in plot 3, and residues in straw specimens ranged between 0.30 - 0.97 mg/kg in plot 2; 0.42 - 2.0 mg/kg in plot 3.

No residues of pyraclostrobin and its metabolite BF 500-3 above the limit of quantitation (0.01 mg/kg) were detected in the untreated specimens of this study. All specimens, apart from the S3 straw specimen from trial S11-00711-02 (L110189), showed that the new formulation BAS 556 03 F does not lead to higher pyraclostrobin residues than the solo formulation BAS 500 06 F.

A summary of residues is presented in Table 6.3.2-24. Details are shown in Table 6.3.2-25.

Table 6.3.2-24: Summary of pyraclostrobin and BF 500-3 residues in the treated wheat specimens after application of BAS 556 03 F or BAS 500 06 F

Crop	Year	Appl.	DALA[1]	BBCH	Matrix	Residues found (mg/kg)		
						BAS 500 F	BF 500-3[2]	Total Residue[3]
Wheat	2011	BAS 556 03 F (EC)	0	69	whole plant[4]	2.0 – 3.8	0.069 – 0.24	2.1 – 4.0
			27-29	77-87	ears	0.11 – 0.31	0.044 – 0.12	0.15 – 0.43
					rest of plant[4]	0.57 – 3.0	0.22 – 1.1	0.79 – 4.1
			34-36	82-87	ears	0.061 – 0.29	0.031 – 0.15	0.092 – 0.44
					rest of plant[4]	0.88 – 1.9	0.28 – 0.75	1.2 – 2.6
			36	83	grain	0.025	0.011	0.037
					straw	2.2	1.0	3.3
			43	85	ears	0.36	0.15	0.051
					rest of plant[4]	1.5	0.67	2.2
			39-42	85-89	grain	<0.01 – 0.013	<0.01	<0.01 – 0.023
					straw	0.49 – 1.5	0.30 – 0.78	0.79 – 2.3
			57	89	grain	<0.01 – 0.020	<0.01	<0.01 – 0.030
					straw	1.3 – 1.9	0.76 – 0.97	2.0 – 2.9
		BAS 500 06 F (EC)	0	69	whole plant[4]	3.9 – 10	0.14 – 0.46	4.1 – 11
			27-29	77-87	ears	0.19 – 0.36	0.078 – 0.17	0.27 – 0.50
					rest of plant[4]	1.3 – 6.1	0.38 – 1.6	1.7 – 7.6
			34-36	82-87	ears	0.16 – 0.35	0.081 – 0.20	0.24 – 0.52
					rest of plant[4]	1.2 – 5.4	0.45 – 1.8	1.6 – 7.1
			36	83	grain	0.025	0.014	0.038
					straw	2.1	0.95	3.1
			43	85	ears	0.50	0.19	0.69
					rest of plant[4]	1.9	0.82	2.7
			39-42	85-89	grain	<0.01 – 0.020	<0.01 – 0.012	<0.01 – 0.033
					straw	0.95 – 5.5	0.42 – 2.0	1.4 – 7.4
			57	89	grain	0.016 – 0.039	<0.01 – 0.023	0.026 – 0.062
					straw	1.9 – 2.5	1.1 – 1.2	3.0 – 3.7

[1] days after last application
[2] as BAS 500 F equivalent (conversion factor is 1.08)
[3] for calculation purposes, "< 0.01" is set 0.01
[4] without roots

III. CONCLUSION

At BBCH 85-89, residues of parent pyraclostrobin analysed in grain ranged between <0.01 - 0.020 mg/kg in plot 2 (treated with the mixed formulation BAS 556 03 F) and between <0.01 and 0.039 mg/kg in plot 3 (treated with BAS 500 06 F); residues in straw specimens ranged between 0.49 and 1.9 mg/kg in plot 2 and were in the range of 0.95 to 5.5 mg/kg in plot 3.

At BBCH 85-89, residues of metabolite 500M07 (BF 500-3) analysed in grain were at <0.01 mg/kg in plot 2 and between <0.01 and 0.023 mg/kg in plot 3; residues in straw specimens ranged between 0.30 and 0.97 mg/kg in plot 2 and ranged between 0.42 and 1.2 mg/kg in plot 3.

Table 6.3.2-25: Residues of pyraclostrobin and BF 500-3 in wheat after applications of BAS 556 03 F or BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[1]	Total[2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	SD (%)
Study code: 401831	Wheat / Julius	Germany	BAS 556 03 F Pyraclostrobin 2 x 0.143	BBCH 69	0	whole plant[3]	3.2	0.22	3.4	**BAS 500 F Method No.:** 535/1				
Doc ID: 2012/1194991					29	ears	0.19	0.064	0.26	whole plant[3]	0.1 /20	8	85	19
Trial No. L110188					29	rest of plant[3]	1.2	0.39	1.6	ears	0.01/ 2.0	8	98	7
GLP: yes					36	ears	0.29	0.104	0.39	grain	0.01 / 1.0	8	94	5
Year: 2011					36	rest of plant[3]	1.5	0.56	2.0	straw	0.01 / 8.0	8	88	17
					43	ears	0.36	0.15	0.51					
					43	rest of plant[3]	1.5	0.67	2.2	**BF 500-3 Method No.:** 535/1				
					57	grain	<0.01	<0.01	<0.01	whole plant[3]	0.1 / 20	8	79	19
					57	straw	1.9	0.97	2.9	ears	0.01 / 2.0	8	91	10
			BAS 500 06 F Pyraclostrobin 2 x 0.25		0	whole plant[3]	5.4	0.28	5.7	grain	0.01 / 1.0	8	90	5
					29	ears	0.36	0.12	0.48	straw	0.01 / 8.0	8	75	15
					29	rest of plant[3]	1.3	0.38	1.7					
					36	ears	0.35	0.12	0.47					
					36	rest of plant[3]	1.4	0.61	2.0					
					43	ears	0.50	0.19	0.69					
					43	rest of plant[3]	1.9	0.82	2.7					
					57	grain	**0.016**	<0.01	0.026					
					57	straw	**1.9**	1.1	3.0					

Table 6.3.2-25: Residues of pyraclostrobin and BF 500-3 in wheat after applications of BAS 556 03 F or BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[1]	Total[2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	SD (%)
Study code: 401831 **Doc ID:** 2012/1194991 **Trial No.** L110189 **GLP:** yes **Year:** 2011	Wheat / Oakley	United Kingdom	BAS 556 03 F Pyraclostrobin 2 x 0.143	BBCH 69	0	whole plant[3]	2.0	0.069	2.1					
					29	ears	0.31	0.12	0.43					
					29	rest of plant[3]	0.82	0.36	1.2					
					36	grain	0.025	0.011	0.037					
					36	straw	2.2	1.0	3.3					
					42	grain	0.013	<0.01	0.023					
					42	straw	1.5	0.78	2.3					
					57	grain	0.020	<0.01	0.030					
					57	straw	1.3	0.76	2.0					
			BAS 500 06 F Pyraclostrobin 2 x 0.25		0	whole plant[3]	3.9	0.14	4.1					
					29	ears	0.33	0.17	0.50					
					29	rest of plant[3]	1.7	0.63	2.3					
					36	grain	0.025	0.014	0.038					
					36	straw	2.1	0.95	3.1					
					42	grain	0.020	0.012	0.033					
					42	straw	**5.5**	1.97	7.4					
					57	grain	**0.039**	0.023	0.062					
					57	straw	2.5	1.2	3.7					

Table 6.3.2-25: Residues of pyraclostrobin and BF 500-3 in wheat after applications of BAS 556 03 F or BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[1]	Total[2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	SD (%)
Study code: 401831 **Doc ID:** 2012/1194991 **Trial No.** L110190 **GLP:** yes **Year:** 2011	Wheat / PR22 R58	France (S)	BAS 556 03 F Pyraclostrobin 2 x 0.143	BBCH 69	0	whole plant[3]	3.0	0.24	3.2					
					28	ears	0.19	0.10	0.29					
					28	rest of plant[3]	3.0	1.1	4.1					
					35	ears	0.29	0.15	0.44					
					35	rest of plant[3]	1.9	0.75	2.6					
					42	grain	<0.01	<0.01	<0.01					
					42	straw	0.49	0.30	0.79					
			BAS 500 06 F Pyraclostrobin 2 x 0.25		0	whole plant[3]	5.7	0.46	6.1					
					28	ears	0.25	0.14	0.40					
					28	rest of plant[3]	6.1	1.6	7.6					
					35	ears	0.31	0.20	0.52					
					35	rest of plant[3]	5.4	1.8	7.1					
					42	grain	**0.011**	<0.01	0.021					
					42	straw	**0.95**	0.42	1.4					

Table 6.3.2-25: Residues of pyraclostrobin and BF 500-3 in wheat after applications of BAS 556 03 F or BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[1]	Total[2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	SD (%)
Study code: 401831 **Doc ID:** 2012/1194991 **Trial No.** L110191 **GLP:** yes **Year:** 2011	Wheat / Bologna	Italy	BAS 556 03 F Pyraclostrobin 2 x 0.143	BBCH 69	0	whole plant[3]	3.8	0.20	4.0					
					27	ears	0.11	0.044	0.15					
					27	rest of plant[3]	0.57	0.22	0.79					
					34	ears	0.061	0.031	0.092					
					34	rest of plant[3]	0.88	0.28	1.2					
					39	grain	<0.01	<0.01	<0.01					
					39	straw	1.2	0.61	1.8					
			BAS 500 06 F Pyraclostrobin 2 x 0.25		0	whole plant[3]	10	0.41	11					
					27	ears	0.19	0.078	0.27					
					27	rest of plant[3]	1.5	0.47	1.9					
					34	ears	0.16	0.081	0.24					
					34	rest of plant[3]	1.2	0.45	1.6					
					39	grain	**<u><0.01</u>**	<0.01	<0.01					
					39	straw	**<u>0.95</u>**	0.42	1.4					

[1] conversion factor for calculation of BF 500 3 to parent BAS 500 F is 1.084
[2] for residues <0.01 mg/kg, value was set to 0.01 mg/kg for calculation of sum
[3] without roots
<u>underlined values</u> are used for MRL calculation

Report:	CA 6.3.2/10 Meyer M., 2013b Study on residue behaviour of Fluxapyroxad (BAS 700 F), Pyraclostrobin (BAS 500 F) in wheat after treatment with either BAS 703 04 F, BAS 700 00 F or BAS 500 06 F under field conditions, Germany, United Kingdom, Spain, Southern France, 2012 2013/1336790
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 703 04 F (EC), BAS 700 00 F (EC), BAS 500 06 F (EC)

Description:	BAS 703 04 F (75 g/L of BAS 700 F and 150 g/L of BAS 500 F, EC) BAS 500 06 F (Pyraclostrobin: 200 g/L of BAS 500 F,EC) BAS 700 00 F (Fluxapyroxad: 62.5 g/L of BAS 700 F, EC)
Lot/Batch #:	BAS 703 04 F: 208088 BAS 500 06 F: 0004863761
Purity:	99.9%
CAS#:	BAS 500 F: 175013-18-0 BF 500-3: 512165-96-7
Development code:	not applicable
Spiking levels:	0.01 - 10 mg/kg

2. Test Commodity: Cereals

Crop:	Wheat
Type:	not applicable
Variety:	Asano, JB Diego, Gallareta, Ingenio
Botanical name:	*Triticum L.*
Crop parts(s) or processed	
Commodity:	Whole plant without roots, ears, grain, rest of plant without roots, straw
Sample size:	0.1-1.0 kg

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2012 growing season, 4 trials in wheat were conducted in different representative growing areas in Southern France, Spain, UK and Germany to determine the residue level of fluxapyroxad (BAS 700 F) and pyraclostrobin (BAS 500 F) in or on raw agricultural commodities (RAC).

Therefore, the fungicidal formulation BAS 703 04 F (fluxapyroxad and pyraclostrobin) was compared to the solo formulations BAS 700 00 F (fluxapyroxad) and BAS 500 06 F (pyraclostrobin). All trials consisted of four plots: plot 1 (control), plot 2 (treated with BAS 703 04 F), plot 3 (treated with BAS 700 00 F) and plot 4 (treated with BAS 500 06 F).

BAS 703 04 F (75 g/L fluxapyroxad and 150 g/L pyraclostrobin, EC) was applied twice at a single rate equivalent to 0.1125 kg as/ha of fluxapyroxad and 0.225 kg as/ha of pyraclostrobin on plot 2. BAS 700 00 F (62.5 g/L fluxapyroxad, EC) was applied twice at a single rate equivalent to 0.125 kg as/ha of fluxapyroxad on plot 3. **BAS 500 06 F** (200 g/L pyraclostrobin, EC) was applied twice at a single rate equivalent to 0.250 kg as/ha of pyraclostrobin on plot 4.

In all trials the applications were made at crop stages BBCH 49 and 69. The spray volume used was 200 L/ha. Wheat “whole plant without roots" specimens were collected directly after the last application. After 28±1, 35±1 and 42±1 days, either ears or rest of plant or grain and straw were taken depending on the crop maturity. In case BBCH growth stage 89 was not yet reached after 42 days, a further sampling was done.

For trial L120235, additional separate samples of grain and straw from the 42 DALA time point were processed and analysed since the absence of fluxapyroxad residues in the straw specimen indicated a possible mix-up in sample labelling. The results from the newly processed specimens were in line with the first indicating that a mislabelling of the straw sample occurred prior to processing.

Commercial harvest (BBCH 89) was reached at 42 to 50 days after the last application (DALA).

The specimens were stored frozen until analysis. The maximum storage interval from harvest until analysis was about 12 months.

Table 6.3.2-26: Application and sampling details for trials conducted in 2012

Region	No. of trials	No. of appl.	F, P, or G	Test item	Active substance	Application		Target timing	
						Rate as (kg/ha)	Water vol. (L/ha)	Appl.	Sampling (DALA)[1]
Northern and Southern Europe	4	2	F	BAS 703 04 F (EC)	BAS 700 F	0.1125	200	BBCH 49 BBCH 69	0 28±1 35±1 42±1 BBCH 89
					BAS 500 F	0.225			
				BAS 700 00 F (EC)	BAS 700 F	0.125			
				BAS 500 06 F (EC)	BAS 500 F	0.250			

[1] days after last application

2. Description of analytical procedures

The specimens were analysed for residues of pyraclostrobin and its metabolite BF 500-3 using BASF method 535/1 (synonym: L0076/01), which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg for pyraclostrobin and its metabolite BF 500-3 in all of the specimen material. The results of procedural recovery experiments were 84.6% for pyraclostrobin and 83.2% for BF 500-3 at fortification levels between 0.01 and 10 mg/kg.
Pyraclostrobin and BF 500-3 residues were extracted from the homogenized specimens with methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. The final determination of pyraclostrobin and BF 500-3 was performed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

Although the application rate of pyraclostrobin was higher in the application of the BAS 500 06 F formulation (2 x 0.250 kg as/ha) compared to the mixed formulation BAS 703 04 F (2 x 0.225 kg as/ha), the residues levels found in both variants were very comparable in wheat matrices.
Directly after the last application, the residues of pyraclostrobin in whole plant without roots ranged between 3.7 and 4.2 mg/kg (mixed formulation) and between 3.0 and 6.7 mg/kg (solo formulation). The residues of pyraclostrobin in wheat grain collected at 36 DALA and later, were between <0.01 and 0.015 mg/kg (mixed formulation) and <0.01 and 0.017 mg/kg (solo formulation). Residues of metabolite BF 500-3 were <0.01 mg/kg.

In wheat straw residues of pyraclostrobin ranged from 0.29 to 1.6 mg/kg (mixed formulation) and 0.29 to 1.5 mg/kg (solo formulation) whereas residues of metabolite BF 500-3 ranged between 0.10 and 0.56 mg/kg (mixed formulation) and from 0.12 to 0.45 mg/kg (solo formulation).

No residues at or above the LOQ of 0.01 mg/kg of pyraclostrobin and its metabolite were present in control specimens.

A summary of residues is presented in Table 6.3.2-27. Details are shown in Table 6.3.2-28.

Table 6.3.2-27: Summary of pyraclostrobin and BF 500-3 residues in the treated wheat specimens after application of BAS 703 04 F and BAS 500 06 F

Crop	Year	Application	DALA[1]	BBCH[2]	Residues found (mg/kg)			
					Matrix	BAS 500 F	BF 500-3[3]	Total Residue[4]
Wheat	2012	BAS 703 04 F (EC)	0	69	whole plant[5]	3.7-4.2	0.025-0.19	3.8-4.2
			28	80-87	ears	0.045-0.48	0.018-0.19	0.063-0.67
			34-35	85-87	ears	<0.01-0.35	<0.01-0.14	<0.02-0.49
			28	80-87	rest of plant[5]	0.26-1.6	0.047-0.41	0.30-2.0
			34-35	85-87	rest of plant[5]	<0.01-1.3	<0.01-0.38	<0.02-1.7
			28	85	grain	<0.01	<0.01	<0.02
			36	87	grain	<0.01	<0.01	<0.02
			42	87-89	grain	<0.01-0.015	<0.01	<0.02-0.025
			50	89	grain	<0.01	<0.01	<0.02
			28	85	straw	1.1	0.21	1.3
			36	87	straw	0.30-1.1	0.10-0.24	0.41-1.3
			42	87-89	straw	0.29-1.6	0.13-0.56	0.42-2.2
			50	89	straw	0.37	0.15	0.53
		BAS 500 06 F (EC)	0	69	whole plant[3]	3.0-6.7	0.031-0.21	3.0-6.8
			28	80-87	rest of plant[3]	0.27-4.3	0.10-1.1	0.37-5.4
			34-35	85-87	rest of plant[3]	0.13-3.9	0.043-1.2	0.17-5.1
			28	80-87	ears	0.068-0.51	0.028-0.23	0.095-0.74
			34-35	85-87	ears	0.050-0.52	0.021-0.26	0.071-0.78
			28	85	grain	0.012	<0.01	0.022
			36	87	grain	<0.01	<0.01	<0.02
			42	87-89	grain	<0.01-0.017	<0.01	<0.02-0.027
			50	89	grain	<0.01	<0.01	<0.02
			28	85	straw	0.74	0.15	0.89
			36	87	straw	0.34-0.84	0.15- 0.21	0.49-1.0
			42	87-89	straw	0.29-1.5	0.12-0.45	0.41-1.9
			50	89	straw	0.41	0.16	0.57

[1] days after last application
[2] BBCH stage at respective sampling
[3] conversion factor for calculation of BF 500-3 to parent equivalent is 1.08
[4] for calculation sum <0.01 mg/kg was set to 0.01 mg/kg
[5] without roots

III. CONCLUSION

Directly after the last application, the residues of pyraclostrobin in whole plant without roots ranged between 3.7 and 4.2 mg/kg (mixed formulation) and between 3.0 and 6.7 mg/kg (solo formulation). The residues of pyraclostrobin in wheat grain collected at 36 DALA and later, were between <0.01 and 0.015 mg/kg (mixed formulation) and between <0.01 and 0.017 mg/kg (solo formulation). Residues of metabolite BF 500-3 were in grain always <0.01 mg/kg.
In wheat straw, residues of pyraclostrobin ranged from 0.29 to 1.6 mg/kg (mixed formulation) and from 0.29 to 1.5 mg/kg (solo formulation) whereas residues of metabolite BF 500-3 ranged between 0.10 and 0.56 mg/kg (mixed formulation) and from 0.12 to 0.45 mg/kg (solo formulation).

Table 6.3.2-28: Residues of pyraclostrobin and BF 500-3 in wheat after applications of BAS 703 04 F or BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[1]	Total[2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 419892	Wheat / Asano	Germany	BAS 703 04 F Pyraclostrobin 2 x 0.225	BBCH 69	0	whole plant[3]	4.0	0.19	4.1	**BAS 500 F Method No.: 535/1 (L0076/01)**				
Doc ID: 2013/1336790					28	ears	0.045	0.018	0.063	whole plant[3]	0.1-10	4	80.4	5.2
Trial No. L120232					28	rest of plant[3]	0.30	0.11	0.41	ears	0.01-1.0	3	88.2	5.3
GLP: yes					34	ears	<0.01	<0.01	<0.02	rest of plant[3]	0.01-1.0	6	83.2	16.6
Year: 2012					34	rest of plant[3]	<0.01	<0.01	<0.02	grain	0.01-1.0	3	92.8	4.2
					42	grain	**<0.01**	<0.01	<0.02	straw	0.1-10	2	79.0	n.a.
					42	straw	0.29	0.13	0.42					
					50	grain	<0.01	<0.01	<0.02					
					50	straw	**0.37**	0.15	0.53	**BF 500-3 Method No.: 535/1 (L0076/01)**				
			BAS 500 06 F Pyraclostrobin 2 x 0.25		0	whole plant[3]	3.7	0.21	3.9	whole plant[3]	0.1-10	4	76.8	8.1
					28	ears	0.068	0.028	0.095	ears	0.01-1.0	3	86.0	6.5
					28	rest of plant[3]	0.27	0.10	0.37	rest of plant[3]	0.01-1.0	6	81.3	14.9
					34	ears	0.050	0.021	0.071	grain	0.01-1.0	3	93.8	3.2
					34	rest of plant[3]	0.13	0.04	0.17	straw	0.1-10	2	81.5	n.a.
					42	grain	**<0.01**	<0.01	<0.02					
					42	straw	0.29	0.12	0.41					
					50	grain	<0.01	<0.01	<0.02					
					50	straw	**0.41**	0.16	0.57					

Table 6.3.2-28: Residues of pyraclostrobin and BF 500-3 in wheat after applications of BAS 703 04 F or BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[1]	Total[2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 419892 **Doc ID:** 2013/1336790 **Trial No.:** L120233 **GLP:** yes **Year:** 2012	Wheat / JB Diego	United Kingdom	BAS 703 04 F Pyraclostrobin 2 x 0.225	BBCH 69	0	whole plant[3]	3.7	0.04	3.0					
					28	grain	<0.01	<0.01	<0.02					
					28	straw	1.1	0.21	1.3					
					36	grain	**<0.01**	<0.01	<0.02					
					36	straw	**1.1**	0.24	1.3					
					42	grain	<0.01	<0.01	<0.02					
					42	straw	1.1	0.25	1.4					
			BAS 500 06 F Pyraclostrobin 2 x 0.25		0	whole plant[3]	3.0	0.03	3.0					
					28	grain	0.012	<0.01	0.022					
					28	straw	0.74	0.15	0.89					
					36	grain	**<0.01**	<0.01	<0.02					
					36	straw	0.84	0.21	1.0					
					42	grain	<0.01	<0.01	<0.02					
					42	straw	**1.3**	0.30	1.6					
Study code: 419892 **Doc ID:** 2013/1336790 **Trial No.** L120234 **GLP:** yes **Year:** 2012	Wheat / Gallareta	Spain	BAS 703 04 F Pyraclostrobin 2 x 0.225	BBCH 69	0	whole plant[3]	4.2	0.045	4.2					
					28	ears	0.35	0.075	0.42					
					28	rest of plant[3]	0.26	0.047	0.30					
					36	grain	**<0.01**	<0.01	<0.02					
					36	straw	0.30	0.10	0.41					
					42	grain	<0.01	<0.01	<0.02					
					42	straw	**0.45**	0.13	0.58					
			BAS 500 06 F Pyraclostrobin 2 x 0.25		0	whole plant[3]	6.7	0.072	6.8					
					28	ears	0.25	0.10	0.35					
					28	rest of plant[3]	0.48	0.22	0.71					
					36	grain	<0.01	<0.01	<0.02					
					36	straw	0.34	0.15	0.49					
					42	grain	**0.010**	<0.01	0.02					
					42	straw	**0.46**	0.22	0.69					

Table 6.3.2-28: Residues of pyraclostrobin and BF 500-3 in wheat after applications of BAS 703 04 F or BAS 500 06 F (EC)

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[1]	Total[2]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 419892 **Doc ID:** 2013/1336790 **Trial No.** L120235 **GLP:** yes **Year:** 2012	Wheat / Ingenio	France (S)	BAS 703 04 F Pyraclostrobin 2 x 0.225	BBCH 69	0	whole plant[3]	3.8	0.025	3.8					
					28	ears	0.48	0.19	0.67					
					28	rest of plant[3]	1.6	0.41	2.0					
					35	ears	0.35	0.14	0.49					
					35	rest of plant[3]	1.3	0.38	1.7					
					42	grain	**0.015**	<0.01	0.025					
					42	straw	**1.6**	0.56	2.2					
			BAS 500 06 F Pyraclostrobin 2 x 0.25		0	whole plant[3]	3.5	0.051	3.5					
					28	ears	0.51	0.23	0.74					
					28	rest of plant[3]	4.3[4]	1.1[4]	5.4[4]					
					35	ears	0.52	0.26	0.78					
					35	rest of plant[3]	3.9[4]	1.2[4]	5.1[4]					
					42	grain	**0.017**	<0.01	0.027					
					42	straw	**1.5**	0.45	1.9					

[1] conversion factor for calculation of 500M07 (BF 500 3) to parent BAS 500 F is 1.084
[2] for residues <0.01 mg/kg, value was set to 0.01 mg/kg for calculation of sum
[3] without roots
[4] mean of three analyses
n.a. not applicable
underlined values are used for MRL calculation

CA 6.3.3 Maize, sweet corn

The use in maize was not part of the previous Annex I inclusion process, it was submitted for the first time in Europe in summer 2010. The dRR was intended to achieve the registration of the mixed formulation BAS 512 16 F (containing the active ingredients pyraclostrobin and epoxiconazole) in maize. Subsequently to this submission, further draft Registration Reports were provided to countries acting as zonal RMS. The trials supporting a registration of the solo formulation BAS 500 06 F were partly included since the trials were performed side-by-side at the same locations.

After submission, Germany has prepared an evaluation report on the MRLs for maize, which was provided to EFSA in summer 2011. Based on the evaluation report EFSA has published a Reasoned Opinion (Feb 2012) in which the use in maize and its impact on the dietary feed burden calculations were investigated.

In order to allow a comprehensive evaluation of the formulation BAS 500 06 F, the old and the new data are summarized in this chapter.

Table 6.3.3-1: Summary of the critical GAP for the proposed use in maize and sweet corn for BAS 500 06 F

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum number of applications	Minimum application interval (days)	Maximum		Minimum PHI (days)
					Rate (kg as/ha)	Water (L/ha)	
Maize	O	65	1	n.a.	0.2	100 -400	F
Sweet corn	O	65	1	n.a.	0.2	100 - 400	F

Table 6.3.3-2: Number of residue trials conducted per geographical region and vegetation period

Crop	Vegetation period	Number of trials					Reference
		EU North	Country	EU South	Country	Total	
Maize, sweet corn	2012	4	DE,UK,NL,BE	4	FR, GR,IT,ES	8	6.3.3/1
	2013	1	FR	-	-	1	6.3.3/2
	2008[1]	4	DE,FR,UK,NL	4	IT,GR,ES,FR	8	6.3.3/3, 6.3.3/4
	2011[2]	1	DE	-	-	1	6.3.3/5
	2009 [1,3]	4	DE,FR,UK,NL	4	IT,GR,ES,FR	8	6.3.3/6
Total number of trials per region		**14**		**12**	**Total number of trials**	**26**	

[1] **BAS 512 04 F: The study has been already evaluated by Germany in context of a previous submission.**
[2] Exploratory Non-GLP trial submitted in context of a rebuttal document to France.
[3] BAS 500 06 F was included for comparison purposes in bridging trials.

Report:	CA 6.3.3/1 Aitken A., 2013a Study on the residue behaviour of Pyraclostrobin (BAS 500 F) in maize following one application of BAS 500 06 F to 8 trials in 2012 - SEU and NEU 2013/1308888
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7525/VI/95 rev. 9 (March 2011), EEC 7029/VI/95 (22 July 1997)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F

Description:	BAS 500 06 F (200 g/l BAS 500 F, pyraclostrobin), EC
Lot/Batch #:	0004863761
Purity:	Not relevant
CAS#:	Pyraclostrobin (BAS 500 F): 175013-18-0 500M07 (BF 500-3): 51265-96-7
Development code:	not applicable
Spiking levels:	0.01 – 20 mg/kg

2. Test Commodity: Cereals

Crop:	Maize
Type:	not relevant
Variety:	Athlet, NK Bull, Pioneer P 8000, LG 30222, Octet, AS 72, Antiss, PR33Y-7U

Crop parts(s) or processed

Commodity:	whole plant without roots, cobs without husks, cobs with husks, rest of plant without roots, grain and straw
Sample size:	> 0.5 kg (12 plants)

B. STUDY DESIGN AND METHODS

1. Test procedure

During the growing season of 2012, eight field trials with maize (field conditions) were conducted in Germany, United Kingdom, The Netherlands, Belgium, South France, Greece, Italy and Spain, in order to determine the magnitude of the residues of pyraclostrobin after a single treatment with BAS 500 06 F.

Each trial consisted of two plots: plot 1 (untreated control) and plot 2 (treated with BAS 500 06 F). The test item BAS 500 06 F (200 g/L pyraclostrobin, EC), was applied to maize once at BBCH 65 at a rate of 0.200 kg as/ha of pyraclostrobin with a water volume of 400 L/ha (with 10% of variation). Whole plant (without roots) specimens were taken at 0 days after the application, cobs without husks, cobs with husks, and the rest of the plant were taken at BBCH 75 and BBCH 85 and grain and straw were taken at BBCH 89. The samples were frozen until analysis. The maximum storage interval was 260 days (the interval from sampling until completion of the analytical phase).

Table 6.3.3-3: Application and sampling details for trials conducted in 2012

Region	No. of trials	No. of appl.	F, G, I[2]	Test item	Active substance	Application		Target timing	
						Rate (kg as/ha)	Water vol. (L/ha)	Appl. (BBCH)	Sampl. (DALA)[1]
EU North & South	8	1	F	BAS 500 06 F (EC)	Pyraclo-strobin (BAS 500 F)	0.200	400	65	0 BBCH 75 BBCH 85 BBCH 89

[1] days after last application
[2] Field, Glasshouse or Indoor

2. Description of analytical procedures

All specimens were analysed for pyraclostrobin and its metabolite BF 500-3 using the BASF method No. 535/1 (L0076/01), which quantifies residues of pyraclostrobin and its metabolite analyte with a limit of quantitation (LOQ) of 0.01 mg/kg per analyte.

The results of procedural recovery experiments obtained with the analytical series averaged at about 93% for pyraclostrobin and at about 92% for BF 500-3 at fortification levels between 0.01 and 20.0 mg/kg.

According to method No. 535/1, the analytes are extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination is performed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

Pyraclostrobin

"Whole plant without roots" specimens collected at 0 DALA (BBCH 65) showed pyraclostrobin residues between 1.5 and 4.2 mg/kg.

At BBCH 75 cobs without husks showed pyraclostrobin residues from <0.010 to 0.021 mg/kg, cobs with husks showed pyraclostrobin residues from <0.010 to 0.075 mg/kg and residues of the rest of plant (without roots) specimens were between 0.15 and 0.71 mg/kg.

At BBCH 85 cobs without husks showed pyraclostrobin residues between <0.010 and 0.039 mg/kg, cobs with husks showed pyraclostrobin residues from <0.010 to 0.052 mg/kg and residues of the rest of plant (without roots) specimens were between 0.20 and 0.76 mg/kg.

Maize straw specimens collected at BBCH 89 showed pyraclostrobin residues between <0.010 and 0.30 mg/kg. The pyraclostrobin residues in maize grain specimens from all trials taken at BBCH 89 were below the LOQ (<0.010 mg/kg).

No residues of pyraclostrobin above the limit of quantitation (0.010 mg/kg) were found in the majority of the untreated specimens of this study. For two whole plants without roots specimens contaminations of pyraclostrobin occurred up to 0.031 mg/kg and in 4 "rest of plant without roots" specimens up to 0.16 mg/kg. In addition, pyraclostrobin was detected in one straw specimen at the limit of quantitation (0.01 mg/kg).

BF 500-3

"Whole plant without roots" specimens collected at BBCH 65 showed BF 500-3 residues between <0.010 and 0.11 mg/kg.

Cobs without husks and cobs with husks specimens collected at BBCH 75 showed BF 500-3 residues below the LOQ (<0.010 mg/kg). Rest of plant (without roots) specimens collected at BBCH 75 showed BF 500-3 residues between 0.038 and 0.23 mg/kg.

Cobs without husks and cobs with husks specimens collected at BBCH 85 showed BF 500-3 residues below the LOQ (<0.010 mg/kg). Rest of plant (without roots) specimens collected at BBCH 85 showed BF 500-3 residues between 0.032 and 0.17 mg/kg.

Maize straw specimens collected at BBCH 89 showed BF 500-3 residues between <0.010 and 0.15 mg/kg. The BF 500-3 residues in maize grain specimens from all trials taken at BBCH 89 were below the LOQ (<0.010 mg/kg).

No residues of BF 500-3 above the limit of quantitation (0.010 mg/kg) were found in the untreated specimens of this study.

Summaries of residues of pyraclostrobin and its metabolite BF 500-3 are given in Table 6.3.3-4, details in Table 6.3.3-5.

Table 6.3.3-4: Summary of pyraclostrobin and BF 500-3 residues in the treated maize specimens after one application of BAS 500 06 F

Region	Matrix	Timing (DALA[1])	Range of residues[4] (mg/kg)		
			BAS 500 F	BF 500-3[2]	Total residue[3]
EU North & South	Whole plant w/o roots	0	1.5 – 4.2	<0.010 – 0.11	1.6 – 4.2
	Cobs without husks	15 - 36	<0.010 – 0.021	<0.010	<0.020 – 0.031
	Cobs with husks		<0.010 – 0.075	<0.010	<0.020 – 0.085
	Rest of plant w/o roots		0.15 – 0.71	0.038 – 0.23	0.22 – 0.92
	Cobs without husks	26 - 56	<0.010 – 0.039	<0.010	<0.020 – 0.049
	Cobs with husks		<0.010 – 0.052	<0.010	<0.020 – 0.062
	Rest of plant w/o roots		0.20 – 0.76	0.032 – 0.17	0.25 – 0.89
	Grain[5]	34 - 82	<0.010	<0.010	<0.020
	Straw[5]		<0.010 – 0.30	<0.010 – 0.15	<0.020 – 0.40

[1] days after last application
[2] conversion factor for calculation of BF 5003 to parent BAS 500 F is 1.084
[3] sum of pyraclostrobin and BF 500-3
[4] for calculation purposes < 0.01 was set to 0.01
[5] In error the farmer harvested the trial plots before the final (S4) sampling could be conducted. Therefore no harvest grain and straw samples were available for analysis.

III. CONCLUSION

The results show, that immediately after the application of formulation BAS 500 06 F at a rate of 0.200 kg as/ha, residues of pyraclostrobin were in "whole plant without root" specimens between 1.5 and 4.2 mg/kg. Residues of metabolite BF 500-3 ranged in those samples from <0.010 to 0.11 mg/kg.
Pyraclostrobin as well as the BF 500-3 (500M07) residues in maize grain specimens from all trials taken at BBCH 89 were below the LOQ (<0.010 mg/kg).

In "cobs without husks" specimens sampled at BBCH 85 (being relevant for extrapolation to sweet corn), pyraclostrobin residues up to 0.039 mg/kg were determined. Residues of metabolite BF 500-3 were in those specimens always below the LOQ of the analytical method applied (0.01 mg/kg).

Table 6.3.3-5: Residues of pyraclostrobin and its metabolite BF 500-3 in maize after one application of BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 422769	Maize / Athlet	Germany	BAS 500 06 F: 1x 0.200 kg pyraclostrobin/ha Spray volume: 400 L/ha	BBCH 65	0	Whole plant[1]	2.0	<0.010	2.00	**BAS 500 F Method No.:535/1 (L0076/01)**				
Doc ID: 2013/1308888					36	Cobs w/o husks	0.013	<0.010	0.023	all sample matrices	0.01 - 20	37	93.0	5.0
Trial No. L120357					36	Cobs with husks	0.012	<0.010	0.022					
GLP: yes					36	Rest of plant[1]	0.27	0.038	0.31	**BF 500-3 Method No.: 535/1 (L0076/01)**				
Year: 2012					56	Cobs w/o husks	**0.015**	<0.010	0.025	all sample matrices	0.01 - 20	37	92.0	5.9
					56	Cobs with husks	<0.010	<0.010	<0.020					
					56	Rest of plant[1]	**0.36**	0.032	0.39					
					78	Maize grain	**<0.010**	<0.010	<0.020					
					78	Maize straw	0.08	0.016	0.096					
Study code: 422769	Maize / NK Bull	UK	BAS 500 06 F: 1x 0.200 kg pyraclostrobin/ha Spray volume: 400 L/ha	BBCH 65	0	Whole plant[1]	4.2	<0.010	4.20					
Doc ID: 2013/1308888					19	Cobs w/o husks	<0.010	<0.010	<0.020					
Trial No. L120358					19	Cobs with husks	0.075	<0.010	0.085					
GLP: yes					19	Rest of plant[1]	**0.25**	0.052	0.30					
Year: 2012					26	Cobs w/o husks	**<0.010**	<0.010	<0.020					
					26	Cobs with husks	0.036	<0.010	0.046					
					26	Rest of plant[1]	0.22	0.037	0.26					
					34	Maize grain	**<0.010**	<0.010	<0.020					
					34	Maize straw	0.14	0.026	0.16					

Table 6.3.3-5: Residues of pyraclostrobin and its metabolite BF 500-3 in maize after one application of BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 422769 **Doc ID:** 2013/1308888 **Trial No.** L120359 **GLP:** yes **Year:** 2012	Maize / Pioneer P 8000	The Nether-lands	BAS 500 06 F: 1x 0.200 kg pyraclostrobin/ha Spray volume: 400 L/ha	BBCH 65	0	Whole plant[1]	3.7	0.11	3.8					
					29	Cobs w/o husks	0.018	<0.010	0.028					
					29	Cobs with husks	0.017	<0.010	0.027					
					29	Rest of plant[1]	**0.71**	0.21	0.92					
					43	Cobs w/o husks	**0.011**	<0.010	0.021					
					43	Cobs with husks	0.022	<0.010	0.032					
					43	Rest of plant[1]	0.47	0.17	0.64					
					71	Maize grain	**<0.010**	<0.010	<0.020					
					71	Maize straw	<0.010	<0.010	<0.020					
Study code: 422769 **Doc ID:** 2013/1308888 **Trial No.** L120360 **GLP:** yes **Year:** 2012	Maize / LG 30222	Belgium	BAS 500 06 F: 1x 0.200 kg pyraclostrobin/ha Spray volume: 400 L/ha	BBCH 65	0	Whole plant[1]	2.7	<0.010	2.7					
					29	Cobs w/o husks	0.021	<0.010	0.031					
					29	Cobs with husks	0.067	<0.010	0.077					
					29	Rest of plant[1]	**0.42**	0.07	0.50					
					44	Cobs w/o husks	**0.039**	<0.010	0.049					
					44	Cobs with husks	0.044	<0.010	0.054					
					44	Rest of plant[1]	0.33	0.06	0.39					
					-	Maize grain[4]	-	-	-					
					-	Maize straw[4]	-	-	-					

Table 6.3.3-5: Residues of pyraclostrobin and its metabolite BF 500-3 in maize after one application of BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 422769 **Doc ID:** 2013/1308888 **Trial No.** L120361 **GLP:** yes **Year:** 2012	Maize / Octet	France (S)	BAS 500 06 F: 1x 0.200 kg pyraclostrobin/ha Spray volume: 400 L/ha	BBCH 65	0	Whole plant[1]	1.8	<0.010	1.8					
					24	Cobs w/o husks	<0.010	<0.010	<0.020					
					24	Cobs with husks	0.068	<0.010	0.078					
					24	Rest of plant[1]	**0.27**	0.049	0.31					
					45	Cobs w/o husks	**<0.010**	<0.010	<0.020					
					45	Cobs with husks	0.052	<0.010	0.062					
					45	Rest of plant[1]	0.20	0.05	0.25					
					82	Maize grain	**<0.010**	<0.010	<0.020					
					82	Maize straw	0.30	0.079	0.38					
Study code: 422769 **Doc ID:** 2013/1308888 **Trial No.** L120362 **GLP:** yes **Year:** 2012	Maize / AS 72	Greece	BAS 500 06 F: 1x 0.200 kg pyraclostrobin/ha Spray volume: 400 L/ha	BBCH 65	0	Whole plant[1]	1.5	0.088	1.6					
					27	Cobs w/o husks	<0.010	<0.010	<0.020					
					27	Cobs with husks	<0.010	<0.010	<0.020					
					27	Rest of plant[1]	0.27	0.078	0.35					
					42	Cobs w/o husks	**<0.010**	<0.010	<0.020					
					42	Cobs with husks	<0.010	<0.010	<0.020					
					42	Rest of plant[1]	**0.76**	0.13	0.89					
					55	Maize grain	**<0.010**	<0.010	<0.020					
					55	Maize straw	0.23	0.058	0.29					

Table 6.3.3-5: Residues of pyraclostrobin and its metabolite BF 500-3 in maize after one application of BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 422769 **Doc ID:** 2013/1308888 **Trial No.** L120363 **GLP:** yes **Year:** 2012	Maize / Antiss	Italy	BAS 500 06 F: 1x 0.200 kg pyraclostrobin/ha Spray volume: 400 L/ha	BBCH 65	0	Whole plant[1]	1.7	<0.010	1.7					
					30	Cobs w/o husks	<0.010	<0.010	<0.020					
					30	Cobs with husks	<0.010	<0.010	<0.020					
					30	Rest of plant[1]	0.15	0.074	0.22					
					44	Cobs w/o husks	**<u><0.010</u>**	<0.010	<0.020					
					44	Cobs with husks	<0.010	<0.010	<0.020					
					44	Rest of plant[1]	**<u>0.21</u>**	0.078	0.28					
					67	Maize grain	**<u><0.010</u>**	<0.010	<0.020					
					67	Maize straw	0.29	0.074	0.36					
Study code: 422769 **Doc ID:** 2013/1308888 **Trial No.** L120364 **GLP:** yes **Year:** 2012	Maize / PR33Y-7U	Spain	BAS 500 06 F: 1x 0.200 kg pyraclostrobin/ha Spray volume: 400 L/ha	BBCH 65	0	Whole plant[1]	2.2	<0.010	2.2					
					15	Cobs w/o husks	<0.010	<0.010	<0.020					
					15	Cobs with husks	0.030	<0.010	0.040					
					15	Rest of plant[1]	**<u>0.60</u>**	0.23	0.82					
					32	Cobs w/o husks	**<u><0.010</u>**	<0.010	<0.020					
					32	Cobs with husks	0.018	<0.010	0.028					
					32	Rest of plant[1]	0.22	0.13	0.34					
					78	Maize grain	**<u><0.010</u>**	<0.010	<0.020					
					78	Maize straw	0.25	0.15	0.40					

[1] without roots
[2] conversion factor for calculation of BF 500-3 to parent BAS 500 F is 1.084
[3] for calculation purposes < 0.01 was set to 0.01
[4] In error the farmer harvested the trial plots before the final (S4) sampling could be conducted. Therefore, no harvest grain and straw samples were available for analysis.
<u>underlined values</u> were used for MRL calculation

Report:	CA 6.3.3/2 Aitken A., 2014b Study on the residue behaviour of Pyraclostrobin (BAS 500 F) in maize following one application of BAS 500 06 F to 1 trial in 2013 – NEU 2014/1001741
Guidelines:	EC 1107/2009 (14 June 2011), EEC 79/117, EEC 91/414, EEC 7525/VI/95 rev. 9 (March 2011), EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	BAS 500 06 F
	Description:	BAS 500 06 F (200 g/l BAS 500 F, pyraclostrobin), EC
	Lot/Batch #:	0005018890
	Purity:	Not relevant
	CAS#:	175013-18-0
	Development code:	not applicable
	Spiking levels:	0.01 – 10 mg/kg
2.	**Test Commodity:**	Cereals
	Crop:	Maize
	Type:	not relevant
	Variety:	Pomeri
	Botanical name:	*Zea mays L.*
	Crop parts(s) or processed	
	Commodity:	whole plant without roots, cobs without husks, cobs with husks, rest of plant without roots, grain and straw
	Sample size:	min. 12 plants (grain: 2.0 kg, straw 0.5 kg, other matrices: 1.0 kg)

B. STUDY DESIGN AND METHODS

1. Test procedure

During the growing season of 2013, one field trial with maize (field conditions) was conducted in Northern France, in order to determine the magnitude of the residues of pyraclostrobin and its metabolite BF 500-3 after a single treatment with BAS 500 06 F. The trial consisted of two plots: plot 1 (control), plot 2 (treated with BAS 500 06 F).

The test item BAS 500 06 F (200 g/L pyraclostrobin), an emulsifiable concentrate (EC), was applied to maize once at BBCH 65 at a rate of 0.200 kg as/ha of pyraclostrobin. The water volume used was 400 L/ha. Whole plant (no roots) specimens were taken at 0 days before application in plot 1 and 0 days after the application in plot 2 (BBCH 65), cobs without husks, cobs with husks and rest of plant were taken at BBCH 75 and BBCH 85 and maize grain and straw were taken at BBCH 89. In order to ensure that the data regarding the residue are as reliable as possible, a minimum of 12 plants were taken.

Table 6.3.3-6: Application and sampling details for trials conducted in 2012

Region	No. of trials	No. of appl.	F, G, I[2]	Test item	Active substance	Application		Target timing	
						Rate (kg as/ha)	Water vol. (L/ha)	Appl. (BBCH)	Sampl. (DALA)[1]
Northern Europe	1	1	F	BAS 500 06 F (EC)	Pyraclo-strobin BAS 500 F	0.200	400	65	0 BBCH 75 BBCH 85 BBCH 89

[1] days after last application
[2] Field, Glasshouse or Indoor

2. Description of analytical procedures

All specimens were analysed for pyraclostrobin and its metabolite BF 500-3 using the BASF method 535/1 (synonym: L0076/01), which quantifies residues of pyraclostrobin and its metabolite analyte with a limit of quantitation (LOQ) of 0.01 mg/kg per analyte.
The results of procedural recovery experiments obtained with the analytical series were at 94.2% for pyraclostrobin and at 99.6% for BF 500-3 at fortification levels between 0.01 and 10.0 mg/kg.
According to method No. 535/1, the analytes are extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination is performed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

Pyraclostrobin:
"Whole plant without roots" specimens collected at 0 DALA (BBCH 65) showed pyraclostrobin residues of 1.6 mg/kg. At BBCH 75 cobs without husks showed pyraclostrobin residues below the LOQ (<0.010 mg/kg), cobs with husks showed pyraclostrobin residues 0.028 mg/kg and the rest of plant (no roots) specimen 0.26 mg/kg. At BBCH 85, cobs without husks showed pyraclostrobin residues below the LOQ (<0.010 mg/kg), cobs with husks showed pyraclostrobin residues of 0.016 mg/kg and the rest of plant (no roots) specimen 0.44 mg/kg. Maize straw specimen collected at BBCH 89 showed pyraclostrobin residues of 0.19 mg/kg. The pyraclostrobin residues in maize grain specimen taken at BBCH 89 were below the LOQ (<0.010 mg/kg). No residues of pyraclostrobin above the limit of quantitation (0.010 mg/kg) were found in the untreated specimens of this study.

Metabolite BF 500-3:
"Whole plant without roots" specimen collected at BBCH 65 showed BF 500-3 residues below the LOQ (<0.010 mg/kg). Cobs without husks and cobs with husks specimens collected at BBCH 75 showed BF 500-3 residues below the LOQ (<0.010 mg/kg). Rest of plant (no roots) specimen collected at BBCH 75 showed BF 500-3 residues of 0.066 mg/kg. Cobs without husks and cobs with husks specimens collected at BBCH 85 showed 500M07 residues below the LOQ (<0.010 mg/kg). Rest of plant (no roots) specimen collected at BBCH 85 showed BF 500-3 residues of 0.071 mg/kg. Maize straw specimens collected at BBCH 89 showed BF 500-3 residues of 0.047 mg/kg. The BF 500-3 residues in Maize grain specimens taken at BBCH 89 were below the LOQ (<0.010 mg/kg). No residues of BF 500 -3 above the limit of quantitation (0.010 mg/kg) were found in the untreated specimens of this study.

Summaries of residues of pyraclostrobin and its metabolite BF 500-3 are given in Table 6.3.2-7, details in Table 6.3.2-8.

Table 6.3.3-7: Summary of pyraclostrobin and BF 500-3 residues in the treated maize specimens after one application of BAS 500 06 F

Region	Matrix	Timing (DALA[1])	Growth stage	Range of residues (mg/kg)		
				BAS 500 F	BF 500-3[2]	Total residue[3]
Northern Europe	Whole plant w/o roots	0	65	1.6	<0.01	1.6
	Cobs without husks	29	75	<0.010	<0.01	<0.020
	Cobs with husks			0.028	<0.01	0.038
	Rest of plant w/o roots			0.26	0.066	0.33
	Cobs without husks	42	85	<0.010	<0.01	<0.020
	Cobs with husks			0.016	<0.01	0.026
	Rest of plant w/o roots			0.44	0.071	0.51
	Grain	85	89	<0.010	<0.01	<0.020
	Straw			0.19	0.047	0.24

[1] days after last application
[2] conversion factor for calculation of BF 5003 to parent BAS 500 F is 1.084
[3] sum of pyraclostrobin and BF 500-3, for calculation purposes < 0.01 was set to 0.01

III. CONCLUSION

Immediately after the application of formulation BAS 500 06 F, "whole plant without root" specimens showed pyraclostrobin residues of 1.6 mg/kg, BF 500-3 residues were below the LOQ (<0.010 mg/kg).
Maize straw specimen collected at BBCH 89 showed pyraclostrobin residues of 0.19 mg/kg and BF 500-3 residues of 0.047 mg/kg. The pyraclostrobin and metabolite BF 500-3 residues in maize grain specimens taken at BBCH 89 were below the LOQ (<0.01 mg/kg).

Table 6.3.3-8: Residues of pyraclostrobin and its metabolite BF 500-3 in maize after one application of BAS 500 06 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[2]	Total[3]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 422769_1	Maize / Pomeri	Northern France	BAS 500 06 F: 1x 0.200 kg pyraclostrobin/ha Spray volume: 400 L/ha	BBCH 65	0	Whole plant[1]	1.60	<0.010	1.60	**BAS 500 F Method No.:535/1 (L0076/01)**				
Doc ID: 2014/1001741					29	Cobs w/o husks	<0.010	<0.010	<0.020	all matrices	0.01 - 10	18	94.2	4.1
Trial No. L130518					29	Cobs with husks	0.028	<0.010	0.038					
GLP: yes					29	Rest of plant[1]	0.26	0.066	0.33	**BF 500-3 Method No.: 535/1 (L0076/01)**				
Year: 2013					42	Cobs w/o husks	**<u><0.010</u>**	<0.010	<0.020	all matrices	0.01 - 10	18	99.6	2.9
					42	Cobs with husks	0.016	<0.010	0.026					
					42	Rest of plant[1]	**<u>0.44</u>**	0.071	0.51					
					85	Maize grain	**<u><0.010</u>**	<0.010	<0.020					
					85	Maize straw	0.19	0.047	0.24					

[1] without roots
[2] conversion factor for calculation of BF 500-3 to parent BAS 500 F is 1.084
[3] for calculation purposes < 0.01 was set to 0.01
<u>underlined values</u> were used for MRL calculation

This study has been already submitted to Germany in context of a previous submission. It was used as basis for the corresponding EFSA Reasoned Opinion (EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606).

Report:	CA 6.3.3/3 Schulz H., Ziske J., 2010a Study on the residue behaviour of Pyraclostrobin and Epoxiconazole in maize after treatment with BAS 512 04 F under field conditions in Germany, Northern France, United Kingdom, the Netherlands, Italy, Greece, Southern France and Spain 2008 2010/1025690
Guidelines:	EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, OECD-ENV/JM/MONO(2002)/9
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Report:	CA 6.3.3/4 Schulz H., 2010a 1st addendum to the report - Study on the residue behaviour of Pyraclostrobin and Epoxiconazole in maize after treatment with BAS 512 04 F under field conditions in DE, N-FR, UK, NL, IT, GR, S-FR and ES, 2008 2010/1080941
Guidelines:	EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, OECD-ENV/JM/MONO(2002)/9
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 512 04 F

Description:	BAS 512 04 F (pyraclostrobin: 133 g/L, epoxiconazole: 50 g/L), SE
Lot/Batch #:	FRE-000369
Purity:	Not relevant
CAS#:	Pyraclostrobin: 175013-18-0 Epoxiconazole: 133855-98-8
Development code:	not applicable
Spiking levels:	Pyraclostrobin: 0.01/1/5 mg/kg

2. Test Commodity: Cereals

Crop:	Maize
Type:	not applicable
Variety:	Delitop, PR 39T13, Justina, Adenzo, 66-77 Dekalb, 3441 Pioneer, Mitic, Tardio 130.
Botanical name:	*Zea mays*
Crop parts(s) or processed	
Commodity:	whole plant (no roots), rest of plant (no roots), cobs with husks, grain
Sample size:	1 kg (12 plants) of whole plant without roots; 1 kg (12 pieces) of cob with husks; 2 kg of grain.

B. STUDY DESIGN AND METHODS

1. Test procedure

During the growing season 2008, eight field trials were conducted in Germany, Northern France, United Kingdom, The Netherlands, Italy, Greece, Southern France and Spain in order to determine the residue levels of pyraclostrobin, its metabolite BF 500-3 and epoxiconazole in maize. The trials were performed with the formulation BAS 512 04 F, a SE formulation containing 133 g/L of pyraclostrobin and 50 g/L of epoxiconazole. BAS 512 04 F was applied once at a rate of 0.1995 kg as/ha of pyraclostrobin and 0.075 kg as/ha of epoxiconazole. The application took place at BBCH 65 with a spray volume of 400 L/ha.
Whole plants without roots were collected from the treated plots immediately after the treatment at BBCH 65. Thereafter specimens consisting of cob with husks or rest of plant without roots were sampled at 7 - 28 DALA (BBCH 71 – 73), at 16 - 42 DALA (BBCH 75 – 79) and at 30 - 70 DALA (BBCH 85). Grain and rest of plant without roots were collected at harvest at 63 - 99 DALA at BBCH 89. All samples were stored deep frozen until analysis. The maximal storage interval was 260 days.

2. Description of analytical procedure

For the analysis of pyraclostrobin and its metabolite BF 500-3 the BASF method no. 535/1 was used. The results of procedural recovery experiment averaged at about 92.5% for pyraclostrobin and 88.5% for BF 500-3 at fortification levels between 0.01 and 5.0 mg/kg. The method achieves a limit of quantitation (LOQ) of 0.01 mg/kg.
The residues of pyraclostrobin and its metabolite BF 500-3 were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination was performed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

Directly after the application (0 DALA) of BAS 512 04 F, pyraclostrobin residues were between 1.93 mg/kg and 3.49 mg/kg in maize whole plant without roots. In the course of the study, the residues of pyraclostrobin declined. At harvest time (66 - 99 DALA) the pyraclostrobin residues in the rest of the plants were between 0.03 mg/kg and 0.27 mg/kg. In grain, no pyraclostrobin residues above the LOQ were obtained.
At 0 DALA, BF 500-3 residues were between 0.01 - 0.14 mg/kg in maize whole plant without roots. At harvest time (66 - 99 DALA) the BF 500-3 residues in the rest of the plants were between 0.01 mg/kg and 0.16 mg/kg. In grain, BF 500-3 residues were between < 0.01 and 0.01 mg/kg.

The results from trial number L080330 showed a different behaviour and are therefore presented separately. Directly after the application (0 DALA) the residues of pyraclostrobin were <0.01 mg/kg in whole plants without roots. At BBCH 89 (63 DALA), pyraclostrobin residues were <0.01 mg/kg in maize grain. In the rest of the plants without roots, pyraclostrobin residues were 0.15 mg/kg.

Summaries of residues of pyraclostrobin and its metabolite BF 500-3 are given in Table 6.3.3-9 and Table 6.3.3-10, details are provided in Table 6.3.2-11.

Table 6.3.3-9: Summary of residues (BAS 500 F and BF 500-3) in maize after one application of BAS 512 04 F (Trials L080323 to L080329)

Matrix	Year	DALA[1]	Range of residues (mg/kg)		
			BAS 500 F	BF 500-3	BAS 500 F, total [2,3]
whole plant[4]	2008	0	1.93 - 3.49	0.01 - 0.14	1.96 - 3.55
cob with husks		7-26	0.02 - 0.09	< 0.01 - 0.02	0.03 - 0.11
rest of plants[4]		7-26	0.19 - 0.84	0.03 - 0.22	0.22 - 1.02
cob with husks		16-42	< 0.01 - 0.06	< 0.01	0.02 - 0.07
rest of plants[4]		16-42	0.09 - 0.21	0.02 - 0.11	0.12 - 0.32
cob with husks		30-70	< 0.01 - 0.09	< 0.01	0.02 - 0.10
rest of plants[4]		30-70	0.05 - 0.16	0.01 - 0.05	0.06 - 0.18
grain		66-99	< 0.01	< 0.01 - 0.01	0.02
rest of plants[4]		66-99	0.03 - 0.27	0.01 - 0.16	0.04 - 0.43

[1] days after last application
[2] as BAS 500 F equivalent
[3] for calculation purposes, "< 0.02" is set 0.02
[4] without root

Table 6.3.3-10: Summary of residues (BAS 500 F and BF 500-3) in maize after one application of BAS 512 04 F (Trial L080330)

Matrix	Year	DALA[1]	Range of residues (mg/kg)		
			BAS 500 F	BF 500-3	BAS 500 F, total [2,3]
whole plant[4]	2008	0	< 0.01	< 0.01	0.02
cob with husks		28	< 0.01	< 0.01	0.02
rest of plants[4]		28	0.08	0.02	0.10
cob with husks		41	< 0.01	< 0.01	0.02
rest of plants[4]		41	< 0.01	< 0.01	0.02
cob with husks		55	0.01	< 0.01	0.02
rest of plants[4]		55	< 0.01	0.02	0.03
grain		63	< 0.01	< 0.01	0.02
rest of plants[4]		63	0.15	0.02	0.17

[1] days after last application
[2] conversion factor for calculation of BF 5003 to parent BAS 500 F is 1.084
[3] for calculation purposes, "< 0.02" is set 0.02
[4] without root

III. CONCLUSION

After the application at BBCH 65, the residues of pyraclostrobin were <0.01 (LOQ), BF 500-3 residues were between <0.01 and 0.01 mg/kg at crop maturity (BBCH 89).

Table 6.3.3-11: Residues (BAS 500 F and BF 500-3) in maize after one application of BAS 512 04 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[3]	Total[4]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 306326 **Doc ID:** 2010/1025690 **Trial No.** L080323 **GLP:** Yes **Year:** 2008	Maize / Delitop	Germany	BAS 512 04 F, Pyraclostrobin: 1x 0.200 kg as/ha	65	0	whole plant without roots	2.26	0.02	2.28	**BAS 500 F Method No.:** 535/1				
					13	cob with husks	0.07	< 0.01	0.08	plant [1]	0.01,1.0,5.0	4	117	24
					13	rest of plant without roots	**0.33**	0.05	0.38	cob [2]	0.01,1.0	13	96	18
					24	cob with husks	0.04	< 0.01	0.05	rest of pl. [1]	0.01,1.0	18	85	21
					24	rest of plant without roots	0.16	0.02	0.18	grain	0.01,1.0	7	92	13
					56	cob with husks	0.02	< 0.01	0.03	**BF 500-3 Method No.:** 445/0				
					56	rest of plant without roots	0.09	0.02	0.11	plant [1]	0.01,1.0,5.0	4	104	11
					99	grain	**< 0.01**	< 0.01	0.02	cob [2]	0.01,1.0	10	93	21
					99	rest of plant without roots	0.16	0.03	0.19	rest of pl. [1]	0.01,1.0	20	82	19
Study code: 306326 **Doc ID:** 2010/1025690 **Trial No.** L080324 **GLP:** Yes **Year:** 2008	Maize / PR 39T13	France (N)	BAS 512 04 F, Pyraclostrobin: 1x 0.200 kg as/ha	65	0	whole plant without roots	2.86	0.03	2.89	grain	0.01,1.0	8	92	12
					12	cob with husks	0.04	< 0.01	0.05					
					12	rest of plant without roots	**0.84**	0.18	1.02					
					42	cob with husks	< 0.01	< 0.01	0.02					
					42	rest of plant without roots	0.14	0.05	0.19					
					55	cob with husks	< 0.01	< 0.01	0.02					
					55	rest of plant without roots	0.13	0.04	0.17					
					71	grain	**< 0.01**	< 0.01	0.02					
					71	rest of plant without roots	0.05	0.02	0.07					
Study code: 306326 **Doc ID:** 2010/1025690 **Trial No.** L080325 **GLP:** Yes **Year:** 2008	Maize / Justina	United Kingdom	BAS 512 04 F, Pyraclostrobin: 1x 0.200 kg as/ha	65	0	whole plant without roots	2.13	0.01	2.14					
					18	cob with husks	0.09	0.02	0.11					
					18	rest of plant without roots	**0.22**	0.04	0.26					
					34	cob with husks	0.06	< 0.01	0.07					
					34	rest of plant without roots	0.17	0.02	0.19					
					70	cob with husks	0.03	< 0.01	0.04					
					70	rest of plant without roots	0.1	0.02	0.12					
					83	grain	**< 0.01**	< 0.01	0.02					
					83	rest of plant without roots	0.17	0.03	0.20					

Table 6.3.3-11: Residues (BAS 500 F and BF 500-3) in maize after one application of BAS 512 04 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[3]	Total[4]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 306326 **Doc ID:** 2010/1025690 **Trial No.** L080326 **GLP:** Yes **Year:** 2008	Maize / Adenzo	The Netherlands	BAS 512 04 F, Pyraclostrobin: 1x 0.200 kg as/ha	65	0	whole plant without roots	3.04	0.05	3.09					
					12	cob with husks	0.02	< 0.01	0.03					
					12	rest of plant without roots	**0.28**	0.08	0.36					
					23	cob with husks	< 0.01	< 0.01	0.02					
					23	rest of plant without roots	0.09	0.03	0.12					
					55	cob with husks	< 0.01	< 0.01	0.02					
					55	rest of plant without roots	0.05	0.01	0.06					
					82	grain	**< 0.01**	< 0.01	0.02					
					82	rest of plant without roots	0.03	0.01	0.04					
Study code: 306326 **Doc ID:** 2010/1025690 **Trial No.** L080327 **GLP:** Yes **Year:** 2008	Maize / Dekalb	Italy	BAS 512 04 F, Pyraclostrobin: 1x 0.200 kg as/ha	65	0	whole plant without roots	1.93	0.03	1.96					
					11	cob with husks	0.04	< 0.01	0.05					
					11	rest of plant without roots	**0.21**	0.05	0.26					
					16	cob with husks	0.02	< 0.01	0.03					
					16	rest of plant without roots	0.17	0.04	0.21					
					30	cob with husks	0.03	< 0.01	0.04					
					30	rest of plant without roots	0.12	0.03	0.15					
					67	grain	**< 0.01**	< 0.01	0.02					
					67	rest of plant without roots	0.09	0.02	0.11					
Study code: 306326 **Doc ID:** 2010/1025690 **Trial No.** L080328 **GLP:** Yes **Year:** 2008	Maize / Pioneer	Greece	BAS 512 04 F, Pyraclostrobin: 1x 0.200 kg as/ha	65	0	whole plant without roots	3.49	0.06	3.55					
					7	cob with husks	0.04	< 0.01	0.05					
					7	rest of plant without roots	**0.57**	0.22	0.79					
					22	cob with husks	0.03	< 0.01	0.04					
					22	rest of plant without roots	0.21	0.11	0.32					
					50	cob with husks	0.01	< 0.01	0.02					
					50	rest of plant without roots	0.10	0.05	0.15					
					66	grain	**< 0.01**	< 0.01	0.02					
					66	rest of plant without roots	0.27	0.16	0.43					

Table 6.3.3-11: Residues (BAS 500 F and BF 500-3) in maize after one application of BAS 512 04 F

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3[3]	Total[4]	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 306326 **Doc ID:** 2010/1025690 **Trial No.** L080329 **GLP:** Yes **Year:** 2008	Maize / Mitic	France (S)	BAS 512 04 F, Pyraclostrobin: 1x 0.200 kg as/ha	65	0	whole plant without roots	2.65	0.14	2.79					
					26	cob with husks	0.05	< 0.01	0.06					
					26	rest of plant without roots	0.19	0.03	0.22					
					33	cob with husks	0.04	< 0.01	0.05					
					33	rest of plant without roots	**0.21**	0.03	0.24					
					54	cob with husks	0.09	< 0.01	0.10					
					54	rest of plant without roots	0.16	0.02	0.18					
					74	grain	**< 0.01**	0.01	0.02					
					74	rest of plant without roots	0.26	0.02	0.28					
Study code: 306326 **Doc ID:** 2010/1025690 **Trial No.** L080330 **GLP:** Yes **Year:** 2008	Maize / Tardio 130	Spain	BAS 512 04 F, Pyraclostrobin: 1x 0.200 kg as/ha	65	0	whole plant without roots	< 0.01	< 0.01	0.02					
					28	cob with husks	< 0.01	< 0.01	0.02					
					28	rest of plant without roots	**0.08**	0.02	0.10					
					41	cob with husks	< 0.01	< 0.01	0.02					
					41	rest of plant without roots	< 0.01	< 0.01	0.02					
					55	cob with husks	0.01	< 0.01	0.02					
					55	rest of plant without roots	< 0.01	0.02	0.03					
					63	grain	**< 0.01**	< 0.01	0.02					
					63	rest of plant without roots	0.15	0.02	0.17					

[1] without roots
[2] with husks
[3] conversion factor for calculation of BF 500-3 to parent BAS 500 F is 1.084
[4] for calculation purposes < 0.01 was set to 0.01
underlined values were used for MRL calculation

Report: CA 6.3.3/5
Fleischer G., 2013a
Study on the residue behaviour of BAS 480 F, BAS 500 F and BF 500-3 (500M07) in corn after treatment with BAS 512 04 F under field conditions in Germany, 2011
2013/1065883
Guidelines: None
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 512 04 F
Description: BAS 512 04 F: 133 g/L pyraclostrobin (BAS 500 F) and 50 g/L epoxiconazole (BAS 480 F), SE
Lot/Batch #: 3191342
Purity: not applicable
CAS#: not applicable
Development code: not applicable
Spiking levels: 0.01-10 mg/kg

2. Test Commodity: Cereals
Crop: Maize
Type: not applicable
Variety: not applicable
Botanical name: *Zea mays*
Crop parts(s) or processed
Commodity: whole plant (no roots), cobs with / without husks, husks
Sample size: not applicable

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2011 growing season, one field trial was conducted in Germany in order to determine the magnitude of residues of pyraclostrobin, BF 500-3 and BAS 480 F in maize after one application of BAS 512 04 F.
The test item BAS 512 04 F (133 g/L BAS 500 F, pyraclostrobin and 50 g/L BAS 480 F epoxiconazole, SE), was foliar applied once with an application rate of 0.2 kg as/ha of pyraclostrobin and 0.075 kg as/ha of epoxiconazole and a spray volume of 400 L/ha at growth stages BBCH 71. “Whole plant no roots” specimens were taken directly after application. Cobs were taken at BBCH 75, 79, 85 and were separated into the portions “cobs with husks”, “cobs without husks” and “husks”.
Samples were stored frozen until analysis. The maximum storage interval from harvest until analysis was 174 days.

Table 6.3.3-12: Application and sampling details for trials conducted in 2011

Region	No. of trials	No. of appl.	F, P, or G	Test item	Active substance	Application		Target timing	
						Rate as (kg/ha)	Water vol. (L/ha)	Application	Sampling (DALA)[1]
Northern Europe	1	1	F	BAS 512 04 F (SE)	BAS 500 F	0.2	400	BBCH 71	BBCH 75 BBCH 79 BBCH 85
					BAS 480 F	0.075			

[1] days after last application

2. Description of analytical procedures

All specimens were analysed for pyraclostrobin and its metabolite BF 500-3 (500M07) using BASF method 535/1 (synonym: L0076/01).
The analytes were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method for pyraclostrobin and its metabolite BF 500-3 was 0.01 mg/kg.
The results of procedural recovery experiments were 91.6% for pyraclostrobin and 87.6% for BF 500-3 at fortification levels between 0.01 and 10 mg/kg.

II. RESULTS AND DISCUSSION

Directly after the application (0 DALA) of BAS 512 04 F, pyraclostrobin residues were 0.98 mg/kg and BF 500-3 residues were 1.02 mg/kg in maize whole plant without roots. The unusual finding of the metabolite immediately after application can be just explained by a contamination in the lab or during sample processing.
In the course of the study, the residues of pyraclostrobin and BF 500-3 declined. In the edible portion “cobs without husks” none of the analytes was found above LOQ throughout the study.

Details of residues are shown in Table 6.3.3-13.

III. CONCLUSION

In the edible portion “cobs without husks” none of the analytes is found above LOQ throughout the study.

Table 6.3.3-13: Residues (BAS 500 F and BF 500-3) in maize after application of BAS 512 04 F

Trial details	Crop	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study: code: NG-500-Mais **Doc ID:** 2013/1065883 **Trial No.** L110466 **GLP:** yes* **Year:** 2011	Maize	Germany	BAS 512 04 F, Pyraclostrobin: 1x 0.200 kg as/ha	71	0	Whole plant[1]	0.98	1.02	2.00	**BAS 500 F Method No.:535/1 (L0076/01)**				
					7	Cobs without husks	<0.01	<0.01	<0.02	all sample matrices	0.01 -10	11	91.6	3.6
					7	Cob with husks	0.03	<0.01	0.04					
					7	Husks	0.13	0.01	0.14					
					22	Cobs without husks	<0.01	<0.01	<0.02					
					22	Cob with husks	0.03	<0.01	0.04	**BF 500-3 Method No.: 535/1 (L0076/01)**				
					22	Husks	0.04	<0.01	0.05	all sample matrices	0.01 -10	11	87.6	6.8
					42	Cobs without husks	**<u><0.01</u>**	<0.01	<0.02					
					42	Cob with husks	0.03	<0.01	0.04					
					42	Husks	0.18	0.07	0.25					

[1] without roots
* only analytical phase
<u>underlined values</u> were used for MRL calculation

This study has been already submitted to Germany in context of a previous submission. It was used as basis for the corresponding EFSA Reasoned Opinion (EFSA Journal 2012; 10(3):2606. [36 pp.] doi:10.2903/j.efsa.2012.2606).

Report:	CA 6.3.3/6 Schulz H., 2010b Residue behaviour of Epoxiconazole and Pyraclostrobin in maize after treatment with BAS 512 04 F and BAS 500 06 F under field conditions in Germany Northern France United Kingdom the Netherlands Italy Greece Southern France and Spain 2009 2010/1039144
Guidelines:	EEC 1607/VI/97 rev. 2 10.06.1999, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, OECD-ENV/JM/MONO(2002)/9
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 500 06 F, BAS 512 04 F

Description:	BAS 500 06 F: 200 g/L pyraclostrobin, EC BAS 512 04 F: 133 g/L pyraclostrobin, 50 g/L epoxiconazole, SE
Lot/Batch #:	BAS 500 06 F: 8265 BAS 512 04 F: FRE-000369
Purity:	not relevant
CAS#:	pyraclostrobin: 175013-18-0 epoxiconazole: 133855-98-8
Development code:	not applicable
Spiking levels:	0.01-10 mg/kg

2. Test Commodity: Cereals

Crop:	Maize
Type:	not relevant
Variety:	Nescio, Maxxis Duo, Ohio, LG 3218, Dekalb 66-77, PR-3345, Anadon, Castellano.
Botanical name:	*Zea mays*
Crop parts(s) or processed Commodity:	whole plant (no roots), rest of plant (no roots), cobs with husks, grain
Sample size:	1 kg (12 plants) of whole plant without roots; 1 kg (12 pieces) of cob with husks; 2 kg of grain.

B. STUDY DESIGN AND METHODS

1. Test procedure

During the growing season 2009, eight field trials were conducted in Germany, Northern France, United Kingdom, The Netherlands, Italy, Greece, Southern France and Spain in order to determine residue levels of pyraclostrobin, its metabolite BF 500-3 and epoxiconazole in maize.
The trials were performed with the formulations BAS 512 04 F (133 g/L of pyraclostrobin and 50 g/L of epoxiconazole, SE). In four out of the eight trials also a treatment with BAS 500 06 F (200 g/L of pyraclostrobin, EC) was included.
Plot 2 of trials L090168, L090170, L090172 and L090174 were treated once with **BAS 512 04 F** at a rate of 1.5 L/ha (0.1995 kg as/ha of pyraclostrobin and 0.075 kg as/ha of epoxiconazole), while on plot 3 of the same trials **BAS 500 06 F** was applied once at a rate of 1 L/ha (0.2 kg as/ha of pyraclostrobin). Consequently, it was possible to compare both treatments in these four trials.
In four additional trials (L090169, L090171, L090173 and L090175) plot 2 were also treated once with BAS 512 04 F at a rate of 1.5 L/ha, but in these trials BAS 500 06 F was not applied.
The application took place at BBCH 65 - 67 with a spray volume of 400 L/ha.
Whole plants without roots were collected from the treated plots immediately after the treatment at BBCH 65 - 67. Thereafter specimens consisting of cob with husks or rest of plant without roots were sampled at 6 - 17 DALA (BBCH 71), at 19 - 36 DALA (BBCH 75 – 77) and at 30 - 64 DALA (BBCH 85). Grain and rest of plant without roots were collected at harvest at 49 - 91 DALA (BBCH 89). The samples were stored until analysis. The maximum storage interval from harvest until analysis was 222 days.

2. Description of analytical procedures

BASF method 535/1 (synonym: L0076/01) was used for the analysis of pyraclostrobin, its metabolite BF 500-3 (500M07) and epoxiconazole. The method achieves a limit of quantitation (LOQ) of 0.01 mg/kg.
Pyraclostrobin, BF 500-3 and BAS 480 F were extracted from plant matrices using a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. An aliquot of the organic phase was evaporated to dryness and dissolved in a mixture of methanol and water. The final determination was performed by HPLC-MS/MS.

II. RESULTS AND DISCUSSION

Trials L090168, L090170, L090172 and L090174
Directly after the last application (0 DALA) of BAS 512 04 F, the pyraclostrobin residues ranged from 1.6 - 2.4 mg/kg in the "whole plant (no roots)" specimens. The residues declined significantly at BBCH 71 to 0.026 - 0.072 mg/kg in "cob with husks" and to 0.18 - 0.66 mg/kg in "rest of plant (no roots)". This decline continued over the remaining sampling stages until at the last one, where <0.01 mg/kg were found in the "grain" and a range of 0.079 - 0.50 mg/kg in the "rest of plant (no roots)" specimens.
Directly after the last application (0 DALA) of BAS 500 06 F, the pyraclostrobin residues ranged from 1.4 - 4.3 mg/kg in the "whole plant (no roots)" specimens. The residues declined significantly at BBCH 71 to 0.025 - 0.053 mg/kg in "cob with husks" and to 0.25 - 0.89 mg/kg in "rest of plant (no roots)". This decline continued over the remaining sampling stages until at the last one, where <0.01 mg/kg were found in the "grain" and a range of 0.083 - 0.50 mg/kg in the "rest of plant (no roots)" specimens.
In the trials which were treated with BAS 512 04 F and BAS 500 06 F, the decline of the residues of pyraclostrobin was similar.

Trials L090169, L090171, L090173 and L090175
In these trials with just one treated plot (treated with BAS 512 04 F), the pyraclostrobin residues ranged from 2.2 - 3.6 mg/kg in the "whole plant (no roots)" specimens at 0 DALA. The residues declined significantly at BBCH 71 to < 0.01 - 0.076 mg/kg in "cob with husks" and to 0.090 - 0.72 mg/kg in "rest of plant (no roots)". This decline continued over the remaining sampling stages until at the last one <0.01 mg/kg were found in the "grain" and a range of 0.045 - 0.67 mg/kg in the "rest of plant (no roots)" specimens.

No residues of the metabolite BF 500-3 (500M07) were found in any of the grain samples.

No residues above the limit of quantitation were found in any of the untreated specimens.

Summaries of residues of pyraclostrobin and its metabolite BF 500-3 are given in Table 6.3.2-14 and Table 6.3.2-15 for trials L090168, L090170, L090172 and L090174. In
Table 6.3.2-16 they are given for trials L090169, L090171, L090173 and L090175, details are shown for all trials in Table 6.3.2-17.

Table 6.3.3-14: Summary of results for BAS 500 F and its metabolite BF 500-3 from plot 2, treated with BAS 512 04 F (Trials L090168, L090170, L090172 and L090174)

Region	Matrix	Timing (DALA[1])	Range of residues (mg/kg)		
			BAS 500 F	BF 500-3[2]	Total residue[3]
EU North and South	Whole plant[4]	0	1.6 - 2.4	0.02 - 0.06	1.6 - 2.5
	Cob with husks	9 - 16	0.026 - 0.072	< 0.010	0.03 - 0.08
	Rest of plant[4]	9 - 16	0.18 - 0.66	0.047 - 0.17	0.23 - 0.83
	Cob with husks	19 - 29	0.020 - 0.058	< 0.010	0.03 - 0.07
	Rest of plants[4]	19 - 29	0.11 - 0.46	0.037 - 0.082	0.16 - 0.5
	Cob with husks	30 - 57	< 0.010 - 0.045	< 0.010	< 0.02 - 0.06
	Rest of plant[4]	30 - 57	0.066 - 0.36	0.017 - 0.044	0.08 - 0.40
	Grain	57 - 91	< 0.010	< 0.010	< 0.02
	Rest of plant[4]	57 - 91	0.079 - 0.5	0.022 - 0.059	0.10 - 0.56

[1] days after last application
[2] conversion factor for calculation of BF 500-3 to parent BAS 500 F is 1.084
[3] for calculation of sum <0.010 was set to 0.010
[4] no roots

Table 6.3.3-15: Summary of results for BAS 500 F and its metabolite BF 500-3 from plot 3, treated with BAS 500 06 F (Trials L090168, L090170, L090172 and L090174)

Region	Matrix	Timing (DALA[1])	Range of residues (mg/kg)		
			BAS 500 F	BF 500-3[2]	Total residue[3]
EU North and South	Whole plant[4]	0	1.4 - 4.3	< 0.010 - 0.048	1.4 - 4.3
	Cob with husks	9 - 16	0.025 - 0.053	< 0.010	0.04 - 0.06
	Rest of plant[4]	9 - 16	0.25 - 0.89	0.044 - 0.11	0.29 - 0.98
	Cob with husks	19 - 29	0.012 - 0.095	< 0.010	0.02 - 0.11
	Rest of plants[4]	19 - 29	0.15 - 0.35	0.043 - 0.060	0.21 - 0.39
	Cob with husks	30 - 57	< 0.010 - 0.022	< 0.010	< 0.02 - 0.03
	Rest of plant[4]	30 - 57	0.12 - 0.28	0.025 - 0.049	0.16 - 0.31
	Grain	57 - 91	< 0.010	< 0.010	< 0.02
	Rest of plant[4]	57 - 91	0.083 - 0.50	0.020 - 0.047	0.10 - 0.54

[1] days after last application
[2] conversion factor for calculation of BF 500-3 to parent BAS 500 F is 1.084
[3] for calculation of sum <0.010 was set to 0.010
[4] no roots

Table 6.3.3-16: Summary of results of additional trials for BAS 500 F and its metabolite BF 500-3 from plot 2, treated with BAS 512 04 F (Trials L090169, L090171, L090173 and L090175)

Region	Matrix	Timing (DALA[1])	Range of residues (mg/kg)		
			BAS 500 F	BF 500-3[2]	Total residue[3]
EU North and South	Whole plant[4]	0	2.2 – 3.6	<0.010 – 0.039	2.2 – 3.7
	Cob with husks	6 – 17	<0.010 – 0.076	<0.010	<0.020 – 0.86
	Rest of plant[4]	6 – 17	0.090 – 0.72	0.015 – 0.21	0.12 – 0.87
	Cob with husks	19 – 36	<0.010 – 0.10	<0.010	<0.020 – 0.11
	Rest of plants[4]	19 – 36	<0.010 – 0.74	<0.010 - 0.20	<0.020 – 0.94
	Cob with husks	35 – 64	<0.010 – 0.051	<0.010	<0.020 – 0.061
	Rest of plant[4]	35 – 64	0.088 – 0.72	<0.010 – 0.25	0.098 – 0.97
	Grain	49 – 86	<0.010	<0.010	<0.020
	Rest of plant[4]	49 – 86	0.045 – 0.67	0.013 – 0.23	0.063 – 0.89

[1] days after last application
[2] conversion factor for calculation of BF 500-3 to parent BAS 500 F is 1.084
[3] for calculation of sum <0.010 was set to 0.010
[4] no roots

III. CONCLUSION

At harvest maturity, the residues of pyraclostrobin and its metabolite BF 500-3 (500M07) were always below the LOQ (0.01 mg/kg)
The results of the study show that the residue behaviour of pyraclostrobin in/on maize specimens after application of BAS 512 04 F is in correspondence to the behaviour found after use of the respective solo formulation BAS 500 06 F.

Table 6.3.3-17: Residues (BAS 500 F and BF 500-3) in maize after one application of BAS 500 06 F and BAS 512 04 F

Trial details	Crop	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 306328 **Doc ID:** 2010/1039144 **Trial No.** L090168 **GLP:** yes **Year:** 2009	Maize / Nescio	Germany	BAS 512 04 F, Pyraclostrobin: 1x 0.1995 kg as/ha	BBCH 67	0	plant w/o roots	2.2	0.02	2.3	**BAS 500 F Method No.:** 535/1				
					13	cob with husks	0.04	< 0.01	0.05	grain	0.01/0.1	2	95.8	n.a.
					13	rest of plant w/o roots	**0.30**	0.07	0.37	cob with husks	0.01/1.0	4	89.6	3.9
					29	cob with husks	0.02	< 0.01	0.03	whole plant[1]	0.01/1.0	2	78.8	n.a.
					29	rest of plant w/o roots	0.20	0.06	0.26	rest of plant[1]	0.01/10	5	88.9	5.5
					57	cob with husks	0.02	< 0.01	0.03					
					57	rest of plant w/o roots	0.13	0.04	0.17	**BF 500-3 Method No.:** 535/1				
					91	grain	**< 0.01**	< 0.01	< 0.02	grain	0.01/0.1	2	92.3	n.a.
					91	rest of plant w/o roots	0.10	0.04	0.14	cob with husks	0.01/1.0	4	92.0	3.1
			BAS 500 06 F, Pyraclostrobin: 1x 0.200 kg as/ha		0	plant w/o roots	1.4	< 0.01	1.4	whole plant[1]	0.01/1.0	2	98.3	n.a.
					13	cob with husks	0.03	< 0.01	0.04	rest of plant[1]	0.01/10	5	89.5	15.0
					13	rest of plant w/o roots	**0.25**	0.04	0.29					
					29	cob with husks	0.01	< 0.01	0.02					
					29	rest of plant w/o roots	0.17	0.05	0.22					
					57	cob with husks	< 0.01	< 0.01	< 0.02					
					57	rest of plant w/o roots	0.12	0.039	0.16					
					91	grain	**< 0.01**	< 0.01	< 0.02					
					91	rest of plant w/o roots	0.09	0.036	0.13					
Study code: 306328 **Doc ID:** 2010/1039144 **Trial No.** L090169 **GLP:** yes **Year:** 2009	Maize / Maxxis Duo	France (N)	BAS 512 04 F, Pyraclostrobin: 1x 0.1995 kg as/ha	BBCH 65	0	plant w/o roots	2.9	0.03	2.9					
					15	cob with husks	< 0.01	< 0.01	< 0.02					
					15	rest of plant w/o roots	0.09	0.03	0.12					
					36	cob with husks	< 0.01	< 0.01	< 0.02					
					36	rest of plant w/o roots	< 0.01	< 0.01	< 0.02					
					43	cob with husks	< 0.01	< 0.01	< 0.02					
					43	rest of plant w/o roots	**0.11**	0.03	0.14					
					79	grain	**< 0.01**	< 0.01	< 0.02					
					79	rest of plant w/o roots	0.05	0.02	0.06					

Table 6.3.3-17: Residues (BAS 500 F and BF 500-3) in maize after one application of BAS 500 06 F and BAS 512 04 F

Trial details	Crop	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 306328 **Doc ID:** 2010/1039144 **Trial No.** L090170 **GLP:** yes **Year:** 2009	Maize / Ohio	United Kingdom	BAS 512 04 F, Pyraclostrobin: 1x 0.1995 kg as/ha	BBCH 65	0	plant w/o roots	1.6	0.03	1.6					
					12	cob with husks	0.07	< 0.01	0.08					
					12	rest of plant w/o roots	**0.66**	0.17	0.83					
					19	cob with husks	0.02	< 0.01	0.03					
					19	rest of plant w/o roots	0.32	0.08	0.40					
					46	cob with husks	< 0.01	< 0.01	< 0.02					
					46	rest of plant w/o roots	0.07	0.02	0.08					
					71	grain	**< 0.01**	< 0.01	< 0.02					
					71	rest of plant w/o roots	0.08	0.02	0.10					
			BAS 500 06 F, Pyraclostrobin: 1x 0.200 kg as/ha		0	plant w/o roots	1.6	0.02	1.7					
					12	cob with husks	0.05	< 0.01	0.06					
					12	rest of plant w/o roots	**0.43**	0.11	0.54					
					19	cob with husks	0.10	< 0.01	0.11					
					19	rest of plant w/o roots	0.22	0.04	0.26					
					46	cob with husks	0.01	< 0.01	0.02					
					46	rest of plant w/o roots	0.16	0.04	0.20					
					71	grain	**< 0.01**	< 0.01	< 0.02					
					71	rest of plant w/o roots	0.08	0.02	0.10					
Study code: 306328 **Doc ID:** 2010/1039144 **Trial No.** L090171 **GLP:** yes **Year:** 2009	Maize / LG 3218	The Netherlands	BAS 512 04 F, Pyraclostrobin: 1x 0.1995 kg as/ha	BBCH 65	0	plant w/o roots	2.2	< 0.01	2.2					
					17	cob with husks	0.08	< 0.01	0.09					
					17	rest of plant w/o roots	**0.21**	0.02	0.23					
					28	cob with husks	0.10	< 0.01	0.11					
					28	rest of plant w/o roots	0.18	0.02	0.19					
					64	cob with husks	0.051	< 0.01	0.06					
					64	rest of plant w/o roots	0.088	< 0.01	0.10					
					86	grain	**< 0.01**	< 0.01	< 0.02					
					86	rest of plant w/o roots	0.09	0.01	0.11					

Table 6.3.3-17: Residues (BAS 500 F and BF 500-3) in maize after one application of BAS 500 06 F and BAS 512 04 F

Trial details	Crop	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 306328 **Doc ID:** 2010/1039144 **Trial No.** L090172 **GLP:** yes **Year:** 2009	Maize / Dekalb 66-77	Italy	BAS 512 04 F, Pyraclostrobin: 1x 0.1995 kg as/ha	BBCH 65	0	plant w/o roots	2.4	0.06	2.5					
					9	cob with husks	0.05	< 0.01	0.06					
					9	rest of plant w/o roots	**0.18**	0.05	0.23					
					20	cob with husks	0.06	< 0.01	0.07					
					20	rest of plant w/o roots	0.11	0.04	0.16					
					30	cob with husks	0.05	< 0.01	0.06					
					30	rest of plant w/o roots	0.09	0.04	0.13					
					57	grain	**< 0.01**	< 0.01	< 0.02					
					57	rest of plant w/o roots	0.11	0.04	0.15					
			BAS 500 06 F, Pyraclostrobin: 1x 0.200 kg as/ha		0	plant w/o roots	1.9	0.04	1.9					
					9	cob with husks	0.05	< 0.01	0.06					
					9	rest of plant w/o roots	**0.29**	0.07	0.35					
					20	cob with husks	0.05	< 0.01	0.06					
					20	rest of plant w/o roots	0.15	0.06	0.21					
					30	cob with husks	0.02	< 0.01	0.03					
					30	rest of plant w/o roots	0.13	0.05	0.18					
					57	grain	**< 0.01**	< 0.01	< 0.02					
					57	rest of plant w/o roots	0.13	0.04	0.17					
Study code: 306328 **Doc ID:** 2010/1039144 **Trial No.** L090173 **GLP:** yes **Year:** 2009	Maize / GC 0645 PR-3345	Greece	BAS 512 04 F, Pyraclostrobin: 1x 0.1995 kg as/ha	BBCH 65	0	plant w/o roots	2.6	0.03	2.6					
					6	cob with husks	0.02	< 0.01	0.03					
					6	rest of plant w/o roots	0.72	0.15	0.87					
					21	cob with husks	< 0.01	< 0.01	< 0.02					
					21	rest of plant w/o roots	**0.74**	0.20	0.94					
					49	cob with husks	< 0.01	< 0.01	< 0.02					
					49	rest of plant w/o roots	0.72	0.25	0.97					
					69	grain	**< 0.01**	< 0.01	< 0.02					
					69	rest of plant w/o roots	0.67	0.23	0.89					

Table 6.3.3-17: Residues (BAS 500 F and BF 500-3) in maize after one application of BAS 500 06 F and BAS 512 04 F

Trial details	Crop	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)				Recovery data				
						Matrix	BAS 500 F	BF 500-3	Total	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 306328 **Doc ID:** 2010/1039144 **Trial No.** L090174 **GLP:** yes **Year:** 2009	Maize / Anadon	France (S)	BAS 512 04 F, Pyraclostrobin: 1x 0.1995 kg as/ha	BBCH 65	0	plant w/o roots	2.4	0.04	2.4					
					16	cob with husks	0.03	< 0.01	0.04					
					16	rest of plant w/o roots	0.49	0.08	0.57					
					27	cob with husks	0.03	< 0.01	0.04					
					27	rest of plant w/o roots	0.46	0.04	0.50					
					49	cob with husks	0.03	< 0.01	0.04					
					49	rest of plant w/o roots	0.36	0.04	0.40					
					77	grain	**< 0.01**	< 0.01	< 0.02					
					77	rest of plant w/o roots	**0.50**	0.06	0.56					
			BAS 500 06 F, Pyraclostrobin: 1x 0.200 kg as/ha		0	plant w/o roots	4.3	0.05	4.3					
					16	cob with husks	0.05	< 0.01	0.06					
					16	rest of plant w/o roots	**0.89**	0.09	0.98					
					27	cob with husks	0.02	< 0.01	0.03					
					27	rest of plant w/o roots	0.35	0.04	0.39					
					49	cob with husks	0.02	< 0.01	0.03					
					49	rest of plant w/o roots	0.28	0.03	0.31					
					77	grain	**< 0.01**	< 0.01	< 0.02					
					77	rest of plant w/o roots	0.50	0.05	0.54					
Study code: 306328 **Doc ID:** 2010/1039144 **Trial No.** L090175 **GLP:** yes **Year:** 2009	Maize / Castellano	Spain	BAS 512 04 F, Pyraclostrobin: 1x 0.1995 kg as/ha	BBCH 65	0	plant w/o roots	3.6	0.04	3.7					
					14	cob with husks	0.01	< 0.01	0.02					
					14	rest of plant w/o roots	**0.34**	0.21	0.54					
					21	cob with husks	< 0.01	< 0.01	< 0.02					
					21	rest of plant w/o roots	0.25	0.09	0.34					
					35	cob with husks	< 0.01	< 0.01	< 0.02					
					35	rest of plant w/o roots	0.34	0.15	0.48					
					49	grain	**< 0.01**	< 0.01	< 0.02					
					49	rest of plant w/o roots	0.27	0.07	0.34					

[1] without roots,
n.a. not applicable
underlined values were used for MRL calculation

CA 6.3.4 Supplemental study supporting the dietary exposure assessment for the metabolite 500M04 (plus conjugates)

Report: CA 6.3.4/1
Morgenthal K., 2014b
500M79: Residue analysis in plant matrices by LC-MS/MS
2014/1001661

Guidelines: EU Regulation Regulation 544/2011 (10 June 2011) implementing Regulation No 1107/2009, EU Regulation 1107/2009 with Regulation 545/2011 (former Annex III), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, SANCO/825/00 rev. 8.1 (16 November 2010), EEC 91/414 Annex II (Part A Section 4), SANCO/3030/99 rev. 4 - Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A Section 4) and Annex III (part A Section 5) of 91/414/EEC, EEC 91/414 Annex III (Part A Section 5), EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method

GLP: yes
(certified by Swiss Federal Office of Public Health, Berne, Switzerland)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin
Description: BAS 536 01 F: 72 g/L BAS 550 F and 40 g/L pyraclostrobin (EC), BAS 516 07 F: 26.7% BAS 510 F and 6.7% pyraclostrobin (WG)
Lot/Batch #: not applicable
Purity: not applicable
CAS#: not applicable
Development code: not applicable
Spiking levels: 0.01-0.1 mg/kg

2. Test Commodity: Brassica vegetables, leaf vegetables and fresh herbs, legume vegetables (fresh), stem vegetables (fresh)
Crop: Broccoli, cauliflower, lettuce, cabbage, leek, spinach, bean
Type: not applicable
Variety: not applicable
Botanical name: Brassica oleracea var. botrytis L., Brassica oleracea italica Plenck , Brassica oleracea var. capitata, Lactuca sativa L., Allium ampeloprasum L., Spinacia oleracea L., Phaseolus vulgaris
Crop parts(s) or processed
Commodity: Broccoli (inflorescences), cauliflower (inflorescences), lettuce (head), cabbage (white, heads), leek (whole plant, no roots), spinach (leaves), bean (pods with seeds, seeds)
Sample size: not applicable

B. STUDY DESIGN AND METHODS

1. Test procedure

The study was conducted to determine residues of 500M79 (Reg. No. 5937091) and 500M04 (BF 500-5, Reg. No. 298327) in plant matrices (broccoli, cauliflower, lettuce, leek cabbage, spinach and bean) derived from the BASF projects No. 365214, 365220_1, 365222, 35216, 365219, 309344 and 309370.

Study Code 365214:
The formulation BAS 536 01 F (EC) was foliar applied in broccoli and cauliflower three times (17±1, 10±1 and 3±1 days before harvest) at a rate of 0.100 kg as/ha pyraclostrobin and 0.180 kg as/ha dimethomorph with a water volume of 400 L/ha. Samples taken 3-4 and 6-8 days after last application (DALA) were analysed for residues of 500M04 and 500M79 in inflorescences.

Study Code 365220_1:
The formulation BAS 536 01 F (EC) was foliar applied three times at a rate of 0.180 kg as/ha dimethomorph and 0.100 kg as/ha pyraclostrobin to lettuce at 28-29, 21 and 14 days before harvest. The nominal spray volume used was 200 L/ha. Samples taken 14 and 20 DALA were analysed for residues of 500M04 and 500M79 in lettuce heads.

Study Code 365222:
The formulation BAS 536 01 F (EC) was foliar applied to lettuce three times at 21±1, 14±1 and 7±1 days before harvest at a rate of 2.5 L/ha of formulated product (0.1 kg as/ha of pyraclostrobin and 0.18 kg as/ha of dimethomorph). The water volume used was 200 L/ha. Samples taken 7 and 13-14 DALA were analysed for residues of 500M04 and 500M79 in lettuce heads.

Study Code 35216:
The formulation BAS 536 01 F (EC) was foliar applied in cabbage (white) three times (17±1, 10±1 and 3±1 days before harvest) at a rate of 0.100 kg as/ha pyraclostrobin and 0.180 kg as/ha dimethomorph in a spray volume of 600 L/ha. Samples taken 3-4 and 7-8 DALA were analysed for residues of 500M04 and 500M79 in cabbage heads.

Study Code 365219:
The formulation BAS 536 01 F (EC) was foliar applied in leek three times (17±1, 10±1 and 3±1 days before harvest) at a rate of 0.100 kg as/ha pyraclostrobin and 0.180 kg as/ha dimethomorph in a spray volume of 200 L/ha. Samples taken 2-3 and 7-8 DALA were analysed for residues of 500M04 and 500M79 in leek whole plants (no roots).

Study Code 309344:
The formulation BAS 516 07 F (WG) was foliar applied in spinach twice at a rate of 0.4005 kg as/ha for boscalid and 0.1005 kg as/ha for pyraclostrobin in a spray volume of 400 L/ha. Application timings were 21 (±1) days before harvest (DBH) for the first and 14 (±1) DBH for the second application. Samples taken 13-14 and 21 DALA were analysed for residues of 500M04 and 500M79 in spinach leaves.

Study Code 309370:
Green beans were treated twice with BAS 516 07 F (WG). In trials L100423 to L100426, BAS 516 07 F was foliar-applied at a rate of 2 x 0.4005 kg as/ha of boscalid and 2 x 0.1005 kg as/ha of pyraclostrobin. For trial L100591 the study plan stated 2 x 0.481 kg as/ha of BAS 510 F and 2 x 0.121 kg as/ha of pyraclostrobin (actual rate was 1 x 0.378 and 1 x 0.396). The application rate of spray solution was 400 L/ha for trials L100423 to L100426 and 150 L/ha for trial L100591. The applications were conducted 14 and 7-8 days before harvest. Residues of 500M04 and 500M79 were analysed in bean pods with seeds taken 3, 7-8 and 13-15 DALA and in seeds taken 7-8 and 13-15 DALA.

2. Description of analytical procedures

Metabolite 500M79 was extracted from plant matrices using a mixture of methanol, Milli-Q water and hydrochloric acid. An aliquot of the extract was evaporated to dryness and re-dissolved in Milli-Q water. The analyte was hydrolysed using enzymatic cleavage to 500M04. Following reversed phase C18 SPE-column clean-up, the final determination of 500M04 was performed by LC-MS/MS.

The results are expressed as 500M04 and as 500M79 equivalent with a Limit of Quantification (LOQ) of 0.005 mg/kg (500M04) and 0.01 mg/kg (500M79).

II. RESULTS AND DISCUSSION

The Limit of Quantitation is 0.005 mg/kg for 500M04 and 0.01 mg/kg for 500M79 in all plant matrices described below. For better readability it is not repeated for each of the plant matrices.

Broccoli

The residues of 500M04 and 500M79 in broccoli inflorescences were investigated in six samples. The residue range in two samples collected 3 days after the last application (DALA) was < 0.005 – 0.007 mg/kg (500M04) and < 0.010 – 0.013 mg/kg (500M79). In two samples collected at 6 DALA, the residue levels were at < 0.005 – 0.026 mg/kg (500M04) and at < 0.010 – 0.047 mg/kg (500M79).
In two untreated samples, no residues of 500M04 or 500M79 above the Limit of Quantitation were present.

Cauliflower

The residues of 500M04 and 500M79 in cauliflower inflorescences were investigated in six samples. The residue range in two samples collected 3 – 4 DALA was < 0.005 – 0.009 mg/kg (500M04) and < 0.010 – 0.017 mg/kg (500M79). In two samples collected 7 – 8 DALA, the residue range increased to < 0.005 – 0.012 mg/kg (500M04) and < 0.010 – 0.022 mg/kg (500M79).
In two untreated samples, no residues of 500M04 or 500M79 above the Limit of Quantitation were present.

Lettuce

The residues of 500M04 and 500M79 in lettuce heads were investigated in fourteen samples. The residue range in four samples collected at 7 DALA was 0.011 – 0.024 mg/kg (500M04) and 0.019 – 0.044 mg/kg (500M79). In six samples collected at 13 – 14 DALA, the residue range was < 0.005 – 0.019 mg/kg (500M04) and < 0.010 – 0.035 mg/kg (500M79). The maximum residues were found in two samples collected at 20 DALA (500M04: 0.009 – 0.018 mg/kg, 500M79: 0.017 – 0.033 mg/kg).
In two untreated samples, no residues of 500M04 or 500M79 above the Limit of Quantitation were present.

Cabbage (White)
The residues of 500M04 and 500M79 in cabbage (white) heads were investigated in nine samples. The residue range in four samples collected at 3 – 4 DALA was < 0.005 – 0.019 mg/kg (500M04) and at < 0.010 – 0.034 mg/kg (500M79). In four samples collected at 7 – 8 DALA, no residues were present at or above the Limit of Quantitation (500M04: < 0.005 mg/kg, 500M79: < 0.010 mg/kg).
In one untreated sample, no residues of 500M04 or 500M79 above the Limit of Quantitation were present.

Leek
The residues of 500M04 and 500M79 in leak whole plants (no roots) were investigated in five samples. The residue range in two samples collected at 2 – 3 DALA was 0.020 – 0.041 mg/kg (500M04) and 0.037 – 0.074 mg/kg (500M79). In two samples collected at 7-8 DALA, the residue range decreased to 0.012 – 0.018 mg/kg (500M04) and to 0.022 – 0.032 mg/kg (500M79).
In one untreated sample, no residues of 500M04 or 500M79 above the Limit of Quantitation were present.

Spinach
The residues of 500M04 and 500M79 in spinach leaves were investigated in seven samples. The residue range in three samples collected at 13 – 14 DALA was 0.026 – 0.039 mg/kg (500M04) and 0.047 – 0.070 mg/kg (500M79). In three samples collected at 21 DALA, the residues ranged between 0.020 and 0.030 mg/kg (500M04) and between 0.036 and 0.055 mg/kg (500M79).
In one untreated sample, no residues of 500M04 or 500M79 above the Limit of Quantitation were present.

Bean
The residues of 500M04 and 500M79 in *bean pods with seeds* were investigated in ten samples. In one sample collected at 3 DALA, no residues of 500M04 or 500M79 at or above the Limit of Quantitation (500M04: 0.005 mg/kg, 500M79: 0.010 mg/kg) were present. The residue range in four samples collected at 7-8 DALA was at < 0.005 – 0.012 mg/kg (500M04) and at < 0.100 – 0.019 mg/kg (500M79). In four samples collected at 13-15 DALA, the residues were found to range between < 0.005 and 0.014 mg/kg (500M04) and between < 0.010 and 0.026 mg/kg (500M79).

The residues of 500M04 and 500M79 in *bean seeds* were investigated in seven samples. The residues ranged in samples collected at 7 DALA between < 0.005 and 0.005 mg/kg (500M04) and were < 0.010 mg/kg (500M79). In samples collected at 13 – 15 DALA, no residues of 500M04 or 500M79 at or above the Limit of Quantitation (500M04: 0.005 mg/kg, 500M79: 0.010 mg/kg) were present.

In none of the untreated samples (total: 2 samples), residues of 500M04 and 500M79 above the Limit of Quantitation were determined.

Residues of 500M04 and 500M79 are summarised in
Table 6.3.4-1, details are shown in Table 6.3.4-2.

Table 6.3.4-1: Summary of Field Specimen Residues

Matrix	Portion analyzed	DALA[1)]	Growth stage	n	Range of 500M04 residues [mg/kg]	Range of 500M79 residues [mg/kg]
Broccoli	Inflorescences	3	49	2	< 0.005 – 0.007	< 0.010 – 0.013
		6	49	2	< 0.005 – 0.026	< 0.010 – 0.047
Cauliflower	Inflorescences	3 – 4	49	2	< 0.005 – 0.009	< 0.010 – 0.017
		7 – 8	49	2	< 0.005 – 0.012	< 0.010 – 0.022
Lettuce	Head	7	47 – 49	4	0.011 – 0.024	0.019 – 0.044
		13 – 14	47 – 49	6	< 0.005 – 0.019	< 0.010 – 0.035
		20	48 – 49	2	0.009 – 0.018	0017 – 0.033
Cabbage (white)	Head	3 – 4	49	4	< 0.005 – 0.019	< 0.010 – 0.034
		7 – 8	49	4	< 0.005	< 0.010
Leek	Whole plant (no roots)	2 – 3	47 – 49	2	0.020 – 0.041	0.037 – 0.074
		7 – 8	48 – 49	2	0.012 – 0.018	0.022 – 0.032
Spinach	Leaves	13 – 14	46 – 49	3	0.026 – 0.039	0.047 – 0.070
		21	47 – 49	3	0.020 – 0.030	0.036 – 0.055
Beans	Pods with seeds	3	82	1	< 0.005	< 0.010
		7–8	77 – 82	4	< 0.005 – 0.012	< 0.010 – 0.019
		13 – 15	79 – 87	4	< 0.005 – 0.014	< 0.010 – 0.026
	Seeds	7	77 – 82	3	< 0.005	< 0.010
		13 – 15	79 – 89	3	< 0.005 – 0.005	< 0.010

[1] days after last application

III. CONCLUSION

In broccoli inflorescences, the residue range of 500M04 and 500M79 in samples collected at 3 days after the last application (DALA) was determined to be < 0.005 – 0.007 mg/kg (500M04) and < 0.010 – 0.013 mg/kg (500M79). In samples collected at 6 DALA, the residues were between < 0.005 and 0.026 mg/kg (500M04) and between < 0.010 and 0.047 mg/kg (500M79).

In cauliflower inflorescences, the residue range of 500M04 and 500M79 in samples collected at 3 - 4 DALA was determined to be < 0.005 – 0.009 mg/kg (500M04) and < 0.010 – 0.017 mg/kg (500M79). In samples collected at 7 – 8 DALA, the residues were on a comparable level with values of < 0.005 – 0.012 mg/kg (500M04) and of < 0.010 – 0.022 mg/kg (500M79).

In lettuce heads, the residue range of 500M04 and 500M79 in samples collected at 7 DALA was determined to be 0.011 – 0.024 mg/kg (500M04) and 0.019 – 0.044 mg/kg (500M79). In samples collected at 20 DALA, the residues were slightly lower with values in the range of 0.009 to 0.018 mg/kg (500M04) and in the range of 0.017 to 0.033 mg/kg (500M79).

In heads of cabbage (white) the residue range of 500M04 and 500M79 in samples collected at 3 – 4 DALA was determined to be < 0.005 - 0.019 mg/kg (500M04) and < 0.010 – 0.034 mg/kg (500M79). In samples collected at 7 – 8 DALA, the residues were always below the Limit of Quantification (LOQ, 500M04: < 0.005 mg/kg, 500M79: < 0.010 mg/kg) in all samples.

In leek samples (whole plants, no roots), the residue range of 500M04 and 500M79 in samples collected at 2 – 3 DALA was determined to be 0.020 – 0.041 mg/kg (500M04) and 0.037 – 0.074 mg/kg (500M79). In samples collected at 7 – 8 DALA, the residues were slightly lower with values in the range of 0.012 to 0.018 mg/kg (500M04) and in the range of 0.022 to 0.032 mg/kg (500M79).

In spinach leaves, the residue range of 500M04 and 500M79 in samples collected at 13 – 14 DALA was determined to be 0.026 – 0.039 mg/kg (500M04) and 0.047 – 0.070 mg/kg (500M79). In samples collected at 21 DALA, the residues were on a comparable level with values ranging between 0.020 and 0.030 mg/kg (500M04) and between 0.036 and 0.055 mg/kg (500M79).

In "bean pods with seed" specimens sampled at 3 DALA, no residues at or above the LOQ (500M04: 0.005 mg/kg, 500M79: 0.010 mg/kg) could be detected. In samples collected at 13 – 15 DALA, the residue range of 500M04 and 500M79 was determined to be < 0.005 – 0.014 mg/kg (500M04) and < 0.010 – 0.026 mg/kg (500M79).

In bean seed specimens sampled at 7 DALA, no residues at or above the LOQ (500M04: 0.005 mg/kg, 500M79: 0.010 mg/kg) could be detected. In samples collected at 13 – 15 DALA, residues of 500M04 were determined to be below or at the LOQ (0.005 mg/kg), residues of 500M79 were in all those samples below the LOQ (0.001 mg/kg).

Table 6.3.4-2: Residues of 500M04 and 500M79 in broccoli, cauliflower, lettuce, cabbage, leek, spinach, bean field specimens

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)			Recovery data				
						Matrix	500M04	500M79	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 365214 **Doc ID:** 2014/1001661 (2012/1157545) **Trial No.** L110141 **GLP:** Yes **Year:** 2011	Broccoli / Marathon	Italy	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	46	3 6	Inflorescences Inflorescences	< 0.005 < 0.005	< 0.010 < 0.010	**BASF Method No. L0220/01 (500M04, 500M79)**				
									Broccoli, inflorescences	0.01-0.1	2	82.0	n. a.
									Cauliflower, inflorescences	0.01-0.1	6	92.0	6.9
									Lettuce, heads	0.01-0.1	2	71.8	n. a.
Study code: 365214 **Doc ID:** 2014/1001661 (2012/1157545) **Trial No.** L110142 **GLP:** Yes **Year:** 2011	Broccoli / Marathon	Greece	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	49	3 6	Inflorescences Inflorescences	0.007 0.026	0.013 0.047					
									Cabbage, heads	0.01-0.1	2	92.1	n. a.
									Leek, whole plants w/o roots	0.01-0.1	2	97.6	n. a.
									Spinach, leaves	0.01-0.1	2	104.7	n. a.
Study code: 365214 **Doc ID:** 2014/1001661 (2012/1157545) **Trial No.** L110143 **GLP:** Yes **Year:** 2011	Cauliflower / Fremont	France	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	47	4 8	Inflorescences Inflorescences	0.009 0.012	0.017 0.022					
									Bean, pods with seeds	0.01-0.1	2	100.9	n. a.
									Bean, pods w/o seeds	0.01-0.1	2	81.9	6.9
									Bean, rest of plant w/o roots	0.01-0.1	2	95.1	7.1
Study code: 365214 **Doc ID:** 2014/1001661 (2012/1157545) **Trial No.** L110144 **GLP:** Yes **Year:** 2011	Cauliflower / Equinox	Spain	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	43	3 7	Inflorescences Inflorescences	< 0.005 < 0.005	< 0.010 < 0.010					
									Bean, seeds	0.01-0.1	2	75.0	n.a.

Table 6.3.4-2: Residues of 500M04 and 500M79 in broccoli, cauliflower, lettuce, cabbage, leek, spinach, bean field specimens

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)			Recovery data				
						Matrix	500M04	500M79	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 365220_1 **Doc ID:** 2014/1001661 (2012/1195474) **Trial No.** L100721 **GLP:** Yes **Year:** 2011	Lettuce / Augusta	France	BAS 536 01 F, Pyraclostrobin: 1x0.111 and 2x0.100 kg as/ha	45-46	14 20	Head Head	< 0.005 0.009	< 0.010 0.017					
Study code: 365222 **Doc ID:** 2014/1001661 (2012/1110543) **Trial No.** L110296 **GLP:** Yes **Year:** 2011	Lettuce / Cavernet	Germany	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	48	7 14	Head Head	0.024 0.009	0.044 0.016					
Study code: 365222 **Doc ID:** 2014/1001661 (2012/1110543) **Trial No.** L110297 **GLP:** Yes **Year:** 2011	Lettuce / Orille-Lollo Rosso	UK	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	45-47	7 14	Head Head	0.018 0.019	0.034 0.035					
Study code: 365222 **Doc ID:** 2014/1001661 (2012/1110543) **Trial No.** L110298 **GLP:** Yes **Year:** 2011	Lettuce / Sansula	Belgium	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	44-46	7 14	Head Head	0.017 0.012	0.030 0.021					

Table 6.3.4-2: Residues of 500M04 and 500M79 in broccoli, cauliflower, lettuce, cabbage, leek, spinach, bean field specimens

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)			Recovery data				
						Matrix	500M04	500M79	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 365222 **Doc ID:** 2014/1001661 (2012/1110543) **Trial No.** L110299 **GLP:** Yes **Year:** 2011	Lettuce / Capira	France (N)	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	39-45	7 13	Head Head	0.011 0.007	0.019 0.012					
Study code: 365220_1 **Doc ID:** 2014/1001661 (2012/1195474) **Trial No.** L110493 **GLP:** Yes **Year:** 2011	Lettuce / Batavia Novelski	Germany	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	46	14 20	Head Head	0.018 0.018	0.033 0.033					
Study code: 365216 **Doc ID:** 2014/1001661 (2012/1157543) **Trial No.** L110137 **GLP:** Yes **Year:** 2011	Cabbage / Impala	France	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	49	3 7	Head Head	0.019 < 0.005	0.034 < 0.010					
Study code: 365216 **Doc ID:** 2014/1001661 (2012/1157543) **Trial No.** L110138 **GLP:** Yes **Year:** 2011	Cabbage / Farao F1	Italy	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	47	3 7	Head Head	< 0.005 < 0.005	< 0.010 < 0.010					

Table 6.3.4-2: Residues of 500M04 and 500M79 in broccoli, cauliflower, lettuce, cabbage, leek, spinach, bean field specimens

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)			Recovery data				
						Matrix	500M04	500M79	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 365216 **Doc ID:** 2014/1001661 (2012/1157543) **Trial No.** L110139 **GLP:** Yes **Year:** 2011	Cabbage / Grandslam	Greece	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	45-47	4 8	Head Head	< 0.005 < 0.005	< 0.010 < 0.010					
Study code: 365216 **Doc ID:** 2014/1001661 (2012/1157543) **Trial No.** L110140 **GLP:** Yes **Year:** 2011	Cabbage / Famosa	Spain	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	49	3 7	Head Head	< 0.005 < 0.005	< 0.010 < 0.010					
Study code: 365219 **Doc ID:** 2014/1001661 (2012/1157544) **Trial No.** L110135 **GLP:** Yes **Year:** 2011	Leek / Kalamopraso	Greece	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	47/49	2 7	Whole plant w/o roots Whole plant w/o roots	0.020 0.018	0.037 0.032					
Study code: 365219 **Doc ID:** 2014/1001661 (2012/1157544) **Trial No.** L110136 **GLP:** Yes **Year:** 2011	Leek / Atal	Spain	BAS 536 01 F, Pyraclostrobin: 3x 0.100 kg as/ha	47	3 8	Whole plant w/o roots Whole plant w/o roots	0.041 0.012	0.074 0.022					

Table 6.3.4-2: Residues of 500M04 and 500M79 in broccoli, cauliflower, lettuce, cabbage, leek, spinach, bean field specimens

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)			Recovery data				
						Matrix	500M04	500M79	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 309344 **Doc ID:** 2014/1001661 (2011/1125587) **Trial No.** L100333 **GLP:** Yes **Year:** 2010	Spinach / Aigle	France (N)	BAS 516 07 F, Pyraclostrobin: 2x 0.100 kg as/ha	19	14 21	Leaves Leaves	0.039 0.030	0.070 0.055					
Study code: 309344 **Doc ID:** 2014/1001661 (2011/1125587) **Trial No.** L100334 **GLP:** Yes **Year:** 2010	Spinach / Marabu	Italy	BAS 516 07 F, Pyraclostrobin: 2x 0.100 kg as/ha	33	14 21	Leaves Leaves	0.026 0.020	0.048 0.036					
Study code: 309344 **Doc ID:** 2014/1001661 (2011/1125587) **Trial No.** L100335 **GLP:** Yes **Year:** 2010	Spinach / Pelican	France (S)	BAS 516 07 F, Pyraclostrobin: 2x 0.100 kg as/ha	19	13 21	Leaves Leaves	0.026 0.025	0.047 0.045					
Study code: 309370 **Doc ID:** 2014/1001661 (2011/1135971) **Trial No.** L100423 **GLP:** Yes **Year:** 2010	Bean / Pedra	France	BAS 516 07 F, Pyraclostrobin: 2x 0.100 kg as/ha	81	3 7	Pods with seeds Seeds	< 0.005 < 0.005	< 0.010 < 0.010					

Table 6.3.4-2: Residues of 500M04 and 500M79 in broccoli, cauliflower, lettuce, cabbage, leek, spinach, bean field specimens

Trial details	Crop / Variety	Country	Formulation, Application rate (kg as/ha)	Crop growth stage	PHI (days)	Residues found (mg/kg)			Recovery data				
						Matrix	500M04	500M79	Matrix	Fortification range (mg/kg)	n	Mean (%)	RSD (%)
Study code: 309370 **Doc ID:** 2014/1001661 (2011/1135971) **Trial No.** L100424 **GLP:** Yes **Year:** 2010	Bean / Etna	Greece	BAS 516 07 F, Pyraclostrobin: 2x 0.100 kg as/ha	77	7 15 7 15	Pods with seeds Pods with seeds Seeds Seeds	0.008 < 0.005 < 0.005 < 0.005	0.014 < 0.010 < 0.010 < 0.010					
Study code: 309370 **Doc ID:** 2014/1001661 (2011/1135971) **Trial No.** L100425 **GLP:** Yes **Year:** 2010	Bean / Flavert	Italy	BAS 516 07 F, Pyraclostrobin: 2x 0.100 kg as/ha	73	7 14 7 14	Pods with seeds Pods with seeds Seeds Seeds	0.012 0.014 < 0.005 0.005	0.019 0.026 < 0.010 < 0.010					
Study code: 309370 **Doc ID:** 2014/1001661 (2011/1135971) **Trial No.** L100426 **GLP:** Yes **Year:** 2010	Bean / Marconi	Spain	BAS 516 07 F, Pyraclostrobin: 2x 0.100 kg as/ha	75	7 14	Pods with seeds Pods with seeds	0.005 < 0.005	< 0.010 < 0.010					
Study code: 309370 **Doc ID:** 2014/1001661 (2011/1135971) **Trial No.** L100591 **GLP:** Yes **Year:** 2010	Bean / Cadillac	Germany	BAS 516 07 F, Pyraclostrobin: 1x 0.378 kg as/ha 1x0.396 kg as/ha	80-81	8 13 13	Pods with seeds Pods with seeds Seeds	< 0.005 0.007 < 0.005	< 0.010 0.013 < 0.010					

n.a. not applicable
w/o without

CA 6.4 Feeding studies

Data/information on poultry and lactating ruminant feeding studies for pyraclostrobin were reviewed during the Annex I inclusion process and were considered to be acceptable. The following section was copied from the Draft Assessment Report, which was prepared by RMS Germany.

Table 6.4-1: Residues from livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

Intakes by livestock ≥ 0.1 mg/kg diet/day:	Ruminant: yes/~~no~~	Poultry: yes/~~no~~	Pig: yes/~~no~~
Muscle	< 0.05	< 0.05	< 0.05
Liver	< 0.05	< 0.05	< 0.05
Kidney	< 0.05	< 0.05	< 0.05
Fat	< 0.05	< 0.05	< 0.05
Milk	< 0.01	Not applicable	Not applicable
Eggs	Not applicable	< 0.05	Not applicable

For the Annex I inclusion process data were submitted covering the safe uses in cereals, grapes and banana (import tolerance). Due to the favourable residue behaviour of pyraclostrobin, where even at the 10 x dose level no residues above LOQ were found, the revised feed burden covering the use in cereals did not result in any new MRL proposal. This was re-confirmed by RMS Germany and in the EFSA publication from February 2012 (see EFSA journal 2012; 10(3): 2606).

CA 6.4.1 Poultry

A hen feeding study is submitted and summarized below, which was performed for registration purposes in the US using the common moiety approach. The study is considered as supplemental information. Based on the results of the poultry metabolism study, no residues above the LOQ of the analytical methods (common moiety, parent) can be expected. This assumption was finally confirmed within the poultry feeding study.

Report:	CA 6.4.1/1 [redacted] 2000a A meat and egg magnitude of the residue study with BAS 500 F in laying hens 2000/5005
Guidelines:	EPA 860.1480
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

I. MATERIAL AND METHODS

Test system
Fifty-six white leghorn laying hens (*Gallus gallus*) aged approximately 64 weeks and in the weight range of 1.404 to 1.770 kg prior to dosing were used in the study. The feeding was performed at Southwest Bio-Labs Animal Carte Facility. Animals were selected for use in the study on the basis of overall health, egg production and body weight. The animals were quarantined for a period of 14 days and then acclimated for two weeks to adjust the hens to laboratory conditions and to determine each hen's suitability for testing.

Feeding and husbandry
The animals were housed individually in 18" x 12" x 16" metabolism cages in a temperature controlled study room. The lighting control was set to a photoperiod of 18 hours light and 6 hours dark and was utilized for the duration of the study. The animals were individually fed a commercial concentrate (EverLay 2000 Crumbles) daily. Additionally, animals in test groups II to IV were dosed with encapsulated pyraclostrobin (solved in methanol) via a balling gun once a day for 30 consecutive days at a target rate equivalent to a dietary exposure of 0.3 (1x), 0.9 (3x) and 3.0 (10x) mg/kg feed, respectively. The control group animals received placebo capsules containing cellulose powder concurrently with the treated animals. The animals were allowed ad libitum access to feed and to fresh potable tap water daily.

Derivation of detected dose levels
The feeding levels were estimated by taking into account the preliminary residue data available at the beginning of this study. The anticipated residues were worst case estimations. The main contributions to the dietary burden were from barley grain and wheat milled by-products. The calculation shown in Table 6.4.1-1 is performed for the estimation of the feeding levels according to US EPA guidelines. The actual US tolerances are very similar to the estimated values and are 0.4 mg/kg for barley grain and 0.04 mg/kg for wheat milled by-products, which leads to a dietary burden of 0.301 mg/kg feed.

Table 6.4.1-1: Calculation of the feed burden for hen

Feed item	% in diet	Tolerance (mg/kg)	Feed burden (mg/kg)
Barley grain	75	0.3	0.225
Wheat milled by-products	25	0.1	0.025
Total diet (mg/kg)			0.250

Therefore, it was concluded that a feeding level of 0.3 mg/kg would reflect a realistic 1x residue situation in feed, leading to feeding levels of 0.9 mg/kg for the 3x level and 3.0 mg/kg for the 10x level.

According to the most recent feed and dietary burden calculations using the EU methodology (see M-CA 6.7.2 for poultry), a maximum dietary burden of about 0.65 mg/kg feed dry matter is calculated. Therefore, based on this calculation the chosen feeding levels 0.3, 0.9 and 3.0 mg/kg feed would represent about 0.5x, 1.5x and 5x of the target rate.

Dose preparation
In order to obtain an accurate dose rate for pyraclostrobin equivalent to the ingested amount of feed, the weight of the feed consumed over a seven-day period was converted from a fresh weight to a dry weight. The appropriate amount of pyraclostrobin was weighed for each test group into a volumetric flask and the flask filled to volume with methanol for dissolving the test substance. The amount of test substance weighed into each flask for the 1x dose (EU: 0.5x), 3x dose (EU: 1.5x) and 10x dose (EU: 5x) was 0.0298 g, 0.0799 g and 0.2896 g, respectively. For each test group 100 µL of the respective solution were pipetted into the capsules.
In addition to the capsules prepared for dosing, two extra capsules were made for analysis (for each dose group and for each week).

Dose administration
Animals in test groups were dosed once per day in the afternoon (AM) following egg collection. The capsules (containing pyraclostrobin solved in methanol) were administered using a balling gun according to the manufacturer's instructions. These animals were dosed over a 30-day period at a target rate equivalent to 0.3, 0.9 and 3.0 mg/kg in the feed (test groups II, III and IV, respectively. Group IV consisting of five sub-groups a-e). The animals in group I served as the control and received placebos (containing cellulose) concurrently with the treated animals.

Egg sampling
Eggs were collected twice daily (AM and PM) beginning on study day -15 and continuing until the scheduled termination for each animal. At each collection period, the number of eggs was recorded. Eggs from PM collection were refrigerated overnight until the next morning when they were pooled with the AM collection for each subgroup. The eggs were removed from their shells, placed into labelled containers, thoroughly mixed using a Tekmar® Tissumizer®, weighed and the weights recorded. The unshelled homogenized eggs from each subgroup were divided into two labelled containers and the weight of the eggs in each container was recorded. All samples were stored frozen at less than -15°C on dry ice.

Terminal procedures
All animals were sacrificed approximately 6.5 hours following the final dose, except for two subgroups which were terminated at three and seven days post dosing to determine residue decline. At sacrifice, a composite of muscle, the entire liver and a composite of mesenteric peripheral fat samples were collected. The tissues were then pooled by subgroup and placed into separate labeled Ziplock® storage bags and transferred to a freezer immediately after collection. All hen tissue samples were processed at Southwest Bio-Labs. After homogenization, samples remained in frozen storage (< -15°C).

Bodyweight
Individual bodyweights were obtained on study days -15, -1, 16 and at scheduled termination for each animal. Prior to dosing, the animals weighed between 1.404 and 1.770 kg. At termination, the animals weighed between 1.376 and 1.837 kg. Many animals lost weight over the course of the treatment period. However, this occurred in treated and in control groups.

Residue analysis
Analyses of the egg and hen tissue samples were carried out with BASF method Number D9902. Method D9902 is a common moiety method and was developed to determine the residues of pyraclostrobin and BF 500-16 (500M77) in hen related matrices (egg, liver, fat and muscle). As part of the method, pyraclostrobin and BF 500-16 are hydrolyzed into BF 500-5 (500M04) and BF 500-9 (Reg.No. 402733, a derivative used for determination only and therefore not included in the metabolite list), respectively, and these analytes are determined by LC/MS/MS. The limit of quantitation of the method is 0.05 mg/kg for each analyte and for all matrices.

II. RESULTS AND DISCUSSION

As shown in Table 6.4.1-2 and Table 6.4.1-3, all pyraclostrobin and BF 500-16 (500M77) residues in eggs or hen tissues at the 5x dose level (EU) were below 0.05 mg/kg for each component of the residue and thus < 0.10 mg/kg for the total residue.

Table 6.4.1-2: Summary of BAS 500 F residues in eggs

Treatment Day	Average residue (mg/kg) 3.0 mg/kg dose (group IV)		
	BAS 500 F	BF 500-16	Total
-1	< 0.05	< 0.05	< 0.10
1	< 0.05	< 0.05	< 0.10
2	< 0.05	< 0.05	< 0.10
4	< 0.05	< 0.05	< 0.10
7	< 0.05	< 0.05	< 0.10
10	< 0.05	< 0.05	< 0.10
14	< 0.05	< 0.05	< 0.10
17	< 0.05	< 0.05	< 0.10
21	< 0.05	< 0.05	< 0.10
24	< 0.05	< 0.05	< 0.10
28	< 0.05	< 0.05	< 0.10
30	< 0.05	< 0.05	< 0.10
32	not analysed [1]		
33	not analysed [1]		
34	not analysed [1]		
36	not analysed [1]		
37	not analysed [1]		

[1] all residues were below LOQ therefore depuration data was not needed

Since there were no detectable residues at the 5x dose level (EU) samples from groups II (EU: 0.5x) and III (EU: 1.5x) and both depuration sub-groups were not analyzed.

Table 6.4.1-3: Summary of BAS 500 F residues in hen tissues

Tissue / Matrix	Average residue (mg/kg) 3.0 mg/kg (group IV)		
	BAS 500 F	BF 500-16	Total
Liver	< 0.05 [1]	< 0.05 [1]	< 0.10
Muscle	< 0.05 [1]	< 0.05 [1]	< 0.10
Fat	< 0.05 [1]	< 0.05 [1]	< 0.10

[1] all residues were below LOQ therefore depuration data was not needed

The efficiency of the analytical method number D9902 was determined by fortifying control samples with pyraclostrobin and its metabolite BF 500-16 (500M77) for each matrix. The average recoveries were 101±16% and 98±14% for pyraclostrobin in eggs and hen tissues, respectively. For BF 500-16, average recoveries were 68%±7 and 76%±22 in eggs and hen tissues, respectively. The detailed recovery results are given in Table 6.4.1-4.

Table 6.4.1-4: Recovery results of BAS 500 F and BF 500-16 in eggs and hen tissues

Matrix	Fortification level [mg/kg]	Average recovery level (%) ± S.D.	
		BAS 500 F (determined as BF 500-5)	BF 500-16 (determined as BF 500-9)
Eggs			
Eggs	0.05	101 ±16	68 ±7
Hen tissues			
Liver	0.05	85	96
Muscle	0.05	112	52
Fat	0.05	96	80
Average recovery [%] ± S.D.		98 ±14	76 ±22
Overall average recovery [%] ± S.D.		100 ±15	71 ±13

III. CONCLUSION

At the 3.0 mg/kg dose level (EU: 5x) residues of pyraclostrobin and its metabolite BF 500-16 (500M77) in eggs and hen tissues were below the method's limit of quantitation (LOQ, 0.05 mg/kg). Based on these results, samples from the 0.3 and the 0.9 mg/kg dose level (EU: 0.5x and 1.5x) as well as the depuration groups were not analyzed.

CA 6.4.2 Ruminants

Data/information on cow feeding studies for pyraclostrobin were reviewed during the Annex I inclusion process and were considered to be acceptable (see M-CA 6.4). No further data have been generated. In order to support the existing study, additional method validation experiments were performed for metabolites being detected in higher amounts in the goat metabolism study. From the fortification experiments performed with 500M04 and 500M85 (see M-CA 4.1), it can be concluded that the common moiety method is suitable for data generation purposes.

CA 6.4.3 Pigs

In case of pyraclostrobin, the metabolism in rats and ruminants are similar. Therefore, no pig feeding study is required.

CA 6.4.4 Fish

According to the Commission Regulations (EU) No 283/2013 (active substances) and 284/2013 (plant protection products) as of 1 March 2013, metabolism studies on fish and fish feeding studies might be required in future (latest by 31 Dec 2015), if residues occur in crops that are intended as feed items for fish. As pyraclostrobin is used in several crops being fed to fish, a fish metabolism study was performed. The study is reported in M-CA 6.2. It is considered as suitable for deriving a MRL proposal despite MRLs for fish matrices will not be set in close future. Due to this fact, but also driven by the lack of any suitable EU guideline / guidance document for the conduct of fish feeding studies, a respective study was not performed.

CA 6.5 Effects of Processing

CA 6.5.1 Nature of the residue

Data/information on processing studies were reviewed during the Annex I inclusion process and were considered acceptable.

The following conclusion was copied from the Draft Assessment Report (section 3, B.7) prepared by RMS Germany according to Directive EEC 91/414.

> Pyraclostrobin (BAS 500 F) was not degraded neither during the simulation of pasteurisation (pH 4, 90°C) nor during the simulation of baking, boiling, brewing (pH 5, 100°C) or during sterilisation (pH 6, 120°C). Because no degradation occurred, no degradation products were observed.

The assessment of Germany was recently confirmed by EFSA during the re-evaluation of established EU MRLs according to 396/2005. The following paragraph is directly copied from the relevant Reasoned Opinion:

The effect of processing on the nature of pyraclostrobin residues was investigated in the framework of the peer review. A study was conducted simulating representative hydrolytic conditions for pasteurization (20 minutes at 90°C, pH 4), boiling/brewing/baking (60 minutes at 100°C, pH 5) and sterilization (20 minutes at 120°C, pH 6). This study demonstrates that food processes such as brewing, cooking, sterilization or pasteurization, will not impact the nature of pyraclostrobin residues. The relevant residue for enforcement and risk assessment in processed commodities is therefore expected to be the same as for primary crops (Germany, 2001).

In 2013, BASF submitted a draft Registration Report to Italy covering the intended uses of the formulation BAS 500 02 F in citrus, kaki (persimmon) and olives. In context of this dRR and for supporting the use in oil olives, a processing study (magnitude of residues, BASF Doc ID 2013/1243223) had been performed. In the study, the relevant process fractions (raw oil, refined oil), but also intermediates and waste products were taken and analyzed for pyraclostrobin and the metabolite 500M07 (BF 500-3). In the final purification step from raw oil to refined oil ("deodorization"), higher temperatures are typically applied than during those steps which are covered by investigations according to OECD 507. As an accumulation of the metabolite 500M07 was observed during this step, the zRMS Italy required the conduct of a ^{14}C processing study simulating oil purification.

Report:	CA 6.5.1/1 Hueben M., 2014a High temperature hydrolysis - Simulated processing of 14C-Pyraclostrobin (14C-BAS 500 F) 2014/1136542
Guidelines:	OECD Test Guideline 507 - Nature of the residues in processed commodities - High temperature hydrolysis, EPA 860.1520, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 7035/VI/95 rev. 5
GLP:	yes (certified by Ministerium fuer Arbeit, Integration und Soziales des Landes Nordrhein-Westfalen, Duesseldorf)

Note: This study was not contained in the application. The reason for submission is described above.

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	BAS 500 F (Pyraclostrobin, Reg No. 304428)
	Description:	[Chlorophenyl-U-^{14}C]-BAS 500 F, specific activity 6.15 MBq/mg [Tolyl-U-^{14}C]-BAS 500 F, specific activity 6.6 MBq/mg
	Lot/Batch #:	[Chlorophenyl-U-^{14}C]-Label: 579-6301 [Tolyl-U-^{14}C]-Label: 566-5101
	Purity:	[Chlorophenyl-U-^{14}C]-Label: 99.2% (radiochemical, CoA) [Tolyl-U-^{14}C]-Label: 99.9% (radiochemical, CoA)
	CAS#:	175013-18-0
	Development code:	not applicable

B. STUDY DESIGN AND METHODS

1. Test procedure

The hydrolytic degradation of pyraclostrobin was investigated at high temperatures in an olive oil / water mixture to simulate the process of olive oil raffination (deodorization step from raw oil to refined oil). The study was performed with ^{14}C-pyraclostrobin labeled at the chlorophenyl or at the tolyl ring.

Figure 6.5.1-1: Structural formula of ^{14}C-BAS 500 F labeled at the chlorophenyl ring

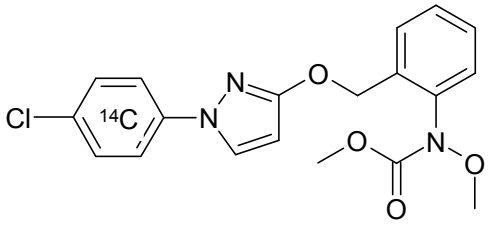
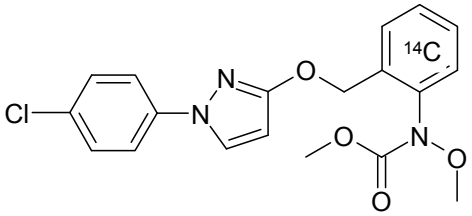


Figure 6.5.1-2: Structural formula of ^{14}C-BAS 500 F labeled at the tolyl ring



The test substance was applied into a mixture of olive oil and aqueous sodium chloride solution (in a ratio of 2:1) at a rate of 1 mg/mL and heated up to 190°C ± 5°C and 240°C ± 5°C for 30 minutes. After the incubation, the mixture was analyzed for the parent compound and possible transformation products. The conditions for the hydrolysis study are shown in Table 6.5.1-1.

Table 6.5.1-1: Simulated conditions for the hydrolysis study

Temperature (°C)	Time (min)	Remarks
190	30	Olive oil / water (2:1)
240	30	Olive oil / water (2:1)

2. Description of analytical procedures

Preparation of Samples for Total Radioactive Analysis

After separation of the phases, an aliquot of the aqueous phase was measured by LSC.

The olive oil phase was extracted four times with acetonitrile and an aliquot of the acetonitrile phase was measured by LSC. A smaller volume of the extracted oil phase was dissolved in acetone and measured by LSC.

In order to release and characterize further radioactivity from the oil phase, a lipase digestion was performed with the extracted oil phases. For phase separation cyclohexane was added to the reaction mixture. The phases were measured by LSC.

Some aliquots of the oil phase were subject to pancreatin and pepsin digestion in order to verify the bioavailability of the test item and its metabolites.

Liquid Chromatography/Mass Spectrometry (HPLC-MS/MS)
Acetonitrile extracts and the aqueous phases were analyzed by HPLC-MS/MS in MRM mode (HPLC Method 2) to scan for parent, known metabolites and to confirm the identification based on radio-TLC analysis. In addition, aqueous phases after enzyme digestion were analyzed to scan for released parent and/or metabolites.
For HPLC Method 2, the column used was a Hypersil Green ENV, the eluent system consisted of 2 mobile phases (A: 0.1% formic acid in water & B: 0.1% formic acid in acetonitrile), which were used applying gradient elution. HPLC Method 3 was used for the analysis of samples by HPLC/MS/MS. A Xevo TQS (Waters) mass spectrometer was used. Two transitions were established for parent and each reference item. Parent pyraclostrobin and 10 reference items were used to tune the HPLC-MS/MS to confirm or to exclude the presence of known metabolites. For HPLC Method 3, the column used was a Waters ACQUITY UPLC BEH C18, the eluent system consisted of 2 mobile phases (A: 0.1% formic acid in water/methanol (90:10, v:v) & B: 0.1% formic acid in methanol), which were used applying gradient elution.

Radio Thin Layer Chromatography (radio TLC)
All sample matrices were analyzed by radio TLC to determine the distribution of the radioactivity and to identify radioactive residues in the extracts. Samples were separated on pure silica with diisopropylether/dichloromethane/acetic acid (50/50/1, v/v/v). Radio-TLC was finally used for quantitation purposes.

II. RESULTS AND DISCUSSION

1. Extraction of residues

The majority of the radioactivity stayed in the olive oil phase during hydrolysis. In the aqueous phase less than 3% AR were found, except the tolyl 240°C samples where 15.77% AR and 13.26% AR were found.
In the 190°C experiments most of the radioactivity was extractable from the oil phase with acetonitrile. In the oil phase after acetonitrile extraction only 0.29% to 2.70% remained. In the 240°C experiments the radioactivity from the oil phase was less extractable. 4.28% to 28.71% of the applied radioactivity stayed in the oil phase after extraction with acetonitrile.
The mass balance for the chlorophenyl-label experiments were close to or above 90% AR. From the tolyl-label all samples were below but very close to 90%. The distribution of radioactivity after incubation is shown in Table 6.5.1-2. Only the results of the replicates 1 and 4 are included in the table below as they were further analyzed.

Table 6.5.1-2: Distribution of radioactivity after incubation at 190°C and 240°C

Sample	Acetonitrile extract oil [% AR]	Aqueous phase [% AR]	oil after extraction [% AR]	Mass balance [% AR]
1 Chloro 240°C	79.58	2.49	8.36	90.4
4 Chloro 240°C	89.56	1.20	4.28	95.0
1 Chloro 190°C	100.11	0.26	1.21	101.6
4 Chloro 190°C	84.73	0.33	2.70	87.8
1 Tolyl 240°C	49.19	15.77	22.09	87.1
4 Tolyl 240°C	45.61	13.26	28.71	87.6
1 Tolyl 190°C	85.20	1.83	2.02	89.0
4 Tolyl 190°C	88.84	0.77	0.29	89.9

2. Identification, characterization and quantitation of extractable residues

At 190°C:
At 190°C pyraclostrobin is degrading. As a major degradation product the metabolite 500M07 was identified at 190°C up to 50.4% AR. Hydrolysis of the parent was not complete at 190°C as residues of up to 46.6% AR of unchanged parent were found. Metabolite 500M07 was further degraded to 500M04 and 500M49. All peaks exceeding 10% AR were identified. The identification of one peak being present in amounts close to 10% AR in both labels failed despite multiple attempts were undertaken. Taking the actual levels of residues in oilseed and oil fruit crops into account (see M-CA 6.7: maximum STMRs at 0.053 mg/kg for oilseeds, 0.065 mg/kg for future use in oil olives), no significant contribution to the dietary risk can be expected.

The high variation of the degradation product composition in the replicates might be caused by even small variations of the test temperatures (± 5°C). The distribution pattern of radioactivity at 190°C is shown in Table 6.5.1-3.

Table 6.5.1-3: Distribution pattern of radioactivity at 190°C, incubation time 30 min

Substance	Replicate	Chlorophenyl-label [% AR]	Tolyl-label [% AR]
BAS 500 F	1	46.6	27.5
	4	0.0	42.2
	mean	23.3	34.8
500M07	1	32.1	36.1
	4	50.4	35.4
	mean	41.3	35.7
500M04	1	13.4	Not detectable
	4	24.6	
	mean	19.0	
500M49	1	Not detectable	10.3
	4		5.7
	mean		8.0
start TLC	1	1.3	4.4
	4	0.8	0.6
	mean	1.1	2.5
TLC unknown peak	1	7.0	8.5
	4	9.0	5.0
	mean	8.0	6.8
TLC unknown unspecific	1	*	0.2
	4	*	*
	mean	*	0.1
not further characterized	1	1.2	2.0
	4	3.0	1.1
	mean	2.1	1.5

*not detected or below detection limit

At 240°C:

At 240°C the degradation of pyraclostrobin is almost complete (only 5% AR were found in one tolyl-label replicate). Metabolite 500M07 is also subject to extensive degradation and only 23.4% AR (compared to 50.4% at 190°C) were found as a maximum concentration. In the chlorophenyl label, the degradation product 500M04 seems to be stable at 240°C and was found in amounts up to 82.3% AR. In contrast, the other cleavage products 500M49 is further degraded under these conditions and was detectable in minor amounts of 4.6% AR.

The terminal products of the high temperature hydrolysis of the tolyl label could only be characterized by chromatographic behavior (SPE, TLC), but was also subjected to enzymatic digestion reactions. None of these characterization reactions revealed distinct peaks. The distribution pattern of radioactivity at 240°C is shown in Table 6.5.1-4.

Table 6.5.1-4: Distribution pattern of radioactivity at 240°C, incubation time 30 min

Substance	Replicate	Chlorophenyl-label [% AR]	Tolyl-label [% AR]
BAS 500 F	1	*	*
	4	*	5.0
	mean	*	2.5
500M07	1	6.6	23.4
	4	6.0	8.5
	mean	6.3	15.9
500M04	1	69.9	Not detectable
	4	82.3	
	mean	76.1	
500M49	1	Not detectable	4.6
	4		*
	mean		2.3
start TLC	1	5.3	36.0
	4	1.3	51.8
	mean	3.3	43.9
TLC unknown peak	1	*	4.9
	4	*	0.0
	mean	*	2.5
TLC unknown unspecific	1	0.3	2.4
	4	*	2.7
	mean	0.2	2.6
not further characterized	1	8.4	15.8
	4	5.5	19.6
	mean	6.9	17.7

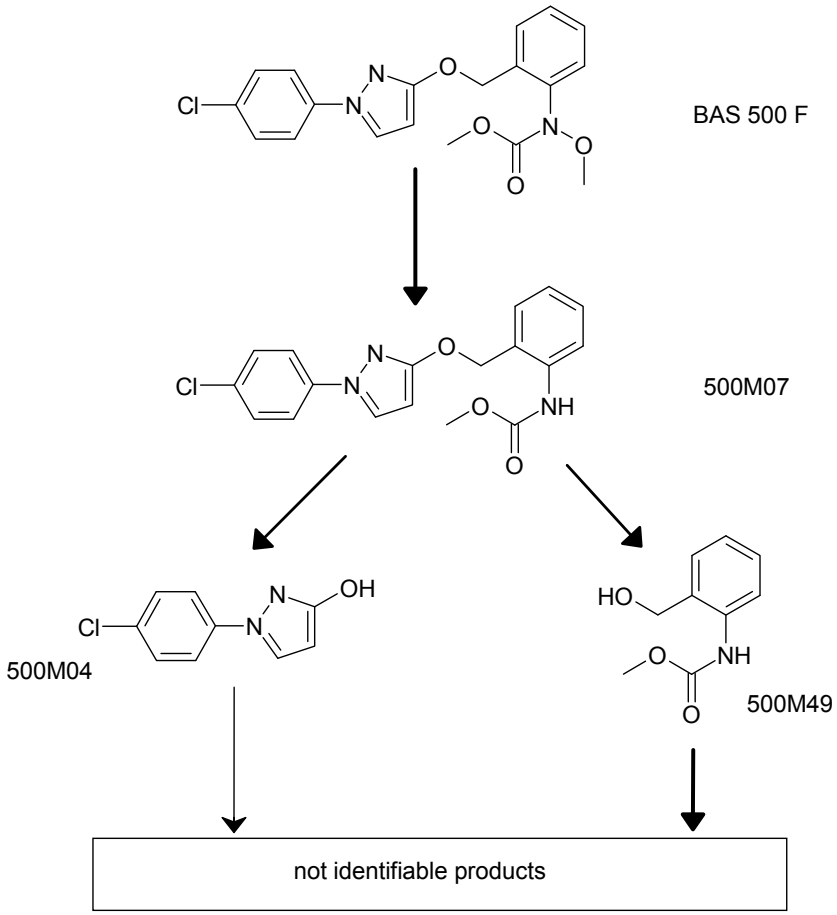
* not detected or below detection limit

3. Proposed degradation pathway

In almost all samples the same metabolites were found in different compositions. Hydrolytic degradation at higher temperatures resulted in the formation of the metabolite 500M07 which is then further degraded to 500M49 and 500M04. Other degradation pathways seem to be of minor importance. At 240°C, the major product is 500M04. At 240°C, the degradation of the tolyl labelled test compound is leading to products which could be only characterized. By enzymatic cleavage reactions it was unambiguously proven this radioactivity is not resulting in pyraclostrobin related metabolites causing consumer concerns.

The proposed degradation pathway is given in Figure 6.5.1-3.

Figure 6.5.1-3: Proposed Degradation Pathway at 190°C and 240°C



4. Storage stability

All extraction, fractionation and HPLC analysis was completed within 30 days, therefore storage stability analysis was not performed.

III. CONCLUSION

In the study, an extensive degradation of the test item was observed at both temperatures. In almost all samples the same metabolites were found in different compositions. Hydrolytic degradation of the parent compound at 190°C resulted in the formation of the metabolite 500M07 which was then degraded to 500M49 and 500M04. At 240°C further degradation occurred; parent and the metabolite 500M07 were only found in minor amounts whereas in the chlorophenyl label, major component was the metabolite 500M04. In the tolyl label, most of the radioactivity present could be only characterized. Intensive characterization attempts were undertaken using different enzymes (lipase, pepsin, pancreatin), but also chromatographic techniques. They did not indicate the presence of any individual or distinct metabolite. The metabolite 500M07 was still the most abundant peak which could be identified followed by metabolite 500M49.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

All representative uses to be evaluated in this dossier (potatoes, cereals and maize) are crops with edible peel only. Therefore, studies on the distribution between peel and pulp are not required.

CA 6.5.3 Magnitude of residues in processed commodities

During the past 15 years, multiple processing studies have been performed with pyraclostrobin containing products covering industrial production, but also household preparations. For the crops listed in Table 6.5.3-1, processing studies exist. The information on the processing steps covered (third column) is limited to those steps being considered as relevant for human consumption. The table does not include the studies submitted in context of this dossier.

Table 6.5.3-1: Overview on available processing studies supporting registered uses of pyraclostrobin containing formulations

EU Crop Group	Crops	Processing steps covered
Citrus fruits	Oranges	Peeling, canning, juice and marmalade production
Pome fruits	Apples	Washing, juice and sauce production
Stone fruits	Cherries	Washing, canning, juice and fruit syrup production
	Plums	Washing, puree and prune production
Berries and small fruits	Grapes	Wine, juice, raisins
	Strawberries	Washing, canning, jam, fruit syrup and distillate production
	Currants	Washing, canning, juice, jam and jelly production
Root and tuber vegetables	Carrots	Washing, peeling, cooking, juice production, canning
Fruiting vegetables	Tomatoes	Washing, canning, juice, puree and paste production
	Melons	Peeling
Brassica vegetables	Cabbage	Washing, cooking, Sauerkraut production, juice production
Leaf vegetables and fresh herbs	Lettuce, spinach	Washing, blanching, cooking
Legume vegetables	Peas	Washing, canning
Oilseeds and oilfruits	Soybean, oilseed rape, cotton, sunflower	Oil production (raw, refined)
Cereals	Barley	Pot barley, beer production
	Wheat	Flour, bran and germ production
Tea, coffee, herbal infusions and cocoa	Coffee	Roasting, brewing of coffee
Hops	Hops	Beer production
Sugar plants	Sugar cane	Sugar production (brown, refined)

Some of the studies were conducted according to the EU guidance document whereas the investigations performed in the USA are following US EPA OPPTS guidelines. The more recent studies are fulfilling the relevant OECD GD and GL. The majority of processing factors is included in the data bases of the German BfR and Dutch RIVM (BfR webpage: BfR compilation of processing factors for pesticide residues (October 2011), http://chemkap.rivm.nl/groente-fruit/processing-factors/). In almost all studies, pyraclostrobin residues did not show any accumulation in food destined for human consumption. Exceptions are prunes and raisins which can be explained by loss of water.

In context of this dossier, only studies for the intended uses are considered. Summaries of the new studies in wheat, oats and maize are provided below. The barley study submitted in the previous Annex I inclusion process is considered as peer reviewed. The results are also included in the EFSA Reasoned Opinion (Review of the existing maximum residue levels (MRLs) for pyraclostrobin according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(8):2344. [92 pp.] doi:10.2903/j.efsa.2011.2344).

Report: CA 6.5.3/1
Plier S., 2013c
Determination of residues of BAS 500 F (Pyraclostrobin) in wheat and its processed products after two applications of BAS 500 06 F in Germany 2012/1067586

Guidelines: IVA Guideline IA-III (1992), EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999, BBA IV 3-4, BBA IV 3-3, OECD 508 Magnitude of the Pesticide Residues in Processed Commodities (2008), OECD 509 Crop Field Trial (2009), Working document of the Commission of the European Communities Directorate General for Agriculture VI B II-1 Appendix B, Working document of the Commission of the European Communities Directorate General for Agriculture VI B II-1 Appendix E, EEC 7029/VI/95 rev. 5, EEC 7035/VI/95 rev. 5

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin, 500M07, BAS 500 06 F

Description:	BAS 500 06F: BAS 500 F (Pyraclostrobin)
Lot/Batch #:	0003223026, Pyraclostrobin: 200.0 g/L
Purity:	BAS 500 F: 99.9% 500M07 (BF 500-3): 99.9%
CAS#:	BAS 500 F: 175013-18-0 500M07 (BF 500-3): 512165-96-7
Development code:	not applicable
Spiking levels:	BAS 500 F: 0.01 – 100 mg/kg 500M07 (BF 500-3): 0.01 – 20 mg/kg

2. Test Commodity: Cereals

Crop:	Wheat
Type:	not applicable
Variety:	JB Asano, Cubus, Akteur
Botanical name:	*Triticum L.*
Crop parts(s) or processed Commodity:	whole plant no roots, wet silage, wilted silage, grain, bran, flour, germ, middlings, shorts, gluten, gluten feed meal, starch, whole meal flour, whole grain bread
Sample size:	For analysis: >0.05 kg For processing: Whole plant no roots >7 kg, grain >40 kg

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2011 growing season, four field trials in Germany were conducted in order to investigate the residue behaviour of BAS 500 F (pyraclostrobin) in wheat and its processed products previously treated with the test item BAS 500 06 F.
Each field trial consisted of a treated plot. Two field trials (L110166 and L110168) also included a control plot. The test item BAS 500 06 F, a 200 g/L EC formulation of pyraclostrobin, was foliar applied twice at an exaggerated target rate of 6.25 L product/ha (1.25 kg a.s./ha) for each application. The applications were made at crop stages BBCH 49 and BBCH 69 using a spray volume of 200 L/ha. Wheat specimens for analysis were sampled on the day of last application as well as at crop stages BBCH 71 and BBCH 89 (on the field). RAC specimens for analysis were taken from the treated specimens directly before the start of processing. The processing of wheat was conducted with specimens taken at crop stages BBCH 71 and BBCH 89. After processing, twelve different fractions of wheat products or intermediates were collected for analysis, namely wet silage, wilted silage, bran, flour, germ, middlings, shorts, gluten, gluten feed meal, starch, whole meal flour and whole grain bread.

2. Description of analytical procedures

The specimens were analysed for residues of BAS 500 F (pyraclostrobin) and its metabolite 500M07 (BF 500-3) using BASF method No. L0076/01 (535/1), which has a limit of quantitation of 0.01 mg/kg for each of the analytes. Pyraclostrobin and 500M07 are extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of pyraclostrobin and 500M07 is performed by HPLC-MS/MS. The mean recovery results for pyraclostrobin and its metabolite 500M07 were 94.6 ± 9.5% and 91.0 ± 13.2%, respectively, at fortification levels of 0.01 and 100 mg/kg (pyraclostrobin) and 0.01 and 20 mg/kg (metabolite 500M07).

II. RESULTS AND DISCUSSION

The residue levels of pyraclostrobin determined in the treated specimens and its processed fractions as well as the calculated transfer factors are presented in Table 6.5.3-2. The mean transfer factors representing the different processing steps were below 1 for pyraclostrobin in wet silage, flour, germ, gluten feed meal, starch, whole meal flour and whole grain bread. Therefore, it can be concluded, that pyraclostrobin is not being accumulated in these processed fractions. The mean transfer factors of wilted silage, bran, middlings, shorts and gluten greater than 1 for pyraclostrobin residues are most likely based on the weight ratio and different water contents between RAC and processing fractions.

Table 6.5.3-2: Summary of BAS 500 F residues in wheat, processed fractions and transfer factors

Matrix	Residue BAS 500 F (mg/kg)				Transfer factor[1] BAS 500 F				Mean transfer factor
Trial [2)]	1	2	3	4	1	2	3	4	
Whole plant no roots, RAC[3]	12.95	10.60	24.80	12.35	1.00	1.00	1.00	1.00	1.00
Wet silage	10.30	8.32	23.68	11.44	0.80	0.78	0.95	0.93	0.87
Wilted silage	15.62	13.30	22.85	9.80	1.21	1.25	0.92	0.79	1.04
Grain, RAC[3]	0.03	0.02	0.11	0.07	1.00	1.00	1.00	1.00	1.00
Bran	0.07	0.08	0.17	0.21	2.33	4.00	1.55	3.00	2.72
Flour	<0.01	<0.01	<0.01	0.022	<0.33	<0.50	<0.09	0.29	<0.30
Germ	0.02	0.01	0.03	0.09	0.67	0.50	0.27	1.29	0.68
Middlings	0.05	0.05	0.09	0.16	1.67	2.50	0.82	2.29	1.82
Shorts	0.08	0.08	0.16	0.21	2.67	4.00	1.45	3.00	2.78
Gluten	0.06	0.05	0.04	0.11	2.00	2.50	0.36	1.57	1.61
Gluten feed meal	0.02	0.02	0.02	0.05	0.67	1.00	0.18	0.71	0.64
Starch	<0.01	<0.01	<0.01	<0.01	<0.33	<0.50	<0.09	0.14	0.27
Whole meal flour	0.03	0.02	0.05	0.06	1.00	1.00	0.45	0.86	0.83
Whole grain bread	<0.01	<0.01	<0.01	0.02	0.33	<0.50	<0.09	0.29	0.30

[1)] Transfer factor = residue in PF / residue in RAC
[2)] Trial 1: L110166, Trial 2: L110167, Trial 3: L110168, Trial 4: L110169
[3)] Specimens were taken directly before start of processing

The residue levels of metabolite 500M07 determined in the treated specimens and its processed fractions as well as the calculated transfer factors are presented in Table 6.5.3-3. The mean transfer factors representing the different processing steps were below 1 for the metabolite 500M07 in flour, gluten feed meal, starch and whole grain bread. Therefore, it can be concluded, that the metabolite 500M07 is not being accumulated in these processed fractions. The mean transfer factors of wet silage, wilted silage, bran, germ, middlings, shorts, gluten and whole meal flour greater 1 for the metabolite 500M07 are most likely based on the weight ratio and different water contents between RAC and processing fractions.

Table 6.5.3-3: Summary of metabolite 500M07 (BF 500-3) residues in wheat, processed fractions and transfer factors

Matrix	Residue 500M07 (BF 500-3)[4] (mg/kg)				Transfer factor[1] 500M07 (BF 500-3)				Mean transfer factor
Trial [2)]	1	2	3	4	1	2	3	4	
Whole plant no roots, RAC[3]	2.81	3.52	7.75	3.02	1.00	1.00	1.00	1.00	1.00
Wet silage	4.32	4.86	7.85	3.65	1.54	1.38	1.01	1.21	1.29
Wilted silage	3.92	4.31	7.22	4.86	1.40	1.22	0.93	1.61	1.29
Grain, RAC[3]	0.01	0.01	0.04	0.04	1.00	1.00	1.00	1.00	1.00
Bran	0.05	0.07	0.07	0.13	5.00	7.00	1.75	3.25	4.25
Flour	<0.01	<0.01	<0.01	0.01	<1.00	<1.00	0.25	0.25	<0.63
Germ	0.02	0.01	0.01	0.05	2.00	1.00	0.25	1.25	1.13
Middlings	0.03	0.03	0.03	0.10	3.00	3.00	0.75	2.50	2.31
Shorts	0.04	0.05	0.05	0.13	4.00	5.00	1.25	3.25	3.38
Gluten	0.03	0.02	0.01	0.05	3.00	2.00	0.25	1.25	1.63
Gluten feed meal	0.01	<0.01	<0.01	0.02	1.00	<1.00	0.25	0.50	0.69
Starch	<0.01	<0.01	<0.01	<0.01	<1.00	<1.00	0.25	<0.25	0.63
Whole meal flour	0.02	0.02	0.02	0.04	2.00	2.00	0.50	1.00	1.38
Whole grain bread	0.01	0.01	0.03	0.03	1.00	1.00	0.75	0.75	0.88

[1] Transfer factor = residue in PF / residue in RAC
[2] Trial 1: L110166, Trial 2: L110167, Trial 3: L110168, Trial 4: L110169
[3] Specimens were taken directly before start of processing
[4] Residues expressed as parent equivalents, conversion factor for calculation of 500M07 (BF 500-3) to parent equivalent is 1.084.

III. CONCLUSION

Pyraclostrobin was concentrated in wilted silage, bran, middlings, shorts and gluten, whereas the metabolite 500M07 (BF 500-3) was concentrated in wet silage, wilted silage, bran, germ, middlings, shorts, gluten and whole meal flour. The concentration is most likely based on the weight ratio and different water contents between RAC and processing fractions. In all further matrices the residues declined during processing.

Report: CA 6.5.3/2
Plier S., 2013d
Determination of residues of BAS 700 F (Fluxapyroxad) and BAS 500 F (Pyraclostrobin) in oat and its processed products after two applications of BAS 703 04 F in Germany, 2012
2013/1037950
Guidelines: OECD-DOC ENV/MC/CHEM (98)17 Paris 1998
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Fluxapyroxad, pyraclostrobin, M700F002, M700F048, M700F008, 500M07 (BF 500-3)

Description:	BAS 703 04 F: BAS 700 F (fluxapyroxad) and BAS 500 F (pyraclostrobin)
Lot/Batch #:	208088, fluxapyroxad: 73.3 g/L; pyraclostrobin: 147.5 g/L
Purity:	BAS 500 F: 99.9% 500M07 (BF 500-3): 99.9%
CAS#:	BAS 500 F: 175013-18-0 500M07 (BF 500-3): 512165-96-7
Development code:	not applicable
Spiking levels:	0.01 – 20 mg/kg

2. Test Commodity: Cereals

Crop:	Oat
Type:	not applicable
Variety:	Flocke
Botanical name:	*Avena sativa* L.
Crop parts(s) or processed Commodity:	Whole plant, grain, flour, groats/rolled oats, husks, dust, bran
Sample size:	For analysis: 0.15-1.84 kg For processing: 21.78-40.01 kg

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2012 growing season, a total of 4 field trails were conducted in Germany to determine the magnitude of the residues of fluxapyroxad (BAS 700 F) and pyraclostrobin (BAS 500 F) in or on oat and processed products.
The formulation BAS 703 04 F was foliar applied twice at an exaggerated rate of 0.450 kg a.s./ha for fluxapyroxad and of 0.900 kg a.s./ha for pyraclostrobin. Application timings were at BBCH 49 and BBCH 69. Oat specimens were collected directly after the application (0 DALA) and at growth stage BBCH 89. Sampling of BBCH 89 specimens was performed twice. One specimen was used for processing into flour, groats/rolled oats, husks, dust and bran.

2. Description of analytical procedures

All specimens were analysed for pyraclostrobin and its metabolite 500M07 (BF 500-3) using BASF Method No. L0076/01 (535/1). The method has a limit of quantitation of 0.01 mg/kg for all analytes.
Pyraclostrobin and its metabolite 500M07 were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS. Mean procedural recoveries were 95.3% for pyraclostrobin and 91.9% for the metabolite 500M07.

II. RESULTS AND DISCUSSION

In the grain specimens used for processing, residues of pyraclostrobin ranged between 0.20 and 0.46 mg/kg and residues of metabolite 500M07 (BF 500-3) ranged between 0.044 and 0.14 mg/kg.
In flour specimens, residues of pyraclostrobin ranged between 0.022 and 0.085 mg/kg and residues of the metabolite 500M07 ranged between <0.01 and 0.023 mg/kg. In groats/rolled oats residues of pyraclostrobin ranged between 0.01 and 0.042 mg/kg and residues of the metabolite 500M07 were between <0.01 and 0.011 mg/kg. Residue data are shown in Table 6.5.3-4.
The transfer factors for parent pyraclostrobin and metabolite 500M07 are shown in Table 6.5.3-5 and Table 6.5.3-6. In husks and dust, pyraclostrobin and its metabolite 500M07 were concentrated (pyraclostrobin: TF 2.34 and 5.23; 500M07: TF 2.42 and 5.45), whereas in all further matrices the residues declined during processing.
No residues of the analytes above the respective limits of quantitation were found in any of the analyzed untreated oat specimens or their processed products.

Table 6.5.3-4: Summary of pyraclostrobin and its metabolite in different oat matrices

Matrix	DALA[1]	Residues as parent equivalents (mg/kg)		
		BAS 500 F	500M07 (BF 500-3) [2]	Total
Whole plant[3]	0	12-18	0.17-0.62	12-19
Grain	43-50	0.23-0.47	0.052-0.14	0.29-0.58
Grain[4]	n.a.	0.20-0.46	0.044-0.14	0.25-0.57
Flour		0.022-0.085	<0.01-0.023	0.032-0.11
Groats/rolled oats		0.010-0.042	<0.01-0.011	0.020-0.053
Husks		0.43-1.2	0.093-0.39	0.53-1.6
Dust		0.93-2.5	0.18-0.92	1.1-3.4
Bran		0.057-0.17	0.011-0.050	0.067-0.22

[1] Days after last application
[2] Conversion factor for calculation of 500M07 (BF 500-3) to parent BAS 500 F is 1.084
[3] Whole plant without roots
[4] Used for processing/at processing start
n.a. not applicable

Table 6.5.3-5: Summary of pyraclostrobin residues in oats, processed fractions and transfer factors

Portion analyzed		Residue BAS 500 F, parent (mg/kg)				Transfer factor [1] BAS 500 F, parent				Mean transfer factor
Trial [2)]		1	2	3	4	1	2	3	4	
RAC	Grain[3]	0.46	0.23	0.43	0.20	1	1	1	1	1
Processed matrices	Flour	0.022	0.024	0.085	0.022	0.05	0.10	0.20	0.11	0.12
	Groats/rolled oats	0.013	0.014	0.042	0.010	0.03	0.06	0.10	0.05	0.06
	Husks	0.71	0.66	1.2	0.43	1.54	2.87	2.79	2.15	2.34
	Dust	0.93	1.4	2.5	1.4	2.02	6.09	5.81	7.00	5.23
	Bran	0.076	0.073	0.17	0.057	0.17	0.32	0.40	0.29	0.30

[1] Transfer factor = residue in PF / residue in RAC
[2] Trial 1: L120191, Trial 2: L120192, Trial 3: L120193, Trial 4: L120194
[3] Used for processing/at processing start

Table 6.5.3-6: Summary of the metabolite 500M07 (BF 500-3) residues in oats, processed fractions and transfer factors

Portion analyzed		Residue 500M07 (BF 500-3)[4] (mg/kg)				Transfer factor[1] 500M07 (BF 500-3)				Mean transfer factor
Trial [2)]		1	2	3	4	1	2	3	4	
RAC	Grain[3]	0.063	0.059	0.14	0.044	1.00	1.00	1.00	1.00	1.00
Processed matrices	Flour	<0.01	<0.01	0.023	<0.01	<0.16	<0.17	0.16	<0.23	<0.18
	Groats/rolled oats	<0.01	<0.01	0.011	<0.01	<0.16	<0.17	0.08	<0.23	<0.16
	Husks	0.13	0.16	0.39	0.093	2.06	2.71	2.79	2.11	2.42
	Dust	0.18	0.34	0.92	0.29	2.86	5.76	6.57	6.59	5.45
	Bran	0.013	0.017	0.050	0.011	0.21	0.29	0.36	0.25	0.28

[1] Transfer factor = residue in PF / residue in RAC
[2] Trial 1: L120191, Trial 2: L120192, Trial 3: L120193, Trial 4: L120194
[3] Used for processing/at processing start
[4] Residues expressed as parent equivalents, conversion factor for calculation of 500M07 (BF 500-3) to parent equivalent is 1.084; for calculation purposes, "< 0.01" is set 0.01

III. CONCLUSION

In husks and dust, pyraclostrobin and its metabolite 500M07 (BF 500-3) were concentrated (pyraclostrobin: TF 2.34 and 5.23, 500M07: TF 2.42 and 5.45), whereas in all further matrices the residues declined during processing.

Report: CA 6.5.3/3
Braun D., 2011a
Determination of residues of BAS 500 F (Pyraclostrobin) and BAS 480 F (Epoxiconazole) in maize and its processed products after one application of BAS 512 04 F in Germany
2010/1144336

Guidelines: EEC 7029/VI/95 rev. 5 Appendix B (July 22 1997), BBA IV 3-3, IVA Guideline IA-II (1992)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin, epoxiconazole, 1,2,4-triazole, triazolylalanine, triazole acetic acid, triazole lactic acid, 500M07 (BF 500-3)

Description: BAS 512 04 F: BAS 500 F (pyraclostrobin), BAS 480 F (epoxiconazole), SE formulation

Lot/Batch #: FRE-000369, pyraclostrobin: 133.4 g/L, epoxiconazole: 50.9 g/L

Purity: BAS 500 F: 99.9%,
500M07 (BF 500-3): 99.9%

CAS#: BAS 500 F: 175013-18-0
500M07 (BF 500-3): 512165-96-7

Development code: not applicable

Spiking levels: 0.01 – 20 mg/kg

2. Test Commodity: Cereals

Crop: Maize

Variety: BKC 2949, DK 243, Ronaldinho, DK 2949

Botanical name: *Zea mays*

Crop parts(s) or processed

Commodity: Whole plant, grain, chopped fodder, silage, refined oil, flour, meal, starch, bran, middlings, gluten, gluten feed meal, steepwater, germs, presscake

Sample size: For processing: 5 kg of whole plant; 20 kg of grain

B. STUDY DESIGN

1. Test procedure

During the growing season 2008, four field trials were conducted in Germany to determine the residue levels of pyraclostrobin and epoxiconazole in maize and their processed products. The trials were performed with the formulation BAS 512 04 F (0.133 kg pyraclostrobin/L and 0.05 kg epoxiconazole/L, SE). The formulation was foliar applied once at BBCH 65 at a rate of 7.5 L/ha (0.9975 kg as/ha of pyraclostrobin and 0.375 kg as/ha of BAS 480 F). Maize specimens for analysis were sampled directly after the last application and in addition 67 and 90 days after last application for trial L080338, 62 and 94 days after last application for trial L080339, 43 and 83 days after last application for trial L080340 as well as 70 and 88 days after last application for trial L080341. The processing of maize was conducted with maize taken at the second sampling event (BBCH 85, sampled as whole plant) and last sampling event (BBCH 89, sampled as grain). After processing, thirteen different fractions of maize products or intermediates were collected for analysis: chopped fodder, silage, refined oil, flour, meal, starch, bran, middlings, gluten, gluten feed meal, steep water, germs and press cake.

2. Description of analytical procedures

All samples were analyzed with BASF method no. 535/1 which quantifies the residues of pyraclostrobin, its metabolite 500M07 (BF 500-3) and epoxiconazole with a limit of quantitation of 0.01 mg/kg each for all matrices.
Pyraclostrobin, 500M07 and epoxiconazole were extracted using a mixture of methanol, water and HCl 2 mol/L. For clean-up a liquid/liquid partition against cyclohexane was used. The final determination of pyraclostrobin, 500M07 and epoxiconazole was performed by HPLC/MS/MS.
Some deviations were proceeded to the BASF Method No 535/1 for refined oil. For pyraclostrobin and 500M07 analysis, in the extraction step the Shaker was used during 50 minutes, instead of Ultra Turrax during 2 minutes. In the partition step, 10 mL of cyclohexane was used and an aliquot of 4 mL was taken. The factor aliquot did not change.
Average procedural recoveries in all sample materials ranged for pyraclostrobin between 73 and 113%, for 500M07 between 73 and 107% and for epoxiconazole between 81 and 109% at fortification levels of 0.01, 0.1, 1.0 and 20 mg/kg.

II. RESULTS AND DISCUSSION

For pyraclostrobin, chopped fodder and silage showed very similar residue levels and only a slight increase compared to whole plant was seen most likely resulting out of some water losses. The mean transfer factors representing the different processing steps were 1 for pyraclostrobin in refined oil, starch, bran, middlings, gluten, gluten feed meal, steep water, germs and press cake, because the residues in all 4 trials in the RAC grain samples were ≤ LOQ. The residue levels of pyraclostrobin determined in the treated specimens and its processed fractions as well as the calculated transfer factors are presented in Table 6.5.3-7.

According to 500M07 (BF 500-3), the transfer factor of chopped fodder and silage indicates that there was a slight increase compared to the residue levels in whole plant, most likely because of a water loss in the samples. The mean transfer factors representing the different processing steps were 1 for BF 500-3 in refined oil, flour, starch, bran, middlings, gluten, gluten feed meal, steep water, germs and press cake, because the residues in the RAC grain samples for all 4 trials were ≤ LOQ. The residue levels of BF 500-3 determined in the treated specimens and its processed fractions as well as the calculated transfer factors are presented in Table 6.5.3-8.

III. CONCLUSION

Concentration factors of process fractions obtained from maize grain were 1 for pyraclostrobin and its metabolite BF 500-3, because the residues in the RAC grain samples were ≤ LOQ.

Table 6.5.3-7: Summary of pyraclostrobin residues and transfer factors

Matrix	BBCH	Residue pyraclostrobin[3] (mg/kg)				Transfer factor pyraclostrobin[1]				
Trial[2]		1	2	3	4	1	2	3	4	Mean
Whole plant	65	7.72	8.48	17.06	10.82	-	-	-	-	-
Whole plant, RAC	85	0.98	0.70	0.91	0.61	1	1	1	1	1
Chopped fodder		1.10	0.67	1.00	1.04	1.12	0.96	1.10	1.70	1.22
Silage		1.50	0.66	1.15	1.09	1.53	0.94	1.26	1.79	1.38
Grain, RAC	89	<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Refined oil		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Flour		0.02[4]	<0.01	<0.01	<0.01	2	1	1	1	1.25
Meal		0.03[4]	<0.01	<0.01	<0.01	3	1	1	1	1.5
Starch		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Bran		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Middlings		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Gluten		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Gluten feed meal		<0.01	<0.01	<0.01	0.01	1	1	1	1	1
Steep water		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Germs		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Press cake		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1

[1] Transfer factor = residue in PF / residue in RAC
[2] Trial 1: L080338, Trial 2: L080339, Trial 3: L080340, Trial 4: L080341
[3] For calculation purposes, "< 0.01" is set 0.01
[4] Matrix impact for analysis

Table 6.5.3-8: Summary of BF 500-3 residues and transfer factors

Matrix	BBCH	Residue BF 500-3[3] (mg/kg)				Transfer factor BF 500-3[1]				
Trial[2]		1	2	3	4	1	2	3	4	Mean
Whole plant	65	0.05	0.07	0.14	0.07	-	-	-	-	-
Whole plant, RAC	85	0.13	0.02	0.19	0.10	1	1	1	1	1
Chopped fodder		0.14	0.05	0.33	0.14	1.08	2.5	1.74	1.4	1.68
Silage		0.28	0.10	0.54	0.25	2.15	5	2.84	2.5	3.12
Grain, RAC	89	0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Refined oil		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Flour		0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Meal		0.04[4]	<0.01	<0.01	<0.01	4	1	1	1	1.75
Starch		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Bran		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Middlings		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Gluten		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Gluten feed meal		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Steep water		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1
Germs		<0.01	<0.01	<0.01	0.01	1	1	1	1	1
Presscake		<0.01	<0.01	<0.01	<0.01	1	1	1	1	1

[1] Transfer factor = residue in PF / residue in RAC
[2] Trial 1: L080338, Trial 2: L080339, Trial 3: L080340, Trial 4: L080341
[3] For calculation purposes, "< 0.01" is set 0.01
[4] Matrix impact for analysis

CA 6.6 Residues in Rotational Crops

A confined rotational crop study (BASF DocID 1999/11829) was submitted and evaluated during the previous Annex I inclusion process. The residue levels and the nature of residues were investigated in three different succeeding crops (radish, lettuce, wheat) at an application rate of 900 g as/ha. In the study the ^{14}C-chlorophenyl and ^{14}C-tolyl labelled pyraclostrobin was applied to bare soil. The study is considered to be still scientifically valid and it is meeting the requirements included in OECD guideline 502.

For pyraclostrobin, the following conclusion was made by the RMS Germany in the course of the Annex I inclusion process. The relevant endpoint was copied from the Draft Assessment Report (2001).

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

30, 120, 365 days plant back interval after application of 0.9 kg as/ha: TRR in the edible parts for human consumption are very low (radish roots, lettuce: < 0.040 mg/kg; wheat grain: < 0.089 mg/kg). No accumulation of pyraclostrobin or its degradation products [radish, lettuce: < 0.0106 mg/kg; wheat straw: < 0.0147 mg/kg; wheat grain: not detectable].

In context of the EU MRL review according to Article 12 (EFSA Journal 2011;9(8):2344) the assessment of Germany way confirmed by EFSA: The following paragraphs were directly copied from the relevant Reasoned Opinion.

Preliminary considerations

The use of pyraclostrobin in permanent crops or in third countries is not considered relevant with regard to the potential occurrence of residues in rotational crops. Within Europe, however, pyraclostrobin is also authorised for use in crops which may be grown in rotations, thus further consideration of pyraclostrobin residues in rotational crops is required. According to the soil degradation studies performed in the framework of the peer review, the highest DT90 value of pyraclostrobin, based on the field and laboratory studies, is 230 and 163 days, respectively. The desmethoxy metabolite (500M07) shows higher persistency in the soil with a DT90f value amounting to 529 days (Germany, 2001).

Nature of residues

In the peer review the metabolism of pyraclostrobin in rotational crops was studied in lettuce, radish and wheat with [tolyl-U-^{14}C]-pyraclostrobin and [chlorophenyl-U-^{14}C]-pyraclostrobin (Germany, 2001). The radiolabelled active substance was applied on a bare soil once at an application rate of 0.9 kg a.s./ha and respective crops were sown or planted at 30, 120 and 365 DAT.

The peer review concluded that the metabolic pathway of pyraclostrobin in rotational crops is similar to that in primary crops and no formation of new metabolites was observed. There is no accumulation of pyraclostrobin or its degradation products (including 500M07) in the parts of plants used for human or animal consumption. The relevant residue in rotational crops therefore should be defined as parent pyraclostrobin.

Magnitude of residues

According to the study results of the ^{14}C study, the total radioactive residues in the edible parts of succeeding crops were very low for all plant back intervals: radish roots, lettuce ≤ 0.04 mg/kg and wheat grain ≤0.089 mg/kg. No accumulation of pyraclostrobin or its residues was observed in rotational crops.

Application rates supported in the framework of this review range between 0.05 and 0.67 kg a.s./ha. Considering the overdosing factor of the above study and the fact that pyraclostrobin was applied to a bare soil (interception of pyraclostrobin by the plants is expected in practice), it is expected that residues of pyraclostrobin resulting from soil uptake will not exceed 0.01 mg/kg. Specific plant-back restrictions related to the use of pyraclostrobin are therefore not required, provided that pyraclostrobin is applied in compliance with the GAPs evaluated in the framework of this review.

CA 6.6.1 Metabolism in rotational crops

In order to prove that no metabolite consisting solely of the pyrazole moiety is formed in soil (see M-CA 7) and taken up by plants, preliminary investigations with the ^{14}C-pyrazole labelled pyraclostrobin were performed. ^{14}C-pyrazole labelled pyraclostrobin was applied at the maximum seasonal rate of 500 g as/ha to bare soil. After an aging period of 30 DAT crops were planted / sown. The use rate of 500 g as/ha corresponds to a realistic worst case GAP for Europe (2 x 250 g as/ha in cereals).

Report:	CA 6.6.1/1 Rabe U., Kalyon B., 2014b Confined indicator rotational crop study with ^{14}C-Pyraclostrobin 2014/1001761
Guidelines:	EPA 860.1850: Confined Accumulation in Rotational Crops, EPA 860.1850: EPA Residue Chemistry Test Guidelines, EPA 860.1000: EPA Residue Chemistry Test Guidelines, OECD 502 Metabolism in Rotational Crops (January 2007), EPA 860.1000: Background - PMRA Section 97.13 (Canada): Residue Chemistry Guidelines Confined Accumulation in Rotational Crops (June 1997)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: ^{14}C-BAS 500 F

Description:	Pyraclostrobin was applied as undiluted ^{14}C-labelled BAS 500 F (labelled at position 3 of the pyrazole ring) - Label: pyrazole-3-C^{14}
Lot/Batch #:	1073-1008
Purity:	90.7% (radiochemical purity: 99.7%)
CAS#:	175013-18-0
Development code:	BAS 500 F
Spiking levels:	not available

2. Test Commodity:

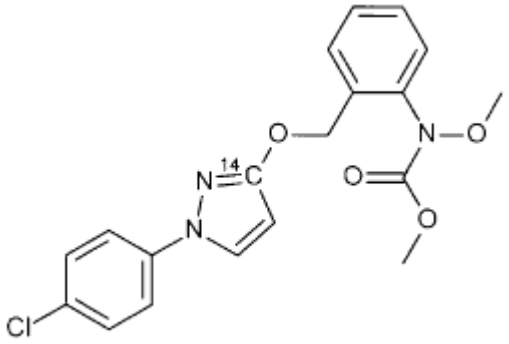
Crop:	radish, wheat, lettuce
Type:	not reported
Variety:	radish: Eiszapfen, wheat: Tassos, lettuce: Hardy
Botanical name:	*Rhaphanus sativus, Triticum L., Lactuca sativa*
Crop parts(s) or processed commodity:	radish: leaf and root wheat: forage lettuce: plant
Sample size:	not applicable

B. STUDY DESIGN

1. Test procedure

The confined indicator rotational crop study was performed with ^{14}C-pyraclostrobin labelled at position 3 of the pyrazole ring.

Figure 6.6.1-1: Structural formula of ^{14}C-BAS 500 F labelled at position 3 of the pyrazole ring



The pure active ingredient was applied to sandy loam soil (USDA scheme) with an application rate of 0.500 kg a.s./ha. After application, the soil was aged for 32 days. After the soil aging period was completed, ploughing was simulated by mixing the treated soil with the help of a concrete mixer. Subsequently the crops radish, wheat and lettuce were sowed or planted. Soil characteristics are given in Table 6.6.1-1.

Table 6.6.1-1: Soil characteristics: soil used for planting

USDA classification	
clay	9.5%
silt	17.4%
sand	73.1%
soil class	sandy loam
DIN classification	
clay	9.5%
silt	21.5%
sand	69.0%
soil class	loamy sand (SL3)
Other soil parameters	
total nitrogen	0.15%
total organic carbon	2.76%
total carbon	2.76%
pH ($CaCl_2$)	7.1
pH (H_2O)	7.9
effective cation exchange capacity	11.5 cmol/kg
max. water holding capacity	25.2 g/100 g dry soil
microbial biomass	35.3 mg C/100 g dry soil
microbial C / organic C	1.3%
bulk density	1344 g/L
dry matter	92.3%

The immature crops were harvested after 42 days, processed and analysed by combustion and radioactivity measurement for the Total Radioactive Residues. In addition, soil samples were taken after ploughing and harvest of the crops.

2. Description of analytical procedures

For the determination of the Extractable Radioactive Residues (**ERR**), the homogenised plant material was extracted three times with methanol and two times with water. After solvent extraction and partition procedures HPLC analyses (Method LC02) were carried out for the concentrated water phases of the methanol extracts with a sufficient level of radioactivity. In context of this HPLC method, a Phenomenex Synergi Polar-RP-column was used (250 x 4.6 mm, particle size 4 µm), operating at a column temperature of 25 °C with a flow rate of 1.0 mL/min. The eluent system consisted of 2 eluents (A: water/acetonitrile/formic acid, 950/50/2.5 & B: water/acetonitrile/formic acid, 50/950/2.5). This system was applied using gradient elution.
The residual radioactive residues after solvent extraction (**RRR**) were characterised by combustion of the dried and homogenised extraction residues.
The Total Radioactive Residues (**TRR**) in *plant* material were obtained by calculating the sum of ERR and RRR (TRR calculated) and additionally by combustion of sample aliquots (TRR Measured). The *soil* sample after aging and ploughing was determined by direct combustion analysis followed by LSC. All calculations throughout the present study were based on the TRR calculated.

II. RESULTS AND DISCUSSION

Total radioactive residues

The Total Radioactive Residues (TRR) of all matrices were very low and ranged from 0.003 mg/kg (radish root) to 0.016 mg/kg (lettuce plant). The TRR determined by combustion analysis showed no major difference to the TRR calculated by summing up the extractable and the residue radioactivity. These results indicate that there was no pronounced translocation of pyraclostrobin or its degradation products from the soil into the plants.

The results of the Total Radioactive Residues (TRR) after ^{14}C-pyraclostrobin treatment in radish, wheat and lettuce are provided in Table 6.6.1-2, the results of the soil samples are shown in Table 6.6.1-3.

Table 6.6.1-2: Total Radioactive Residues in rotational crops after ^{14}C-BAS 500 F treatment

Matrix	Days after sowing / planting DAP	TRR measured[1)] [mg/kg]	TRR calculated[2)] [mg/kg]
Plant back interval: 32 DAT			
Radish leaf	42	0.011	0.010
Radish root	42	0.003	0.003
Wheat forage	42	0.015	0.014
Lettuce plant	42	0.017	0.016

[1)] TRR was determined by direct combustion
[2)] TRR was calculated as the sum of ERR (extraction with methanol and water) + RRR

Due to the very low TRR level in soil samples already after ploughing at 32 DAT, the soil samples collected at 74 DAT after harvest of the mature crops were not further analysed.

Table 6.6.1-3: Total Radioactive Residues in soil samples after ^{14}C-BAS 500 F treatment

Soil samples (Days After Treatment DAT)	Days after sowing DAP	TRR measured[1)] [mg/kg]
Plant back interval: 32 DAT		
After ploughing		
32 DAT	0	0.140
After harvest of mature crops		
Radish (74 DAT)	42	n.a.
Wheat (74 DAT)	42	n.a.
Lettuce (74 DAT)	42	n.a.

1) TRR was determined by direct combustion
n.a. not analysed due to the low TRR level already after ploughing at 32 DAT

Extraction behaviour

For all rotational crop matrices the methanol extract and the water extract were summarised as ERR.
The extractabilities with methanol and water are summarised in Table 6.6.1-4. The major portions of the radioactive residues were extracted with methanol (46.9 to 63.3% TRR). The extraction with water released additional 3.9 to 8.5% TRR.

Table 6.6.1-4: Extractability of Radioactive Residues in rotational crops after ^{14}C-BAS 500 F treatment

Matrix	TRR calculated[1)]	Methanol extract		Water extract		ERR[2)]		RRR	
	[mg/kg]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Plant back interval: 32 DAT									
Radish leaf	0.010	0.006	63.3	0.001	8.5	0.007	71.8	0.003	28.2
Radish root	0.003	0.002	46.9	< 0.001	8.0	0.002	55.0	0.002	45.0
Wheat forage	0.014	0.008	59.0	0.001	3.9	0.009	62.9	0.005	37.1
Lettuce plant	0.016	0.008	50.8	0.001	8.0	0.009	58.8	0.006	41.2

1) TRR was calculated as the sum of ERR (extraction with methanol and water) + RRR and set at 100% for all further calculations.
2) Extractable Radioactive Residue (ERR) was calculated as the sum of methanol and water extract.

The extractability of radish leaf with methanol was good and accounted for 63.3% TRR. Subsequent extraction with water released only minor amounts of 8.5% TRR. Thus, the Extractable Radioactive Residue (ERR) from radish leaf was 71.8% TRR. The residue after solvent extraction (RRR) accounted for 0.003 mg/kg and was therefore not further investigated.

The extractability of radish root with methanol accounted for 46.9% TRR. Subsequent extraction with water released only minor amounts of 8.0% TRR. Thus, the Extractable Radioactive Residue (ERR) from radish root was 55.0% TRR. The residue after solvent extraction (RRR) accounted for 0.002 mg/kg. The extracts and the residue after solvent extraction were not further investigated due to low levels of radioactivity.

The extractability of wheat forage with methanol accounted for 59.0% TRR. Subsequent extraction with water released only minor amounts of 3.9% TRR. Thus, the Extractable Radioactive Residue (ERR) from wheat forage was 62.9% TRR. The residue after solvent extraction (RRR) accounted for 0.005 mg/kg and was therefore not further investigated.

The extractability of lettuce plant with methanol accounted for 50.8% TRR. Subsequent extraction with water released only minor amounts of 8.0% TRR. Thus, the Extractable Radioactive Residue (ERR) from lettuce plant was 58.8% TRR. The residue after solvent extraction (RRR) accounted for 0.006 mg/kg and was therefore not further investigated.

Partition behaviour

In order to characterise the radioactive residues in the methanol extract as organo-soluble or water-soluble, liquid / liquid partitions of the methanol extracts from radish leaf, wheat forage and lettuce plant were carried out using iso-hexane as organic solvent. In wheat forage and lettuce plant, higher portions of the radioactive residues were water-soluble, and lower portions were detected in the organic phase. In radish leaf, comparable portions were detected in the organic and in the water phase. Results are summarised in Table 6.6.1-5.

Table 6.6.1-5: Partition characteristics of Radioactive Residues extracted with methanol from rotational crop samples

Matrix	Methanol extract		Isohexane partition				Recovery [1)]
			Organo-soluble		Water-soluble		
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[%]
Radish leaf	0.006	63.3	0.003	29.9	0.003	26.9	89.726
Radish root	0.002	46.9	n.a.				-
Wheat forage	0.008	59.0	0.002	17.3	0.005	32.0	83.576
Lettuce plant	0.008	50.8	0.002	15.2	0.006	38.6	106.087

[1)] Recovery calculated as (Organo-soluble + Water-soluble) [mg/kg] x 100 / Methanol Extract [mg/kg]
n.a. = not applied

Identification and characterization of extractable radioactivity

The concentrated water phases of the methanol extracts of radish leaf, wheat forage and lettuce plant were analysed by HPLC and resulted in a metabolite pattern of one polar peak at a retention time of approximately 4.3 min and three peaks at approximately 20.2 min, 21.7 min and 22.8 min. An assignment of the peaks to a structure was not possible due to the low levels of radioactive residues. All peaks were below the trigger of 0.01 mg/kg. The Residual Radioactive Residues (RRR) were low with values ranging from 0.002 mg/kg to 0.006 mg/kg.
The summaries of characterised residues from radish leaf, wheat forage and lettuce plant are compiled in the tables below.

Radish leaf

The methanol extract of radish leaf was extracted with isohexane, which resulted in a isohexane phase and a water phase. Quantitative analysis of the concentrated water phase using HPLC method LC02 yielded one polar peak at 4.3 min (< 0.001 mg/kg or 4.7% TRR) and three peaks at 20.2 min (0.001 mg/kg or 8.3% TRR), 21.7 min (0.001 mg/kg or 10.5% TRR) and 22.8 min (< 0.001 mg/kg or 3.4% TRR). Furthermore, 0.003 mg/kg or 29.9% TRR were characterised by their solubility in the isohexane phase of the methanol extract and 0.001 mg/kg or 8.5% TRR by their extractability with water. The residue after solvent extraction (RRR) was not further investigated due to low levels of radioactivity (0.003 mg/kg or 28.2% TRR).
In total, 65.3% of the TRR were characterised from the ERR by HPLC or by their extractability. Summarised with the Residual Radioactive Residue, the radioactive residues accounted for 0.010 mg/kg or 93.5% TRR.

Table 6.6.1-6: Summary of identified and characterised Radioactive Residues extracted from radish leaf

Designation	[mg/kg]	[% TRR]
Total Radioactive Residue (TRR)	***0.010***	***100.0***
Characterised from Concentrated Water Phase of Methanol Extract by HPLC		
Peak at 4.3 min	< 0.001	4.7
Peak at 20.2 min	0.001	8.3
Peak at 21.7 min	0.001	10.5
Peak at 22.8 min	< 0.001	3.4
Total Characterised from ERR by HPLC	**0.003**	**26.9**
Isohexane Phase of Methanol Extract	0.003	29.9
Water Extract	0.001	8.5
Total Characterised from ERR	**0.007**	**65.3**
Residual Radioactive Residue (RRR)	0.003	28.2
Sum of RRR and Total Characterised from ERR	**0.010**	**93.5**

Wheat forage

The methanol extract of wheat forage was extracted with isohexane, which resulted in a isohexane phase and a water phase. Quantitative analysis of the concentrated water phase using HPLC method LC02 yielded the polar peak at 4.3 min (0.001 mg/kg or 3.7% TRR) and the two peaks at 20.2 min (0.001 mg/kg or 7.9% TRR) and 21.7 min (0.003 mg/kg or 20.4% TRR). Although the peak at 22.8 min was not integrated in the chromatogram, it was present as a shoulder of peak at 21.7 min. Furthermore, 0.002 mg/kg or 17.3% TRR were characterised by their solubility in the isohexane phase of the methanol extract and 0.001 mg/kg or 3.9% TRR by their extractability with water. The residue after solvent extraction (RRR) was not further investigated due to low levels of radioactivity (0.005 mg/kg or 37.1% TRR).

In total, 53.2% of the TRR were characterised from the ERR by HPLC or by their extractability. Summarised with the Residual Radioactive Residue, the radioactive residues accounted for 0.013 mg/kg or 90.3% TRR.

Table 6.6.1-7: Summary of identified and characterised Radioactive Residues extracted from wheat forage

Designation	[mg/kg]	[% TRR]
Total Radioactive Residue (TRR)	***0.014***	***100.0***
Characterised from Concentrated Water Phase of Methanol Extract by HPLC		
Peak at 4.3 min	0.001	3.7
Peak at 20.2 min	0.001	7.9
Peak at 21.7 min	0.003	20.4
Total Characterised from ERR by HPLC	**0.005**	**32.0**
Isohexane Phase of Methanol Extract	0.002	17.3
Water Extract (Lab0011)	0.001	3.9
Total Characterised from ERR	**0.008**	**53.2**
Residual Radioactive Residue (RRR)	0.005	37.1
Sum of RRR and Total Characterised from ERR	**0.013**	**90.3**

Lettuce plant

The methanol extract of lettuce plant was extracted with isohexane, which resulted in a isohexane phase and a water phase. Quantitative analysis of the concentrated water phase using HPLC method LC02 yielded the polar peak at 4.3 min (0.001 mg/kg or 7.3% TRR) and the three peaks at 20.2 min (0.001 mg/kg or 9.0% TRR), 21.7 min (0.003 mg/kg or 17.4% TRR) and 22.8 min (0.001 mg/kg or 5.0% TRR). Furthermore, 0.002 mg/kg or 15.2% TRR were characterised by their solubility in the isohexane phase of the methanol extract and 0.001 mg/kg or 8.0% TRR by their extractability with water. The residue after solvent extraction (RRR) was not further investigated due to low levels of radioactivity (0.006 mg/kg or 41.2% TRR).
In total, 61.8% of the TRR were characterised from the ERR by HPLC or by their extractability. Summarised with the Residual Radioactive Residue, the radioactive residues accounted for 0.015 mg/kg or 103.0% TRR.

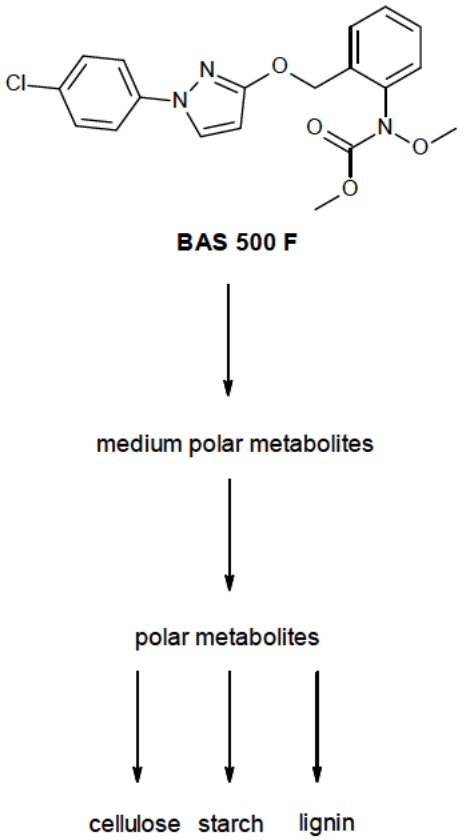
Table 6.6.1-8: Summary of identified and characterised Radioactive Residues extracted from lettuce plant

Designation	[mg/kg]	[% TRR]
Total Radioactive Residue (TRR)	***0.016***	***100.0***
Characterised from Concentrated Water Phase of Methanol Extract by HPLC		
Peak at 4.3 min	0.001	7.3
Peak at 20.2 min	0.001	9.0
Peak at 21.7 min	0.003	17.4
Peak at 22.8 min	0.001	5.0
Total Characterised from ERR by HPLC	**0.006**	**38.6**
Isohexane Phase of Methanol Extract	0.002	15.2
Water Extract	0.001	8.0
Total Characterised from ERR	**0.009**	**61.8**
Residual Radioactive Residue (RRR)	0.006	41.2
Sum of RRR and Total Characterised from ERR	**0.015**	**103.0**

Metabolic pathway

In all matrices analysed by HPLC, the same peaks were detected in the chromatograms. Due to the low levels of radioactivity in the plants, no structure could be assigned to the peaks. Therefore, a metabolic pathway with designated metabolites could not be evaluated.
It can be assumed, that the results from this indicator study with ^{14}C-pyraclostrobin labelled in the pyrazole ring are in good accordance with the results of the confined rotational crop study performed with Pyraclostrobin ^{14}C-labelled in the tolyl and the phenyl ring systems (Veit, P.: Confined Rotational Crop Study with ^{14}C-pyraclostrobin, BASF Doc ID 1999/11829).

Figure 6.6.1-2: Metabolic pathway of BAS 500 F in rotational crops



III. CONCLUSION

In all rotational crop matrices (radish, wheat, lettuce) low levels of radioactive residues were determined. The calculated Total Radioactive Residues (TRR) in radish leaf accounted for 0.010 mg/kg, for radish root 0.003 mg/kg, for wheat forage 0.014 mg/kg and for lettuce plant 0.016 mg/kg. The measured TRR of the top soil layer after aging and ploughing was 0.140 mg/kg.

The extractability of the radioactive residues with methanol and water was good. The major portions of the radioactive residues were extracted with methanol (46.9% to 63.3% TRR). The extractable residues in water were ≤ 8.5% TRR.
After liquid / liquid partitions of the methanol extracts from radish leaf, wheat forage and lettuce plant using isohexane as organic solvent, higher portions of the radioactive residues extracted were water-soluble, and lower portions were found in the organic phase in wheat forage and lettuce plant. In radish leaf, comparable portions were found in the organic and in the water phase.

HPLC analysis of the concentrated water phases of the methanol extracts of radish leaf, wheat forage and lettuce plant resulted in a metabolite pattern of one polar peak at a retention time of approximately 4.3 min and three peaks at approximately 20.2 min, 21.7 min and 22.8 min. An assignment of the peaks to structures was not possible due to the low levels of radioactive residues. All peaks were below the trigger of 0.01 mg/kg. The Residual Radioactive Residues (RRR) were low with values ranging from 0.002 mg/kg to 0.006 mg/kg.

The results indicate that there was no significant translocation of pyraclostrobin and / or its degradation products from the soil.

CA 6.6.2 Magnitude of residues in rotational crops

According to Reg. 283/2013, studies on the magnitude of residues in rotational crops are required under the following circumstances:

If the metabolism studies indicate that residues of the active substance or of relevant metabolites or breakdown products either from plant or soil metabolism may occur (> 0.01 mg/kg), limited field studies and, if necessary, field trials shall be carried out.

Studies shall not be required in the following cases:

- no metabolism studies on rotational crops are to be performed, or
- metabolism studies on rotational crops show that no residues of concern are to be expected in rotational crops

Due to the favorable residue behavior of pyraclostrobin in succeeding crops which has been demonstrated in two studies with different ring labels and under worst case conditions (bare soil application with partly exaggerated rates), no residues are expected. Consequently, no higher tier study is required.

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

The residue definitions currently established in the EU and supported in future are compiled in Table 6.7.1-1. In the subsequent section, detailed justifications for BASF's proposal are provided. The proposal is based on a careful evaluation of all studies being available at the time point of submission. Consequently, it includes considerations for all those crops for which an EU MRL is established. It is not limited to the intended uses in potatoes, cereals and maize.

Table 6.7.1-1: Residue definition - pyraclostrobin

End-Point	**Active Substance: Pyraclostrobin**	
	EU agreed endpoints (SANCO/1420/2001; Monograph 12945/ECCO/BBA/01, Vol. 1, list of endpoints)	Residue definitions proposed in the context of this dossier
Residue definition in plant matrices for risk assessment	Pyraclostrobin (parent)	Pyraclostrobin (parent)
Residue definition in plant matrices for monitoring	Pyraclostrobin (parent)	Pyraclostrobin (parent)
Residue definition in animal matrices for risk assessment	Pyraclostrobin (parent) except: Liver (except poultry liver) and milk fat only: Pyraclostrobin and its metabolites analysed as the hydroxypyrazoles BF 500-5 * and BF 500-8 **, sum expressed as pyraclostrobin	Pyraclostrobin and its metabolites analysed as the hydroxypyrazoles BF 500-5 * and BF 500-8 **, sum expressed as pyraclostrobin
Residue definition in animal matrices for monitoring	Pyraclostrobin (parent)	Pyraclostrobin (parent)
Conversion factors between residue definitions (animal)		Liver (w/o poultry): 4 All other: 1

* synonym: 500M04
** synonym: 500M85

For deriving appropriate residue definitions for monitoring and risk assessment purposes the principles described in the following document were considered:

- OECD GUIDANCE DOCUMENT ON THE DEFINITION OF RESIDUE (as revised in 2009), SERIES ON TESTING AND ASSESSMENT No. 63 and SERIES ON PESTICIDES No. 31 (ENV/JM/MONO(2009)30)
- EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, EFSA Journal 2012;10(07): 2799

The first document covers both aspects whereas the purpose of the PPR Scientific Opinion is limited to the residue definition for risk assessment purposes. The corresponding EU guidance document is in preparation and will be available earliest by end of 2015.

Plant Matrices

For deriving a suitable residue definition for food of plant origin, the following studies were considered:

- Crop metabolism studies
- Confined rotational crop studies
- Hydrolysis studies at exaggerated temperatures simulating processing

For pyraclostrobin, plant metabolism studies have been performed in fruits, root & tuber vegetables, leafy vegetables and cereals (wheat, paddy rice) covering both, foliar and seed treatment applications. During these investigations, samples/matrices were analyzed which serve either as animal feed or human food. Two confined rotational crop studies were additionally conducted; after application of pyraclostrobin to bare soil, representative crops (typically small grain, leafy crops and root vegetables) were investigated at different replant intervals (emergency plant back, fall plant back, annual plant back).

In general, pyraclostrobin follows a common pathway in all crops and independent from the type of application. The following metabolic conversion steps were observed in the plant metabolism and succeeding crop studies:

- Desmethoxylation of the side chain resulting in the metabolite 500M07
- Cleavage between the ring systems resulting in the metabolites 500M04 (followed by conjugation reactions)
- Cleavage between the ring systems resulting in the metabolites 500M24 and 500M72 (tryptophan)
- Hydroxylation of the chlorophenyl pyrazole moiety (followed by conjugation)
- Hydroxylation of the tolyl moiety (followed by conjugation)
- Photolytic rearrangement reaction resulting in the metabolite 500M76.

In almost all samples investigated from metabolism studies, the parent molecule forms by far the predominant residue followed by the metabolite 500M07. All other metabolites are present in significantly lower amounts, but also not consistently in all crops or commodities.

As non- systemic fungicide pyraclostrobin, but also its degradation products formed during soil metabolism (500M01, 500M02) are not taken up by plants. Even at exaggerated rates, the succeeding crop study did not indicate any likelihood for the presence of detectable residues.

To simulate processing, hydrolysis studies were performed at exaggerated temperatures. Radiolabelled test items of pyraclostrobin were incubated under the conditions considered as representative for boiling, baking, brewing, sterilization and pasteurization. Pyraclostrobin was found to be stable under all test conditions applied. At higher temperatures (up to 240°C), which might only occur during one step of oil processing (deodorization step), degradation to the metabolites 500M07 and the subsequent cleavage products 500M04 / 500M49 (anthranilic acid derivative) was observed.

Residue definition for monitoring purposes

According to the OECD Guidance Document, the residue definition for tolerance/MRL enforcement purposes should focus on those analytes which would indicate a possible misuse of the pesticide and which can be easily detected /measured by a broad base of national laboratories (use of a multi-residue method). The analyte(s) to be selected for monitoring purposes should occur in large quantities, and should be common to all commodities in which residues are expected. Ideally, a monitoring method should be based on one single analyte (‘marker or indicator compound’).

In case of pyraclostrobin and its metabolites in food of plant origin, there is only one component which meets all criteria listed in the OECD guidance document. Based on the studies available, the following residue definition is proposed for monitoring purposes in plant commodities (including process fractions thereof):

Pyraclostrobin, parent only

Residue definition for data generation / risk assessment purposes

The derivation of a suitable residue definition for risk assessment purposes is much more complex; according to the OECD guidance document the contribution of each metabolite/ degradates to a potential dietary risk need to be considered.

In general, two factors must be addressed:

- **Potential for exposure** to the metabolite/degradate in the human diet;
- **Relative toxicity** of the metabolite/degradate to the parent.

Metabolites / degradates with higher potential exposures and toxicities are more likely to be included in the dietary assessment. The OECD guidance document provides a first hint how an indicative risk assessment can be performed if metabolites are not readily available as reference substances. For such cases, the document recommends to calculate ratios from the metabolism studies and to apply these ratios in a second step to the residue level being measured during e.g. supervised field trials. The approach is described much more in detail in the EFSA Scientific Opinion 2799 (2012) which also includes the concept of the threshold of toxicological concern (TTC) as screening tool for pesticide metabolites. Main "purpose" of the TTC concept is to check whether there is negligible exposure.

In order to propose a suitable residue definition for risk assessment purposes, the pyraclostrobin metabolites found in plant metabolism studies and during processing were grouped in total into six different groups.

- Group 1: Desmethoxy metabolite 500M07 (synonym: BF 500-3)
- Group 2: Chlorphenyl pyrazole derivatives
- Group 3: Anthranilic acid derivatives 500M24 and 500M49
- Group 4: Hydroxylated metabolites (chlorphenyl pyrazole moiety)
- Group 5: Hydroxylated metabolites (tolyl moiety)
- Group 6: Photo metabolite 500M76

The dietary exposure for each group was assessed separately for identifying the contributions of the plant metabolites to the total dietary risk. Due to obvious reasons no assessment has been performed for the metabolite 500M72 which has been identified as the natural amino acid tryptophan. Due to their absence in succeeding and root crops even at exaggerated rates, the same applies for the aerobic soil metabolites 500M01 and 500M02. The assessments were limited to those target crops from which a contribution to the dietary risk could be expected.

For deriving residue levels to be used in chronic and acute exposure assessments, the following studies were additionally considered:

- Magnitude of residue studies in target crops (supervised field trials)
- Magnitude of residues in processed commodities

In case of group 1 and 2, residue data from supervised field trials are available, whereas for other groups the assessments are based on metabolite / parent ratios. The relevant chronic and acute exposure assessments for plant commodities are summarized more in detail in section 6.9. For groups 1, 4 and 5 the exposure was assessed against the endpoints of the parent molecule as the metabolites were found in significant amounts in the rat metabolism study (see M-CA 5.1). For group 2, several toxicological studies have been performed with the metabolite 500M04. For groups 3 and 6, the assessment is based on acute and chronic Cramer class III endpoints after proving the absence of genotoxicity (see M-CA 5.8). The individual contributions of the metabolites to the dietary risk are summarized in the table below.

Table 6.7.1-2: Contribution of pyraclostrobin plant metabolites to chronic dietary risk

Metabolite group / Metabolites	ADI used [mg/kg bw / day]	ADI utilization [%]	Comment
1 - 500M07	0.03	< 2%	Indicative assessment covering all target crops (worst case)
2 - 500M04 and conjugated	0.52	< 0.1%	
3 - 500M24	0.0015 (TTC)	< 9%	Cereal grain
3 - 500M49	0.0015 (TTC)	0.1%	Oilseeds
3 - 500M51	0.0015 (TTC)	n.a.	Metabolite only found in livestock metabolism studies
4 - Hydroxylated metabolites (chlorphenyl pyrazole moiety)	0.03	0.6%	
5 - Hydroxylated metabolites (tolyl moiety)	0.03	0.6%	
6 - 500M76	0.0015 (TTC)	0.2%	

Table 6.7.1-3: Contribution of pyraclostrobin plant metabolites to acute dietary risk

Metabolite group / Metabolites	ARfD used [mg/kg bw / day]	ARfD utilization [%]	Comment
1 - 500M07	0.03	Max. 30% (celery, onions)	Refined for table grapes, apples and scarole
2 - 500M04 and conjugated	n.a.	n.a.	
3 - 500M24	0.005 (TTC)	Not relevant	Metabolite only found in wheat metabolism study (grain)
3 - 500M49	0.005 (TTC)	0%	
3 - 500M51	0.005 (TTC)	n.a.	Metabolite only found in livestock metabolism studies
4 - Hydroxylated metabolites (chlorphenyl pyrazole moiety)	0.03	8% (melons) 7% (water melons) 5% (table grapes)	Refined for table grapes, apples and scarole
5 - Hydroxylated metabolites (tolyl moiety)	0.03	11% kale 8% (melons) 7% (water melons)	Refined for table grapes, apples and scarole
6 - 500M76	0.005 (TTC)	15% (kale) 9% (globe artichokes) 8% (Chinese cabbage)	Refined for scarole

The data show that the contributions of the metabolites to the dietary risk are small even under unrealistic worst case assumptions. None of the groups should be included in the residue definition for dietary risk assessment. Due to the favorable outcome, no further refinement than indicated above was performed (e.g. by inclusion of processing factors for vegetable crops which are always cooked prior to consumption).

Following an indicative assessment based on an in-depth analysis of all metabolism, residue and processing fraction studies, the residue definition shown below is proposed for risk assessment in plant commodities (including process fractions thereof):

Pyraclostrobin, parent only

Animal matrices

For deriving a suitable residue definition for food of animal origin, the following studies were considered:

- Metabolism study in hens
- Metabolism study in goats (ruminants)
- Metabolism studies in fish (dietary)

For pyraclostrobin, all relevant studies are available.

In general, pyraclostrobin follows a common pathway in different livestock species, which is also comparable to the one observed in rats. As in plant matrices, the following metabolic conversion steps were observed in the relevant studies:

- Desmethoxylation of the side chain resulting in the metabolite 500M07
- Cleavage between the ring systems resulting in the metabolites 500M04, 500M05 and 500M85
- Cleavage between the ring systems resulting in the metabolites 500M49 and 500M51
- Hydroxylation of the chlorophenyl pyrazole moiety (followed by conjugation)
- Hydroxylation of the tolyl moiety (followed by conjugation)

Besides these steps, in fish the metabolite 500M89 was identified in very low amounts; it is formed by desmethylation.

In the majority of samples investigated (poultry fat / eggs, goat milk / meat / fat), the parent molecule forms a predominant part of the residue followed by the metabolite 500M07. In milk, the cleavage products 500M04 / 500M05 are in equal amounts present than parent. Especially in goat liver, but also kidney, relatively high amounts of radioactivity were incorporated into tissues which could be at least partly released by enzymatic techniques. After applying harsh conditions (refluxing with hydrochloric acid), the metabolites 500M04 and 500M85 were detected in the hydrolyzate.
All other metabolites are present in significantly lower amounts, but also not consistently in all livestock commodities.

Residue definition for monitoring purposes

For the residue definition in animal commodities the same criteria apply as for plants. The analyte(s) to be selected for monitoring purposes should occur in large quantities, and should be common to all commodities in which residues are expected. Ideally, the monitoring method should be based on one single analyte (‘marker or indicator compound’).

In case of pyraclostrobin and its metabolites in food of animal origin, there is only one component which meets most of the criteria listed in the OECD guidance document. Based on the studies available where unchanged pyraclostrobin formed a considerable part of the residue in food items being highly consumed (milk for small children, meat / fat for all subpopulation groups), the following residue definition is proposed for monitoring purposes in animal commodities:

Pyraclostrobin, parent only

Residue definition for data generation / risk assessment purposes

As in plants, the derivation of a suitable residue definition for risk assessment purposes is much more complex; according to the OECD guidance document the contribution of each metabolite/ degradates to a potential dietary risk need to be considered.

In order to propose a suitable residue definition for risk assessment purposes, the pyraclostrobin metabolites found in livestock metabolism studies were grouped in total into five different groups. Except group 6 ("photo metabolite"), the grouping followed the same principles as for plants.

- Group 1: Desmethoxy metabolite 500M07 (synonym: BF 500-3)
- Group 2: Chlorphenyl pyrazole derivatives
- Group 3: Anthranilic acid derivatives 500M49 and 500M51
- Group 4: Hydroxylated metabolites (chlorphenyl pyrazole moiety)
- Group 5: Hydroxylated metabolites (tolyl moiety)

The dietary exposure for each group was assessed separately for identifying the contributions of the livestock metabolites to the total dietary risk. For deriving residue levels to be used in chronic and acute exposure assessments, the following studies were additionally considered:

- Magnitude of residue study in cows
- Magnitude of residue study in hens

For deriving the residue levels for risk assessment purposes, the dietary feed burden calculations were fully considered (see M-CA 6.7.2). As the metabolite 500M89 was only found in trace amounts in fish (about 0.01 mg/kg at a 8 x feeding level), no exposure assessment has been carried out.

In case of groups 1 and 2 (plus partly 5), residue data from the livestock feeding studies are available. In the cow feeding study, the samples were analyzed with a "parent method", but also a method including a common moiety approach. The common moiety approach is based on the hydrolytic degradation of pyraclostrobin and its metabolites to 500M04 and 500M85. In poultry, a similar approach was selected; however, it covers a different hydroxylation pattern.

For the other groups the assessments are based on metabolite / parent ratios. The relevant chronic and acute exposure assessments for plant commodities are summarized more in detail in M-CA 6.9. For groups 1, 4 and 5 the exposure was assessed against the endpoints of the parent molecule as the metabolites were found in significant amounts in the rat metabolism study (see M-CA 5.1). For group 2, several toxicological studies have been performed with the metabolite 500M04. For group 3, the assessment is based on acute and chronic Cramer class III endpoints after proving the absence of genotoxicity (see M-CA 5.8). The individual contributions of the metabolites to the dietary risk are summarized in the table below.

Table 6.7.1-4: Contribution of pyraclostrobin livestock metabolites to chronic dietary risk

Metabolite group / Metabolites	ADI used [mg/kg bw / day]	ADI utilization [%]	Comment
1 - 500M07	0.03	< 0.1%	
2 - 500M04 and conjugates	0.52	< 0.1%	
3 - 500M24	0.0015 (TTC)	n.a.	Metabolite only found in wheat metabolism study (grain)
3 - 500M49	0.0015 (TTC)	0%	
3 - 500M51	0.0015 (TTC)	0%	
4 - Hydroxylated metabolites (chlorphenyl pyrazole moiety)	0.03	0.1%	
5 - Hydroxylated metabolites (tolyl moiety)	0.03	< 0.1%	
6 - 500M76	0.0015 (TTC)	n.a.	Metabolite only found in wheat metabolism study (forage)

Table 6.7.1-5: Contribution of pyraclostrobin livestock metabolites to acute dietary risk

Metabolite group / Metabolites	ARfD used [mg/kg bw / day]	ARfD utilization [%]	Comment
1 - 500M07	0.03		
2 - 500M04 and conjugates	n.a.	n.a.	
3 - 500M24	0.005 (TTC)	n.a.	Metabolite only found in wheat metabolism study (grain)
3 - 500M49	0.005 (TTC)	Max. 0.2% (poultry liver)	
3 - 500M51	0.005 (TTC)	Max. 0.2% (bovine kidney)	
4 - Hydroxylated metabolites (chlorphenyl pyrazole moiety)	0.03	< 1% (milk)	
5 - Hydroxylated metabolites (tolyl moiety)	0.03	< 0.5% (milk)	
6 - 500M76	0.005 (TTC)	n.a.	Metabolite only found in wheat metabolism study (forage)

The data demonstrate that the contributions of the individual metabolites to the dietary risk at realistic dose levels are even smaller than for parent.

The proposal below is based on the indicative assessment, but also takes the findings of the cow feeding study into account where residues of 500M04 and 500M85 were found in liver (realistic feed burden), milk and kidney (10 x to 20 x). Accordingly, the following residue definition is proposed for risk assessment in animal commodities:

Pyraclostrobin and its metabolites analysed as the hydroxypyrazoles 500M04 (synonym BF 500-5) and 500M85 (synonym BF 500-8), sum expressed as pyraclostrobin

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

The following table shows the current EU MRLs for pyraclostrobin (mg/kg) as of May 7, 2014 (source: DG Sanco website http://ec.europa.eu/sanco_pesticides/public/index.cfm?event =substance.resultat&s=1**)**. * indicates the lower limit of analytical determination and (ft) that a footnote is given at the end of the table.

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
100000	1. FRUIT FRESH OR FROZEN NUTS	
110000	(i) Citrus fruit	
110010	Grapefruit (Shaddocks, pomelos, sweeties, tangelo (except mineola), ugli and other hybrids)	1
110020	Oranges (Bergamot, bitter orange, chinotto and other hybrids)	2
110030	Lemons (Citron, lemon, Buddha's hand (Citrus medica var. sarcodactylis))	1
110040	Limes	1
110050	Mandarins (Clementine, tangerine, mineola and other hybrids tangor (Citrus reticulata x sinensis))	1
110990	Others	1
120000	(ii) Tree nuts	
120010	Almonds	0.02*
120020	Brazil nuts	0.02*
120030	Cashew nuts	0.02*
120040	Chestnuts	0.02*
120050	Coconuts	0.02*
120060	Hazelnuts (Filbert)	0.02*
120070	Macadamia	0.02*
120080	Pecans	0.02*
120090	Pine nuts	0.02*
120100	Pistachios	1
120110	Walnuts	0.02*
120990	Others	0.02*

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
130000	(iii) Pome fruit	0.5
130010	Apples (Crab apple)	0.5
130020	Pears (Oriental pear)	0.5
130030	Quinces	0.5
130040	Medlar	0.5
130050	Loquat	0.5
130990	Others	0.5
140000	(iv) Stone fruit	
140010	Apricots	1
140020	Cherries (Sweet cherries, sour cherries)	3
140030	Peaches (Nectarines and similar hybrids)	0.3
140040	Plums (Damson, greengage, mirabelle, sloe, red date/Chinese date/Chinese jujube (Ziziphus zizyphus))	0.8
140990	Others	0.02*
150000	(v) Berries & small fruit	
151000	(a) Table and wine grapes	
151010	Table grapes	1 (ft)
151020	Wine grapes	2
152000	(b) Strawberries	1.5
153000	(c) Cane fruit	
153010	Blackberries	3
153020	Dewberries (Loganberries, tayberries, boysenberries, cloudberries and other Rubus hybrids)	2
153030	Raspberries (Wineberries, arctic bramble/raspberry, (Rubus arcticus), nectar raspberries (Rubus arcticus x Rubus idaeus))	3
153990	Others	2

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
154000	(d) Other small fruit & berries	
154010	Blueberries (Bilberries)	4
154020	Cranberries (Cowberries/red bilberries (V. vitis-idaea))	3
154030	Currants (red, black and white)	3
154040	Gooseberries (Including hybrids with other Ribes species)	3
154050	Rose hips	3
154060	Mulberries (Arbutus berry)	3
154070	Azarole (mediteranean medlar) (Kiwiberry (Actinidia arguta))	3
154080	Elderberries (Black chokeberry/appleberry, mountain ash, buckthorn/sea sallowthorn, hawthorn, serviceberries, and other treeberries)	3
154990	Others	3
160000	(vi) Miscellaneous fruit	
161000	(a) Edible peel	0.02*
161010	Dates	0.02*
161020	Figs	0.02*
161030	Table olives	0.02*
161040	Kumquats (Marumi kumquats, nagami kumquats, limequats (Citrus aurantifolia x Fortunella spp.))	0.02*
161050	Carambola (Bilimbi)	0.02*
161060	Persimmon	0.02*
161070	Jambolan (java plum) (Java apple/water apple, pomerac, rose apple, Brazilean cherry, Surinam cherry/grumichama (Eugenia uniflora))	0.02*
161990	Others	0.02*
162000	(b) Inedible peel, small	0.02*
162010	Kiwi	0.02*
162020	Lychee (Litchi) (Pulasan, rambutan/hairy litchi, longan, mangosteen, langsat, salak)	0.02*
162030	Passion fruit	0.02*
162040	Prickly pear (cactus fruit)	0.02*
162050	Star apple	0.02*
162060	American persimmon (Virginia kaki) (Black sapote, white sapote, green sapote, canistel/yellow sapote, mammey sapote)	0.02*
162990	Others	0.02*

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
163000	(c) Inedible peel, large	
163010	Avocados	0.02*
163020	Bananas (Dwarf banana, plantain, apple banana)	0.02*
163030	Mangoes	0.05
163040	Papaya	0.07
163050	Pomegranate	0.02*
163060	Cherimoya (Custard apple, sugar apple/sweetsop, ilama (Annona diversifolia) and other medium sized Annonaceae fruits)	0.02*
163070	Guava (Red pitaya/dragon fruit (Hylocereus undatus))	0.02*
163080	Pineapples	0.02*
163090	Bread fruit (Jackfruit)	0.02*
163100	Durian	0.02*
163110	Soursop (guanabana)	0.02*
163990	Others	0.02*
200000	2. VEGETABLES FRESH OR FROZEN	
210000	(i) Root and tuber vegetables	
211000	(a) Potatoes	0.02*
212000	(b) Tropical root and tuber vegetables	0.02*
212010	Cassava (Dasheen, eddoe/Japanese taro, tannia)	0.02*
212020	Sweet potatoes	0.02*
212030	Yams (Potato bean/yam bean, Mexican yam bean)	0.02*
212040	Arrowroot	0.02*
212990	Others	0.02*

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
213000	(c) Other root and tuber vegetables except sugar beet	
213010	Beetroot	0.1
213020	Carrots	0.5
213030	Celeriac	0.3
213040	Horseradish (Angelica roots, lovage roots, gentiana roots)	0.3
213050	Jerusalem artichokes (Crosne)	0.06
213060	Parsnips	0.3
213070	Parsley root	0.1
213080	Radishes (Black radish, Japanese radish, small radish and similar varieties, tiger nut (Cyperus esculentus))	0.5
213090	Salsify (Scorzonera, Spanish salsify/Spanish oysterplant, edible burdock)	0.1
213100	Swedes	0.02*
213110	Turnips	0.02*
213990	Others	0.02*
220000	(ii) Bulb vegetables	
220010	(a) Garlic	0.3
220020	(b) Onions (Other bulb onions, silverskin onions)	1.5
220030	(c) Shallots	0.3
220040	(d) Spring onions and welsh onions (Other green onions and similar varieties)	1.5
220990	(e) Others	0.02*
230000	(iii) Fruiting vegetables	
231000	(a) Solanacea	
231010	Tomatoes (Cherry tomatoes, Physalis spp., gojiberry, wolfberry (Lycium barbarum and L. chinense), tree tomato)	0.3
231020	Peppers (Chilli peppers)	0.5
231030	Aubergines (egg plants) (Pepino, antroewa/white eggplant (S. macrocarpon))	0.3
231040	Okra (lady's fingers)	0.02*
231990	Others	0.02*

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
232000	(b) Cucurbits — edible peel	0.5
232010	Cucumbers	0.5
232020	Gherkins	0.5
232030	Courgettes (Summer squash, marrow (patisson), lauki (Lagenaria siceraria), chayote, sopropo/bitter melon, snake gourd, angled luffa/teroi)	0.5
232990	Others	0.5
233000	(c) Cucurbits-inedible peel	0.5
233010	Melons (Kiwano)	0.5
233020	Pumpkins (Winter squash, marrow (late variety))	0.5
233030	Watermelons	0.5
233990	Others	0.5
234000	(d) Sweet corn (Baby corn)	0.02*
239000	(e) Other fruiting vegetables	0.02*
240000	(iv) Brassica vegetables	
241000	(a) Flowering brassica	0.1
241010	Broccoli (Calabrese, Broccoli raab, Chinese broccoli)	0.1
241020	Cauliflower	0.1
241990	Others	0.1
242000	(b) Head brassica	
242010	Brussels sprouts	0.3
242020	Head cabbage (Pointed head cabbage, red cabbage, savoy cabbage, white cabbage)	0.2
242990	Others	0.02*
243000	(c) Leafy brassica	1.5
243010	Chinese cabbage (Indian or Chinese) mustard, pak choi, Chinese flat cabbage/ai goo choi), choi sum, Peking cabbage/pe-tsai)	1.5
243020	Kale (Borecole/curly kale, collards, Portuguese Kale, Portuguese cabbage, cow cabbage)	1.5
243990	Others	1.5
244000	(d) Kohlrabi	0.02*

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
250000	(v) Leaf vegetables & fresh herbs	
251000	(a) Lettuce and other salad plants including Brassicacea	
251010	Lamb's lettuce (Italian corn salad)	10
251020	Lettuce (Head lettuce, lollo rosso (cutting lettuce), iceberg lettuce, romaine (cos) lettuce)	2
251030	Scarole (broad-leaf endive) (Wild chicory, red-leaved chicory, radicchio, curly leaf endive, sugar loaf (C. endivia var. crispum/C. intybus var. foliosum), dandelion greens)	0.4
251040	Cress (Mung bean sprouts, alfalfa sprouts)	10
251050	Land cress	10
251060	Rocket, Rucola (Wild rocket (Diplotaxis spp.))	10
251070	Red mustard	10
251080	Leaves and sprouts of Brassica spp, including turnip greens (Mizuna, leaves of peas and radish and other babyleaf crops, including brassica crops (crops harvested up to 8 true leaf stage), kohlrabi leaves)	10
251990	Others	10
252000	(b) Spinach & similar (leaves)	
252010	Spinach (New Zealand spinach, amaranthus spinach (pak-khom, tampara), tajer leaves, bitterblad/bitawiri)	0.5
252020	Purslane (Winter purslane/miner's lettuce, garden purslane, common purslane, sorrel, glassworth, agretti (Salsola soda))	0.02*
252030	Beet leaves (chard) (Leaves of beetroot)	0.5
252990	Others	0.02*
253000	(c) Vine leaves (grape leaves) (Malabar nightshade, banana leaves, climbing wattle (Acacia pennata))	0.02*
254000	(d) Water cress (Morning glory/Chinese convolvulus/water convolvulus/water spinach/kangkung (Ipomea aquatica), water clover, water mimosa)	0.02*
255000	(e) Witloof	0.02*

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
256000	(f) Herbs	2
256010	Chervil	2
256020	Chives	2
256030	Celery leaves (Fennel leaves, coriander leaves, dill leaves, caraway leaves, lovage, angelica, sweet cisely and other Apiacea leaves, culantro/stinking/long coriander/stink weed (Eryngium foetidum))	2
256040	Parsley (leaves of root parsley)	2
256050	Sage (Winter savory, summer savory, Borago officinalis leaves)	2
256060	Rosemary	2
256070	Thyme (Marjoram, oregano)	2
256080	Basil (Balm leaves, mint, peppermint, holy basil, sweet basil, hairy basil, edible flowers (marigold flower and others), pennywort, wild betel leaf, curry leaves)	2
256090	Bay leaves (laurel) (Lemon grass)	2
256100	Tarragon (Hyssop)	2
256990	Others	2
260000	(vi) Legume vegetables (fresh)	0.02*
260010	Beans (with pods) (Green bean/French beans/snap beans, scarlet runner bean, slicing bean, yard long beans, guar beans, soya beans)	0.02*
260020	Beans (without pods) (Broad beans, flageolets, jack bean, lima bean, cowpea)	0.02*
260030	Peas (with pods) (Mangetout/sugar peas/snow peas)	0.02*
260040	Peas (without pods) (Garden pea, green pea, chickpea)	0.02*
260050	Lentils	0.02*
260990	Others	0.02*

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
270000	(vii) Stem vegetables (fresh)	
270010	Asparagus	0.02*
270020	Cardoons (Borago officinalis stems)	0.02*
270030	Celery	0.02* (ft)
270040	Fennel	0.02*
270050	Globe artichokes (Banana flower)	2
270060	Leek	0.7
270070	Rhubarb	0.02*
270080	Bamboo shoots	0.02*
270090	Palm hearts	0.02*
270990	Others	0.02*
280000	(viii) Fungi	0.02*
280010	Cultivated fungi (Common mushroom, oyster mushroom, shiitake, fungus mycelium (vegetative parts))	0.02*
280020	Wild fungi (Chanterelle, truffle, morel, cep)	0.02*
280990	Others	0.02*
290000	(ix) Sea weeds	0.02*
300000	3. PULSES, DRY	
300010	Beans (Broad beans, navy beans, flageolets, jack beans, lima beans, field beans, cowpeas)	0.3
300020	Lentils	0.5
300030	Peas (Chickpeas, field peas, chickling vetch)	0.3
300040	Lupins	0.05
300990	Others	0.3

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
400000	4. OILSEEDS AND OILFRUITS	
401000	(i) Oilseeds	
401010	Linseed	0.2
401020	Peanuts	0.04
401030	Poppy seed	0.2
401040	Sesame seed	0.2
401050	Sunflower seed	0.3
401060	Rape seed (Bird rapeseed, turnip rape)	0.2
401070	Soya bean	0.05
401080	Mustard seed	0.2
401090	Cotton seed	0.3
401100	Pumpkin seeds (Other seeds of Cucurbitaceae)	0.02*
401110	Safflower	0.2
401120	Borage (Purple viper's bugloss/Canary flower (Echium plantagineum), Corn Gromwell (Buglossoides arvensis))	0.2
401130	Gold of pleasure	0.2
401140	Hempseed	0.02*
401150	Castor bean	0.2
401990	Others	0.02*
402000	(ii) Oilfruits	0.02*
402010	Olives for oil production	0.02*
402020	Palm nuts (palmoil kernels)	0.02*
402030	Palmfruit	0.02*
402040	Kapok	0.02*
402990	Others	0.02*

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
500000	5. CEREALS	
500010	Barley	1
500020	Buckwheat (Amaranthus, quinoa)	0.02*
500030	Maize	0.02*
500040	Millet (Foxtail millet, teff, finger millet, pearl millet)	0.02*
500050	Oats	1
500060	Rice (Indian/wild rice (Zizania aquatica))	0.02*
500070	Rye	0.2
500080	Sorghum	0.5
500090	Wheat (Spelt, triticale)	0.2
500990	Others (Canary grass seeds (Phalaris canariensis))	0.02*
600000	6. TEA, COFFEE, HERBAL INFUSIONS AND COCOA	
610000	(i) Tea	0.1*
620000	(ii) Coffee beans	0.3 (ft)
630000	(iii) Herbal infusions (dried)	0.1*
631000	(a) Flowers	0.1*
631010	Camomille flowers	0.1*
631020	Hybiscus flowers	0.1*
631030	Rose petals	0.1*
631040	Jasmine flowers (Elderflowers (Sambucus nigra))	0.1*
631050	Lime (linden)	0.1*
631990	Others	0.1*
632000	(b) Leaves	0.1*
632010	Strawberry leaves	0.1*
632020	Rooibos leaves (Ginkgo leaves)	0.1*
632030	Maté	0.1*
632990	Others	0.1*
633000	(c) Roots	0.1*
633010	Valerian root	0.1*
633020	Ginseng root	0.1*
633990	Others	0.1*
639000	(d) Other herbal infusions	0.1*
640000	(iv) Cocoabeans (fermented or dried)	0.1*
650000	(v) Carob (st johns bread)	0.1*
700000	7. HOPS (dried)	15

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
800000	8. SPICES	
810000	(i) Seeds	0.1*
810010	Anise	0.1*
810020	Black caraway	0.1*
810030	Celery seed (Lovage seed)	0.1*
810040	Coriander seed	0.1*
810050	Cumin seed	0.1*
810060	Dill seed	0.1*
810070	Fennel seed	0.1*
810080	Fenugreek	0.1*
810090	Nutmeg	0.1*
810990	Others	0.1*
820000	(ii) Fruits and berries	0.1*
820010	Allspice	0.1*
820020	Sichuan pepper (Anise pepper, Japan pepper)	0.1*
820030	Caraway	0.1*
820040	Cardamom	0.1*
820050	Juniper berries	0.1*
820060	Pepper, black, green and white (Long pepper, pink pepper)	0.1*
820070	Vanilla pods	0.1*
820080	Tamarind	0.1*
820990	Others	0.1*
830000	(iii) Bark	0.1*
830010	Cinnamon (Cassia)	0.1*
830990	Others	0.1*
840000	(iv) Roots or rhizome	
840010	Liquorice	0.1*
840020	Ginger	0.1*
840030	Turmeric (Curcuma)	0.1*
840040	Horseradish	(ft)
840990	Others	0.1*
850000	(v) Buds	0.1*
850010	Cloves	0.1*
850020	Capers	0.1*
850990	Others	0.1*

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
860000	(vi) Flower stigma	0.1*
860010	Saffron	0.1*
860990	Others	0.1*
870000	(vii) Aril	0.1*
870010	Mace	0.1*
870990	Others	0.1*
900000	9. SUGAR PLANTS	
900010	Sugar beet (root)	0.2
900020	Sugar cane	0.02*
900030	Chicory roots	0.02*
900990	Others	0.02*
1000000	10. PRODUCTS OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS	
1010000	(i) Tissue	0.05*
1011000	(a) Swine	0.05*
1011010	Muscle	0.05*
1011020	Fat	0.05*
1011030	Liver	0.05*
1011040	Kidney	0.05*
1011050	Edible offal	0.05*
1011990	Others	0.05*
1012000	(b) Bovine	0.05*
1012010	Muscle	0.05*
1012020	Fat	0.05*
1012030	Liver	0.05*
1012040	Kidney	0.05*
1012050	Edible offal	0.05*
1012990	Others	0.05*
1013000	(c) Sheep	0.05*
1013010	Muscle	0.05*
1013020	Fat	0.05*
1013030	Liver	0.05*
1013040	Kidney	0.05*
1013050	Edible offal	0.05*
1013990	Others	0.05*

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
1014000	(d) Goat	0.05*
1014010	Muscle	0.05*
1014020	Fat	0.05*
1014030	Liver	0.05*
1014040	Kidney	0.05*
1014050	Edible offal	0.05*
1014990	Others	0.05*
1015000	(e) Horses, asses, mules or hinnies	0.05*
1015010	Muscle	0.05*
1015020	Fat	0.05*
1015030	Liver	0.05*
1015040	Kidney	0.05*
1015050	Edible offal	0.05*
1015990	Others	0.05*
1016000	(f) Poultry chicken, geese, duck, turkey and Guinea fowl, ostrich, pigeon	0.05*
1016010	Muscle	0.05*
1016020	Fat	0.05*
1016030	Liver	0.05*
1016040	Kidney	0.05*
1016050	Edible offal	0.05*
1016990	Others	0.05*
1017000	(g) Other farm animals (Rabbit, kangaroo, deer)	0.05*
1017010	Muscle	0.05*
1017020	Fat	0.05*
1017030	Liver	0.05*
1017040	Kidney	0.05*
1017050	Edible offal	0.05*
1017990	Others	0.05*
1020000	(ii) Milk	0.01*
1020010	Cattle	0.01*
1020020	Sheep	0.01*
1020030	Goat	0.01*
1020040	Horse	0.01*
1020990	Others	0.01*

Table 6.7.2-1: EU MRLs set for the uses of pyraclostrobin

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
1030000	(iii) Bird eggs	0.05*
1030010	Chicken	0.05*
1030020	Duck	0.05*
1030030	Goose	0.05*
1030040	Quail	0.05*
1030990	Others	0.05*
1040000	(iv) Honey (Royal jelly, pollen, honey comb with honey (comb honey))	0.05*
1050000	(v) Amphibians and reptiles (Frog legs, crocodiles)	0.05*
1060000	(vi) Snails	0.05*
1070000	(vii) Other terrestrial animal products (Wild game)	0.05*

Footnotes:

Pyraclostrobin (F)
(F) = Fat soluble

0151010 Table grapes
The European Food Safety Authority identified some information on residue trials as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 13 July 2015, or, if that information is not submitted by that date, the lack of it.

0270030 Celery
The European Food Safety Authority identified some information on residue trials as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 13 July 2015, or, if that information is not submitted by that date, the lack of it.

0620000 (ii) Coffee beans
The European Food Safety Authority identified some information on analytical methods as unavailable. When re-viewing the MRL, the Commission will take into account the information referred to in the first sentence, if it is submitted by 13 July 2015, or, if that information is not submitted by that date, the lack of it.

0840040 Horseradish
The applicable maximum residue level for horseradish (Armoracia rusticana) in the spice group (code 0840040) is the one set for horseradish (Armoracia rusticana) in the vegetables category, root and tuber vegetables group (code 0213040) taking into account changes in the levels by processing (drying) according to Art. 20 (1) of Regulation (EC) No 396/2005.

Plant Matrices

For pyraclostrobin MRLs are established in multiple crops. In order to support the renewal of approval for pyraclostrobin, additional residue trials have been performed in the intended uses (potatoes, cereals, maize). In the chapters below, the new data are evaluated using statistical means and compared with the data being included in the most recent EFSA Reasoned Opinions. For the majority of crops, the assessments performed in context of the Reasoned Opinions are covering the new data. In case of cereal feed items and sweet corn, higher residues were detected and considered in the feed burden calculation and the dietary exposure assessment.

Potatoes

In 2003, in total six trials were performed with the formulation BAS 516 00 F. In these trials, an application rate of 20 g as/ha was applied 4 times.

In the growing season 2005, in total ten field trials were performed in Northern Europe (Germany, Belgium, Denmark and Northern France) and Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulation BAS 536 01 F according to the critical GAP of this formulation (± 25%).

In the growing season 2015, in total four field trials were performed in Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulation BAS 516 07 F according to the critical GAP of this formulation (± 25%).

For deriving a MRL proposal, the more critical GAP which is applied with BAS 536 01 F was considered.

The following parent compound residues were found in potato tuber samples at a PHI of 7 days (Northern EU) and at a PHI of 3 days (Southern EU):

Northern Europe (n=5): 5x <0.02 mg/kg

Southern Europe (n=5): 5x <0.02 mg/kg

In none of the trials any detectable residue above the limit of quantitation (LOQ) was found. As all residue levels in potato tubers were below the LOQ of 0.02 mg/kg, it is not needed to perform any statistical calculation to derive an MRL.

Based on the findings of the residue trials it is proposed to keep the established EU MRL for potato tubers at the limit of quantitation of the enforcement method which corresponds to 0.02 mg/kg.

Code number 0211000 (potatoes): 0.02 mg/kg (default MRL)

Barley

In the growing seasons 2009 - 2012, in total 28 field trials comprising 36 plots were performed in Northern Europe (Germany, The Netherlands, Denmark, Northern France and The United Kingdom) and Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulations BAS 500 06 F, BAS 702 01 F and BAS 703 04 F according to the critical GAP of formulation BAS 500 06 F (± 25%).

The following parent compound residues were found in barley grain samples at a PHI of 35 days or later:

Northern Europe (n=18): 0.02, 0.039, 0.043, 0.056, 0.06, 0.065, 0.07 (2x), 0.08, 0.09 (2x), 0.10, 0.11, 0.21, 0.22, 0.25, 0.35, 0.82 mg/kg

Southern Europe (n=18): <0.01 (3x), 0.02 (2x), 0.029, 0.03 (3x), 0.039, 0.06, 0.09, 0.11, 0.13, 0.14, 0.15, 0.27, 0.54 mg/kg

For MRL calculation the current version of the OECD MRL calculator was used. In order to derive the most robust MRL, all trials were considered.

Table 6.7.2-2: MRL calculation for barley grain in the EU for pyraclostrobin based on parent residues (mg/kg)

	Pyraclostrobin [mg/kg]
	North (n=18)
STMR	0.085
HR	0.82
OECD	**0.9**
	South (n=18)
STMR	0.035
HR	0.54
OECD	**0.7**

The data show, that the calculated MRL for pyraclostrobin in barley grain is covered by the current MRL of 1.0 mg/kg. Therefore, BASF proposes to keep the current EU MRL of pyraclostrobin at 1.0 mg/kg for barley grain.

Code number 0500010 (barley): 1.0 mg/kg

Based on the extrapolation rules of the EU guidance document "Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs" (SANCO 7525/VI/95 rev. 9, March 2011), the same MRL should apply for oat grain:

Code number 0500050 (oats): 1.0 mg/kg

Barley straw (feed item)

Despite MRLs are not yet set for animal feed, the residue data summarized in M-CA 6.3 has been used for deriving a *"pseudo MRL"*. The relevant STMR and HR vales can be used for deriving a feed burden and subsequently, a MRL proposal for food of animal origin.

In the growing seasons 2009 - 2012, in total 28 field trials comprising 36 plots were performed in Northern Europe (Germany, The Netherlands, Denmark, Northern France and The United Kingdom) and Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulations BAS 500 06 F, BAS 702 01 F and BAS 703 04 F according to the critical GAP of formulation BAS 500 06 F (± 25%).

The following parent compound residues were found in barley straw samples at a PHI of 35 days or later:

Northern Europe (n=18): 0.50, 0.93, 1.17, 1.28, 1.4, 1.9, 2.09, 2.1, 2.42, 2.5, 2.61, 2.75, 2.8, 2.9, 3.98, 4.1, 6.3, 8.73 mg/kg

Southern Europe (n=18): <0.01, 0.56, 0.77, 0.79, 0.81, 0.98, 1.04, 1.2, 1.3, 1.94, 2.1, 2.42, 2.87, 3.05, 3.36, 3.53, 3.79, 4.24 mg/kg

For MRL calculation the current version of the OECD MRL calculator was used.

Table 6.7.2-3: MRL calculation for barley straw in the EU for pyraclostrobin based on parent residues (mg/kg)

	Pyraclostrobin [mg/kg]
	North (n=18)
STMR	2.46
HR	8.73
OECD	**15**
	South (n=18)
STMR	1.62
HR	4.24
OECD	**7**

The data show, that the calculated MRL for pyraclostrobin in barley straw is covered by the current *"pseudo MRL"* of 15 mg/kg (see MRL review for pyraclostrobin according to Article 12, EFSA Journal 2011;9(8):2344). Therefore, BASF proposes to keep the current value of pyraclostrobin at 15 mg/kg for barley and oat straw.

The following values were published by EFSA, see EFSA Journal 2011;9(8):2344 (page 37):

Northern EU → STMR = 2.20 mg/kg
HR = 6.0 mg/kg
MRL Proposal = 10 mg/kg

Southern EU → STMR = 3.38 mg/kg
HR = 6.92 mg/kg
MRL Proposal = 15 mg/kg

Sweet corn (extrapolation from maize "cobs without husks")

According to the commodity definition of sweet corn (= cobs without husks) as defined in Regulation (EC) No 396/2005, the relevant residue values from cobs without husks specimens sampled at about BBCH 85 were considered for MRL calculation. Sweet corn is defined as a minor crop (SANCO 7525/VI/95 rev.9) requiring a minimum of 4 trials per residue zone.

In the growing seasons 2012 and 2013, six trials were performed in Northern Europe (Germany, The Netherlands, The United Kingdom, Northern France and Belgium) and four were performed in Southern Europe (Italy, Spain, Greece and Southern France). The following parent compound residues were found in the maize cobs without husks samples collected at about BBCH 85:

Northern Europe (n=6): <0.01 (3x), 0.011, 0.015, 0.039 mg/kg

Southern Europe (n=4): <0.01 (4x) mg/kg

For MRL calculation the current version of the OECD MRL calculator was used.

Table 6.7.2-4: MRL calculation for sweet corn in the EU for pyraclostrobin based on parent residues (mg/kg)

	Pyraclostrobin [mg/kg]
	North (n=6)
STMR	0.011
HR	0.039
OECD	**0.07 ***
	South (n=4)
STMR	0.01 (all residue values <0.01 mg/kg)
HR	0.01 (all residue values <0.01 mg/kg)
OECD	**0.01 (all residue values <0.01 mg/kg)**

* High uncertainty of MRL estimate due to small dataset

The data show, that the calculated MRL for pyraclostrobin in sweet corn after application of formulation BAS 500 06 F is not covered by the current MRL of 0.02 mg/kg (default MRL). Therefore, BASF proposes to establish an EU MRL of pyraclostrobin at 0.07 mg/kg for sweet corn.

Code number 0234000 (sweet corn): 0.07 mg/kg

Maize

In the period from 2008 to 2009, in total 16 residue trials were performed in maize in the EU; 8 trials were located in the Northern Region and 8 in the Southern Region. The formulations BAS 512 04 F (133 g/L pyraclostrobin and 50 g/L epoxiconazole, SE) and BAS 500 06 F (200 g/L of pyraclostrobin, EC) were applied once at a rate of 1.5 L/ha and 1 L/ha, respectively according to GAP ±25%. Maize grain was collected at crop maturity (BBCH 89) corresponding to 49 - 99 DALA.

Additionally, in the growing seasons 2012 and 2013, five trials were performed in Northern Europe (Germany, The Netherlands, The United Kingdom, Northern France and Belgium) and four were performed in Southern Europe (Italy, Spain, Greece and Southern France). In these trials, formulation BAS 500 06 F was applied according to the critical GAP. Maize grain was collected at crop maturity (BBCH 89) corresponding to 34 - 85 DALA.

The trials fulfill the requirements concerning the geographical and the seasonal distribution. The evaluation of the residue data led to the conclusion that there is no significant difference of the residue behavior regarding different seasons, formulations or regions in the North and South EU.

In none of the trials any detectable residue above the limit of quantitation (LOQ) was found.
As all residue levels in maize grain were below the LOQ of 0.01 mg/kg, it is not needed to perform any statistical calculation to derive an MRL.

Based on the findings of the residue trials it is proposed to keep the MRL for maize grain at the limit of quantitation of the enforcement method which corresponds to 0.02 mg/kg. This MRL is also covering potential imports of maize grain from the NAFTA region.

Code number 0500030: 0.02 mg/kg (default MRL)

Maize (feed item)

Despite MRLs are not yet set for animal feed, the residue data summarized in M-CA 6.3 has been used for deriving a *"pseudo MRL"*. The relevant STMR and HR vales can be used for deriving a feed burden and subsequently, a MRL proposal for food of animal origin.

In the period from 2008 to 2009, in total 16 residue trials were performed in maize in the EU; 8 trials were located in the Northern Region and 8 in the Southern Region. The formulations BAS 512 04 F (133 g/L pyraclostrobin and 50 g/L epoxiconazole, SE) and BAS 500 06 F (200 g/L of pyraclostrobin, EC) were applied once at a rate of 1.5 L/ha and 1 L/ha, respectively according to GAP ±25%. Rest of plant without roots were sampled at 7 - 28 DALA at BBCH 71 - 73, at 16 - 42 DALA at BBCH 75 - 79 and at 30 - 70 DALA at BBCH 85.

Additionally, in the growing seasons 2012 and 2013, five trials were performed in Northern Europe (Germany, The Netherlands, The United Kingdom, Northern France and Belgium) and four were performed in Southern Europe (Italy, Spain, Greece and Southern France). In these trials, formulation BAS 500 06 F was applied according to the critical GAP. Rest of plant without roots were sampled at 15 - 36 DALA at BBCH 75 and at 26 - 56 DALA at BBCH 85.

For the calculation, the highest residues of each trial from all sampling stages except of the 0 DALA sample were used. This approach can be considered as worst case assumption covering also drying effects which might occur during ripening of the crop. The following pyraclostrobin parent residues were used for calculation purposes:

Northern Europe (n=15): 0.11, 0.21, 0.22, 0.25 (2x), 0.28, 0.30, 0.33, 0.36, 0.42, 0.43, 0.44, 0.66, 0.71, 0.84 mg/kg

Southern Europe (n=14): 0.15, 0.18, 0.21 (2x), 0.26, 0.27, 0.29, 0.34, 0.50, 0.57, 0.60, 0.74, 0.76, 0.89 mg/kg

For MRL calculation the current OECD calculator was used.

Table 6.7.2-5: MRL calculation for maize - feed items in the EU for pyraclostrobin based on parent residues (mg/kg)

	Pyraclostrobin [mg/kg]
	North (n=15)
STMR	0.33
HR	0.84
OECD	**1.5**
	South (n=14)
STMR	0.315
HR	0.89
OECD	**1.5**

As a conclusion it is proposed to keep the established pseudo MRL for pyraclostrobin of 1.5 mg/kg in maize feed items (silage), see EFSA Journal 2012;10(3):2606. The residue situation is very comparable in the Northern and in the Southern EU.

The following values were published by EFSA, see EFSA Journal 2012;10(3):2606 (page 11 and 12):

Northern EU → STMR = 0.29 mg/kg
HR = 0.84 mg/kg
MRL Proposal = 1.5 mg/kg

Southern EU → STMR = 0.32 mg/kg
HR = 0.89 mg/kg
MRL Proposal = 1.5 mg/kg

Wheat

In the growing seasons 2010 - 2012, in total 24 field trials comprising 28 plots were performed in Northern Europe (Germany, The Netherlands, Denmark, Northern France and The United Kingdom) and Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulations BAS 500 06 F and BAS 703 04 F according to the critical GAP of formulation BAS 500 06 F (± 25%).

The following parent compound residues were found in wheat grain samples at a PHI of 35 days or later:

Northern Europe (n=14): <0.01 (7x), 0.016, 0.02 (3x), 0.03, 0.039, 0.14 mg/kg

Southern Europe (n=14): <0.01 (7x), 0.01 (2x), 0.011, 0.015, 0.017, 0.02 (2x) mg/kg

For MRL calculation the current version of the OECD MRL calculator was used.

Table 6.7.2-6: MRL calculation for wheat grain in the EU for pyraclostrobin based on parent residues (mg/kg)

	Pyraclostrobin [mg/kg]
	North (n=14)
STMR	0.013
HR	0.14
OECD	**0.2**
	South (n=14)
STMR	0.01
HR	0.02
OECD	**0.03**

The data show, that the calculated MRL for pyraclostrobin in wheat grain is covered by the current MRL of 0.2 mg/kg. Therefore, BASF proposes to keep the current EU MRL of pyraclostrobin at 0.2 mg/kg for wheat grain which covers triticale as well.

Code number 0500090 (wheat): 0.2 mg/kg

Based on the extrapolation rules of the EU guidance document "Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs" (SANCO 7525/VI/95 rev. 9, March 2011), the same MRL should apply for rye grain:

Code number 0500070 (rye): 0.2 mg/kg

Wheat straw (feed item)

Despite MRLs are not yet set for animal feed, the residue data summarized in M-CA 6.3 has been used for deriving a *"pseudo MRL"*. The relevant STMR and HR vales can be used for deriving a feed burden and subsequently, a MRL proposal for food of animal origin.

In the growing seasons 2010 - 2012, in total 24 field trials comprising 28 plots were performed in Northern Europe (Germany, The Netherlands, Denmark, Northern France and The United Kingdom) and Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulations BAS 500 06 F and BAS 703 04 F according to the critical GAP of formulation BAS 500 06 F (± 25%).

The following parent compound residues were found in wheat straw samples at a PHI of 35 days or later:

Northern Europe (n=14): 0.30, 0.37, 0.41, 0.51, 1.1, 1.3 (2x), 1.74, 1.9, 1.98, 3.01, 5.43, 5.5, 5.76 mg/kg

Southern Europe (n=14): 0.45, 0.46, 0.85, 0.95 (2x), 1.5, 1.55, 1.6, 2.26, 4.08, 4.17, 4.18, 4.40, 6.96 mg/kg

For MRL calculation the current version of the OECD MRL calculator was used.

Table 6.7.2-7: MRL calculation for wheat straw in the EU for pyraclostrobin based on parent residues (mg/kg)

	Pyraclostrobin [mg/kg]
	North (n=14)
STMR	1.52
HR	5.76
OECD	**10**
	South (n=14)
STMR	1.575
HR	6.96
OECD	**10**

The data show, that the calculated MRL for pyraclostrobin in wheat straw is covered by the current *"pseudo MRL"* of 10 mg/kg (see MRL review for pyraclostrobin according to Article 12, EFSA Journal 2011;9(8):2344). Therefore, BASF proposes to keep the current value of pyraclostrobin at 10 mg/kg for wheat and rye straw.

The following values were published by EFSA, see EFSA Journal 2011;9(8):2344 (page 38):

Northern EU → STMR = 1.65 mg/kg
HR = 3.14 mg/kg
Proposal = 5 mg/kg

Southern EU → STMR = 1.85 mg/kg
HR = 5.68 mg/kg
Proposal = 10 mg/kg

Animal matrices

A worst case diet was derived for different livestock species according to the table in Appendix G (Lundehn document 7031/VI/95 rev.4. July 1996). It is assumed that from each group of crops/commodities the item with the highest potential residue contribution on a dry matter basis is chosen. Then, the total diet is composed beginning with the group representing the highest contribution and filling the rest with feed from the other groups in descending order.
The evaluation is based on the following formula:

$$\text{Uptake [mg/kg bw/day]} = \frac{\text{Total intake of dry matter [kg/animal/day]} \times \text{\% of diet} \times \text{Residue in feed item [mg/kg]}}{\text{Dry matter content of feed item [\%]} \times \text{Bodyweight [kg]}}$$

In 2011, the most recent calculation of the overall feed burden has been performed by EFSA in context of the MRL re-evaluation according to EEC 396/2005, § 12. This calculation was used as basis, since it already includes maize grain (based on a NAFTA import tolerance). The contribution of "maize - silage" was additionally considered. For estimating the feed burden, the procedure of EFSA (Profile file) has been applied.

Since then, additional uses of pyraclostrobin containing products with new STMR and HR values were published and accepted by EFSA, e.g. for the use in kale (EFSA Journal 2012;10(3):2606).

The following input values were used for calculation of median and maximum dietary burden:

Table 6.7.2-8: Input values for the revised dietary burden calculation (EU methodology)

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Cabbage [1]	0.02	STMR	0.09	HR
Kale [2]	0.19	STMR	0.70	HR
Sugar beet tops [1]	0.07	STMR	0.18	HR
Orange pomace [4]	1.10	STMR x 2.25	1.10	STMR x 2.25
Grapefruit pomace [1]	0.49	STMR x 2.5	0.49	STMR x 2.5
Lemon pomace [1]	0.49	STMR x 2.5	0.49	STMR x 2.5
Lime pomace [1]	0.49	STMR x 2.5	0.49	STMR x 2.5
Mandarin pomace [1]	0.49	STMR x 2.5	0.49	STMR x 2.5
Apples pomace [1]	0.25	STMR x 2.5	0.25	STMR x 2.5
Barley and oat grain [3]	0.35	STMR	0.35	STMR
Wheat and rye grain [1]	0.02	STMR	0.02	STMR
Maize grain [1]	0.02	STMR	0.02	STMR
Wheat and rye bran [1]	0.16	STMR x 8	0.16	STMR x 8
Barley and oat straw	3.38[1]	STMR	8.73[5]	HR
Wheat and rye straw	1.85[1]	STMR	6.96[5]	HR
Peas (dry)/beans (dry) [1]	0.04	STMR	0.04	STMR
Potatoes [1]	0.02	STMR	0.02	HR
Sugar beet roots [1]	0.04	STMR	0.11	HR
Rape seed [4]	0.035	STMR	0.035	STMR
Rape seed meal [4]	0.07	STMR x 2	0.07	STMR x 2
Cotton seed [4]	0.03	STMR	0.03	STMR
Cotton seed meal [4]	0.04	STMR x 1.3	0.04	STMR x 1.3
Linseed [4]	0.04	STMR (combined rape seed and sunflower seed)	0.04	STMR (combined rape seed and sunflower seed)
Linseed meal [4]	0.07	STMR (combined rape seed and sunflower seed) x 1.3	0.07	STMR (combined rape seed and sunflower seed) x 1.3
Peanuts [1]	0.02	STMR	0.02	STMR
Peanuts meal [1]	0.04	STMR x 2	0.04	STMR x 2
Sunflower seed [4]	0.053	STMR	0.053	STMR
Sunflower seed meal [4]	0.106	STMR x 2	0.106	STMR x 2
Soya bean [1]	0.02	STMR	0.02	STMR
Soya bean meal [1]	0.03	STMR x 1.3	0.03	STMR x 1.3

[1] see EFSA Journal 2011;9(8):2344
[2] see EFSA Journal 2012;10(3):2606
[3] see EFSA Journal 2014;12(5):3685
[4] see EFSA Journal 2011;9(3):2120
[5] derived in the context of this dossier

The following tables show the calculation of the maximum dietary burden for each relevant livestock species, which are based on the highest and the median residue levels of pyraclostrobin. It should be noted that the doses assume that the diet completely consists of plant material which had been treated with pyraclostrobin. Furthermore, some default processing factors have been included in the calculation. As shown in M-CA 6.5.3, the factor of 8 for bran is extremely conservative. From the trials performed, a PF of 2.7 has been derived.

Table 6.7.2-9: Detailed results of the livestock dietary burden calculations for dairy ruminants

Maximum

Commodity	*DM intake (%)*	*Residue intake over DM intake*	*Actual contribution to total DM intake (%)*	*Actual contribution to total residue intake (mg/kg bw/d)*	*Total DM intake (%)*	*Total residue intake (mg/kg bw/d)*
Barley straw	20	0.003691	20	0.073827	20	0.073827
Kale	35	0.001818	35	0.063636	55	0.137463
Sugar beets	30	0.000200	30	0.006000	85	0.143463
Barley grain	40	0.000148	15	0.002220	100	0.145683

Maximum dietary burden: 0.145683 mg/kg bw/d

Median

Commodity	*DM intake (%)*	*Residue intake over DM intake*	*Actual contribution to total DM intake (%)*	*Actual contribution to total residue intake (mg/kg bw/d)*	*Total DM intake (%)*	*Total residue intake (mg/kg bw/d)*
Orange pomace	10	0.001739	10	0.017391	10	0.017391
Barley straw	20	0.001429	20	0.028584	30	0.045975
Barley grain	40	0.000148	40	0.005920	70	0.051894
Sugar beets	30	0.000073	30	0.002182	100	0.054076

Median dietary burden: 0.054076 mg/kg bw/d

Table 6.7.2-10: Summarised results of the livestock dietary burden calculations for dairy ruminants

Dietary Burden of Dairy Ruminants	
Maximum dietary burden (mg/kg bw/d):	0.145683
Maximum dietary burden (mg/kg feed DM):	4.006279
Highest contributing commodity:	Barley straw
Median dietary burden (mg/kg bw/d):	0.054076
Median dietary burden (mg/kg feed DM):	1.487098
Highest contributing commodity:	Barley straw

Table 6.7.2-11: Detailed results of the livestock dietary burden calculations for meat ruminants

Maximum

Commodity	*DM intake (%)*	*Residue intake over DM intake*	*Actual contribution to total DM intake (%)*	*Actual contribution to total residue intake (mg/kg bw/d)*	*Total DM intake (%)*	*Total residue intake (mg/kg bw/d)*
Barley straw	50	0.004350	50	0.217525	50	0.217525
Kale	35	0.002143	35	0.075000	85	0.292525
Sugar beets	60	0.000236	15	0.003536	100	0.296061

Maximum dietary burden: 0.296061 mg/kg bw/d

Median

Commodity	*DM intake (%)*	*Residue intake over DM intake*	*Actual contribution to total DM intake (%)*	*Actual contribution to total residue intake (mg/kg bw/d)*	*Total DM intake (%)*	*Total residue intake (mg/kg bw/d)*
Orange pomace	30	0.002050	30	0.061491	30	0.061491
Barley straw	50	0.001684	50	0.084219	80	0.145710
Barley grain	80	0.000174	20	0.003488	100	0.149198

Median dietary burden: 0.149198 mg/kg bw/d

Table 6.7.2-12: Summarised results of the livestock dietary burden calculations for meat ruminants

Dietary Burden of Meat Ruminants	
Maximum dietary burden (mg/kg bw/d):	0.296061
Maximum dietary burden (mg/kg feed DM):	6.908081
Highest contributing commodity:	Barley straw
Median dietary burden (mg/kg bw/d):	0.149198
Median dietary burden (mg/kg feed DM):	3.481294
Highest contributing commodity:	Barley straw

Table 6.7.2-13: Detailed results of the livestock dietary burden calculations for poultry

Maximum

Commodity	*DM intake (%)*	*Residue intake over DM intake*	*Actual contribution to total DM intake (%)*	*Actual contribution to total residue intake (mg/kg bw/d)*	*Total DM intake (%)*	*Total residue intake (mg/kg bw/d)*
Kale	5	0.003158	5	0.015789	5	0.015789
Sugar beets	20	0.000347	20	0.006947	25	0.022737
Barley grain	70	0.000257	70	0.017993	95	0.040729
Sunflower seed meal	10	0.000078	5	0.000389	100	0.041119

Maximum dietary burden: 0.041119 mg/kg bw/d

Median

Commodity	*DM intake (%)*	*Residue intake over DM intake*	*Actual contribution to total DM intake (%)*	*Actual contribution to total residue intake (mg/kg bw/d)*	*Total DM intake (%)*	*Total residue intake (mg/kg bw/d)*
Kale	5	0.000857	5	0.004286	5	0.004286
Barley grain	70	0.000257	70	0.017993	75	0.022278
Sugar beets	20	0.000126	20	0.002526	95	0.024805
Sunflower seed meal	10	0.000078	5	0.000389	100	0.025194

Median dietary burden: 0.025194 mg/kg bw/d

Table 6.7.2-14: Summarised results of the livestock dietary burden calculations for poultry

Dietary Burden of Poultry	
Maximum dietary burden (mg/kg bw/d):	0.041119
Maximum dietary burden (mg/kg feed DM):	0.651047
Highest contributing commodity:	Barley grain
Median dietary burden (mg/kg bw/d):	0.025194
Median dietary burden (mg/kg feed DM):	0.398904
Highest contributing commodity:	Barley grain

Table 6.7.2-15: Detailed results of the livestock dietary burden calculations for pigs

Maximum

Commodity	*DM intake (%)*	*Residue intake over DM intake*	*Actual contribution to total DM intake (%)*	*Actual contribution to total residue intake (mg/kg bw/d)*	*Total DM intake (%)*	*Total residue intake (mg/kg bw/d)*
Kale	15	0.002000	15	0.030000	15	0.030000
Sugar beets	60	0.000220	60	0.013200	75	0.043200
Barley grain	80	0.000163	25	0.004070	100	0.047270

Maximum dietary burden: 0.047270 mg/kg bw/d

Median

Commodity	*DM intake (%)*	*Residue intake over DM intake*	*Actual contribution to total DM intake (%)*	*Actual contribution to total residue intake (mg/kg bw/d)*	*Total DM intake (%)*	*Total residue intake (mg/kg bw/d)*
Kale	15	0.000543	15	0.008143	15	0.008143
Barley grain	80	0.000163	80	0.013023	95	0.021166
Sugar beets	60	0.000080	5	0.000400	100	0.021566

Median dietary burden: 0.021566 mg/kg bw/d

Table 6.7.2-16: Summarised results of the livestock dietary burden calculations for pigs

Dietary Burden of Pigs	
Maximum dietary burden (mg/kg bw/d):	0.047270
Maximum dietary burden (mg/kg feed DM):	1.181744
Highest contributing commodity:	Kale
Median dietary burden (mg/kg bw/d):	0.021566
Median dietary burden (mg/kg feed DM):	0.539153
Highest contributing commodity:	Barley grain

Additionally, the most recent version of the OECD feed burden calculator (using the OECD methodology) was applied. The information is provided as supplemental information. Main purpose is to prove that the low dose feeding level of the goat metabolism study is considered as more relevant for metabolite considerations.

The following input values were used for calculation of the Results for Reasonable Worst Case Feeding Levels (RWCFL):

Table 6.7.2-17: Input values for the revised dietary burden calculation (OECD methodology)

Commodity	RWCFL EU	
	Input value (mg/kg)	Comment
Forages		
Barley, straw [6]	8.73	HR
Beet, sugar, tops [1]	0.18	HR
Cabbage, heads [1]	0.09	HR
Kale [2]	0.70	HR
Oat, straw [6]	8.73	HR
Rye, straw [6]	6.96	HR
Wheat, straw [6]	6.96	HR
Roots & Tubers		
Carrot, culls [4]	0.24	HR
Potatoes, culls [1]	0.02	HR
Cereal Grain/Crops Seeds		
Barley, grain [3]	0.35	STMR
Bean, seed [1]	0.04	STMR
Corn, field, grain [1]	0.02	STMR
Corn, pop, grain [1]	0.02	STMR
Lupin, seed [1]	0.02	STMR
Oat, grain [3]	0.35	STMR
Pea, seed [1]	0.04	STMR
Rye, grain [1]	0.02	STMR
Sorghum, grain [4]	0.025	STMR
Soybean, seed [5]	0.02	STMR
Wheat, grain [1]	0.02	STMR
By-Products		
Apples pomace [1]	0.25	STMR x 2.5
Beet, sugar, dried pulp [1]	0.10	STMR x 2.5
Beet, sugar, ensiled pulp [1]	0.04	STMR
Citrus, dried pulp [1]	0.49	STMR x 2.5
Cotton, meal [5]	0.04	STMR x 1.3
Peanut, meal [1]	0.04	STMR x 2
Rape, meal [5]	0.07	STMR x 2
Soybean, meal [1]	0.03	STMR x 1.3
Sunflower, meal [5]	0.106	STMR x 2
Wheat, milled byproducts [1]	0.16	STMR x 8

[1] see EFSA Journal 2011;9(8):2344
[2] see EFSA Journal 2012;10(3):2606
[3] see EFSA Journal 2014;12(5):3685
[4] HR/STMR in context of a JMPR submission leading to CODEX MRL, which was later adopted by EFSA
[5] see EFSA Journal 2011;9(3):2120
[6] derived in context of this dossier

The results of the total maximum dietary burdens are presented below.

Table 6.7.2-18: Summary of the results for RWCFL (EU)

	Cattle Beef	Cattle Dairy	Sheep Ram/Ewe	Sheep Lamb	Swine Breeding	Swine Finishing	Poultry Broiler	Poultry Layer	Poultry Turkey
Regions	EU	EU	EU	EU	EU	EU	EU	EU	EU
Body weight (kg)	500	650	75	40	260	100	1.7	1.9	7
Daily intake (kg DM)	12	25	2.5	1.7	6	3	0.12	0.13	0.5
Dietary Burden (mg/kg bw)	**0.083**	**0.134**	**0.212**	**0.271**	**0.028**	**0.024**	**0.036**	**0.079**	**0.031**
Feed Burden (mg/kg DM)	**3.461**	**3.483**	**6.365**	**6.365**	**1.225**	**0.798**	**0.515**	**1.149**	**0.435**

Table 6.7.2-19: Detailed results for RWCFL (EU): cattle (beef)

EU								
Category	Crop	Feedstuff	% of Diet	Dietary Contribution (mg/kg bw)	Cumulative % Diet	Adjusted % Diet	Adjusted Dietary Contribution (mg/kg bw)	Feed Burden (mg/kg DM)
Forages	Barley	straw	30	0.071	30	30	0.071	2.943
Roots/tubers	Carrot	culls	15	0.007	45	15	0.007	0.300
Cereal grain	Barley	grain	70	0.007	100	55	0.005	0.219
By-products	Apple	pomace, wet	20	0.003	100	0	0.000	0.000
Total			**135**	**0.088**	**-**	**100**	**0.083**	**3.461**

Table 6.7.2-20: Detailed results for RWCFL (EU): cattle (dairy)

EU								
Category	Crop	Feedstuff	% of Diet	Dietary Contribution (mg/kg bw)	Cumulative % Diet	Adjusted % Diet	Adjusted Dietary Contribution (mg/kg bw)	Feed Burden (mg/kg DM)
Forages	Barley	straw	30	0.113	30	30	0.113	2.943
Roots/tubers	Carrot	culls	15	0.012	45	15	0.012	0.300
Cereal grain	Barley	grain	40	0.006	85	40	0.006	0.159
By-products	Citrus	dried pulp	20	0.004	100	15	0.003	0.081
Total			**105**	**0.135**	**-**	**100**	**0.134**	**3.483**

Table 6.7.2-21: Detailed results for RWCFL (EU): sheep (ram/ewe)

EU								
Category	Crop	Feedstuff	% of Diet	Dietary Contribution (mg/kg bw)	Cumulative % Diet	Adjusted % Diet	Adjusted Dietary Contribution (mg/kg bw)	Feed Burden (mg/kg DM)
Forages	Barley	straw	60	0.196	60	60	0.196	5.885
Roots/tubers	Carrot	culls	20	0.013	80	20	0.013	0.400
Cereal grain	Barley	grain	40	0.005	100	20	0.003	0.080
By-products	Wheat	milled bypdts	40	0.002	100	0	0.000	0.000
Total			**160**	**0.217**	**-**	**100**	**0.212**	**6.365**

Table 6.7.2-22: Detailed results for RWCFL (EU): sheep (lamb)

EU								
Category	Crop	Feedstuff	% of Diet	Dietary Contribution (mg/kg bw)	Cumulative % Diet	Adjusted % Diet	Adjusted Dietary Contribution (mg/kg bw)	Feed Burden (mg/kg DM)
Forages	Barley	straw	60	0.250	60	60	0.250	5.885
Roots/tubers	Carrot	culls	20	0.017	80	20	0.017	0.400
Cereal grain	Barley	grain	60	0.010	100	20	0.003	0.080
By-products	Wheat	milled bypdts	50	0.004	100	0	0.000	0.000
Total			**190**	**0.281**	**-**	**100**	**0.271**	**6.365**

Table 6.7.2-23: Detailed results for RWCFL (EU): swine (breeding)

EU								
Category	Crop	Feedstuff	% of Diet	Dietary Contribution (mg/kg bw)	Cumulative % Diet	Adjusted % Diet	Adjusted Dietary Contribution (mg/kg bw)	Feed Burden (mg/kg DM)
Roots/tubers	Carrot	culls	25	0.012	25	25	0.012	0.500
Forages	Kale	leaves	10	0.011	35	10	0.011	0.467
Cereal grain	Barley	grain	80	0.007	100	65	0.006	0.259
By-products	Wheat	milled bypdts	50	0.002	100	0	0.000	0.000
Total			**165**	**0.032**	**-**	**100**	**0.028**	**1.225**

Table 6.7.2-24: Detailed results for RWCFL (EU): swine (finishing)

EU								
Category	Crop	Feedstuff	% of Diet	Dietary Contribution (mg/kg bw)	Cumulative % Diet	Adjusted % Diet	Adjusted Dietary Contribution (mg/kg bw)	Feed Burden (mg/kg DM)
Roots/tubers	Carrot	culls	25	0.015	25	25	0.015	0.500
Cereal grain	Barley	grain	80	0.010	100	75	0.009	0.298
By-products	Wheat	milled bypdts	50	0.003	100	0	0.000	0.000
Forages	Wheat	silage	0	0.000	100	0	0.000	0.000
Total			**155**	**0.027**	**-**	**100**	**0.024**	**0.798**

Table 6.7.2-25: Detailed results for RWCFL (EU): poultry (broiler)

EU								
Category	Crop	Feedstuff	% of Diet	Dietary Contribution (mg/kg bw)	Cumulative % Diet	Adjusted % Diet	Adjusted Dietary Contribution (mg/kg bw)	Feed Burden (mg/kg DM)
Cereal grain	Barley	grain	70	0.020	70	70	0.020	0.278
Roots/tubers	Carrot	culls	10	0.014	80	10	0.014	0.200
By-products	Wheat	milled bypdts	20	0.003	100	20	0.003	0.036
Forages	Wheat	silage	0	0.000	100	0	0.000	0.000
Total			**100**	**0.036**	**-**	**100**	**0.036**	**0.515**

Table 6.7.2-26: Detailed results for RWCFL (EU): poultry (layer)

EU								
Category	Crop	Feedstuff	% of Diet	Dietary Contribution (mg/kg bw)	Cumulative % Diet	Adjusted % Diet	Adjusted Dietary Contribution (mg/kg bw)	Feed Burden (mg/kg DM)
Forages	Wheat	straw	10	0.054	10	10	0.054	0.791
Cereal grain	Barley	grain	100	0.027	100	90	0.024	0.358
Roots/tubers	Carrot	culls	10	0.014	100	0	0.000	0.000
By-products	Wheat	milled bypdts	20	0.002	100	0	0.000	0.000
Total			**140**	**0.098**	**-**	**100**	**0.079**	**1.149**

Table 6.7.2-27: Detailed results for RWCFL (EU): poultry (turkey)

EU								
Category	Crop	Feedstuff	% of Diet	Dietary Contribution (mg/kg bw)	Cumulative % Diet	Adjusted % Diet	Adjusted Dietary Contribution (mg/kg bw)	Feed Burden (mg/kg DM)
Roots/tubers	Carrot	culls	10	0.014	10	10	0.014	0.200
Cereal grain	Barley	grain	50	0.014	60	50	0.014	0.199
By-products	Wheat	milled bypdts	20	0.003	80	20	0.003	0.036
Forages	Wheat	silage	0	0.000	80	0	0.000	0.000
Total			**80**	**0.031**	**-**	**80**	**0.031**	**0.435**

In addition to the feed burden calculation for those livestock species for which MRLs are already established, the potential feed burden has been also derived for fish (trout, carp). For calculation purposes, the procedure specified in the EU Working Document SANCO/11187/2013 on the nature of pesticide residues in fish (as of 31 January 2013) has been used.

According to the Working Document, fish diet of trout and carp mainly consists of cereals, pulses, oilseeds and processed fractions thereof. Due to the broad use of pyraclostrobin, the contribution of fish meal was not further considered:

The calculation resulted in the following approximate maximum feed burdens:

- Trout: 0.6 mg/kg feed DM
- Carp: 1.3 mg/kg feed DM

Proposed animal MRLs

The doses to be used when estimating the maximum residues in products of animal origin are for

dairy cattle 0.146 mg/kg bw/d
beef cattle 0.296 mg/kg bw/d
chicken 0.041 mg/kg bw/d
pig 0.047 mg/kg bw/d

Cattle (sheep, goat) products

A residue transfer study with pyraclostrobin was conducted in cows. The animals were dosed with 7, 21 and 70 mg/kg feed (dry matter) equal to 140, 420 and 1400 mg/animal and day for a period of 28 days. In the dose group relevant under normal agricultural conditions (7 mg/kg feed), no residues could be detected in milk, meat, fat, kidney and tissues. Low residues of pyraclostrobin metabolites may occur in liver. The revised feed burden calculation presented above indicates that the 1 x dose level is the most appropriate for covering the uses of pyraclostrobin.

In the cow feeding study samples were analysed according to both residue definitions. The analysis according to the residue definition for MRL setting (parent only) resulted in residues below the LOQ of the relevant method (corresponding to 0.01 mg/kg for milk and 0.05 mg/kg for tissues).

Consequently, the current EU MRLs for milk and tissues are re-confirmed.
The analysis of the samples according to the definition of the relevant residue for risk assessment purposes (common moiety method) resulted in residues below 0.1 mg/kg, except for liver where 0.2 mg/kg were found.

Pig products

No separate feeding study with pigs has been performed since common metabolic pathways have been observed in rats and goats and therefore significant differences in the metabolic pathways from pigs as compared to ruminants are very unlikely. The proposals for maximum residue levels for pig products can therefore be derived from the cattle feeding study. The 1 x dose level of the cow feeding study was considered despite the dose level corresponds to 5 x of the pig feed burden. The samples were analysed according to both residue definitions. The residue levels found at the 1 x dose level were all below the LOQ of the monitoring method (0.05 mg/kg). The analytical method following the common moiety concept (corresponding to: definition of the relevant residue for risk assessment) indicates the presence of pyraclostrobin metabolites only in liver at a level of 0.2 mg/kg. All other matrices were below 0.1 mg/kg.

Poultry products

Based on the results of the hen metabolism study and the revised feed burden calculation indicating a very low feed burden with highest contribution of barley, no feeding study in hen would be required. The poultry feeding study which was performed for US submission is provided as supplemental information within this dossier. It confirms the favourable residue behaviour in poultry matrices. The MRLs in poultry tissues and eggs, which are established at the limit of quantitation of the enforcement method (corresponding to 0.05 mg/kg) are re-confirmed.

Fish products

The actual feed burden is compared with the feeding levels and the results of the fish metabolism study in which parent was identified as main residue (see M-CA 6.2). As worst case the feed burden of carp was taken into account. Based on the overdosing factor and the pyraclostrobin levels measured, a preliminary proposal for a future EU MRL for fish can be derived.

Table 6.7.2-28: Expected residue levels of pyraclostrobin in fish matrices

Matrix analyzed	Total radioactive residue [mg/kg]*	Pyraclo strobin [mg/kg]	Feed burden fish metabolism study [mg/kg feed DM]	Feed burden calculated [mg/kg feed DM]	Overdosing factor fish metabolism	Pyraclo strobin calculated [mg/kg]
Fish filet	0.282	0.262	11	1.3	8.5	0.03
Fish skin	0.232	0.21	11	1.3	8.5	0.02
Fish liver	0.556	0.409	11	1.3	8.5	0.05

* highest value from both labels

For further evaluation the following EU MRLs were used:

0.05 mg/kg for all animal matrices (including fish, except milk)
0.01 mg/kg for milk

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

The MRLs listed in M-CA 6.7.2 include domestic uses, but also values for imported crops. Prior to final approval, the import tolerances have been carefully evaluated by Germany (RMS) and EFSA. Parts of them are resulting from the adoption of CODEX MRLs. In the meantime, pyraclostrobin and its crops have been assessed three times by JMPR.

CA 6.8 Proposed safety intervals

Residue trials have been conducted with applications made at the latest recommended crop growth stage with harvest taking place at the time of crop maturity following good agricultural practice.

Pre-harvest interval
For potatoes the application is intended at growth stages BBCH 41-89 with a pre-harvest interval of 0-3 days. For cereals (wheat, barley, oats, rye and triticale) application is intended at growth stages BBCH 25-69 (corresponding to an approximate minimum period of 35 to 42 days until harvest). For maize incl. sweet corn, latest application is at growth stage BBCH 65. Except for potatoes, the preharvest interval is fixed by the intended use (growth stage at last application ("F")).

Re-entry period for livestock to areas to be grazed
Because pyraclostrobin is not intended to be used in areas to be grazed, no re-entry period for livestock has to be defined.

Re-entry period for man to treated crops
Re-entry assessments are given for the representative uses in the supplemental product dossiers (M-CP 7.2). Re-entry is possible after the spray deposits on the crops have dried given the worker is wearing adequate work clothing.

Withholding period for animal feed stuffs
Treated cereals and maize feed items may be used as fodder for livestock and fish. Pyraclostrobin derived residues in those feed items are assessed in M-CA 6.7 by providing updated calculations of livestock and fish dietary burdens and deriving suitable MRLs for animal products covering the intended uses. There is no additional withholding period needed for animal feeds with regard to pyraclostrobin derived residues.
Due to the favorable residue situation in potatoes with residues consistently below LOQ no withholding period is needed.

Waiting period between application and crop sowing or planting the crop to be protected
No waiting period is necessary since pyraclostrobin is not intended in a pre-emergence use.

Waiting period between application and handling treated produce
This is not relevant here since a post-harvest treatment is not intended for potatoes, cereals and maize incl. sweet corn.

Waiting period between last application and sowing or planting succeeding crops
Due to the favorable residue behavior at all replant intervals investigated, no replant restrictions are needed. Even at exaggerated rates, the residue levels of pyraclostrobin and its metabolites are very low (see M-CA 6.6). No detectable residues above the MRLs can be expected when crops are sown or planted after a previous pyraclostrobin application.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

Assessments of the potential chronic and acute dietary consumer risk due to exposure to residues of pyraclostrobin were performed using the EFSA model for chronic and acute risk assessment - rev. 2_0 (Model PRIMo). The EFSA model was used since it considers all the different diets in the EU and all consumer groups. In order to check the contribution of fish to the diet, an assessment according to the most recent version of the German model is also included.

The ADI and ARfD for the active substance pyraclostrobin are summarized in the table below. Due to their presence in significant amounts in the rat metabolism study, the same toxicological endpoints are also applying to the metabolites 500M07 (BF 500-3) and the metabolites resulting from simple ring hydroxylation reactions (plants: 500M34, 500M54, 500M56, 500M68, 500M70, 500M71; livestock: 500M08, 500M32, 500M39, 500M45, 500M64, 500M66, 500M67). The use of the same ADI value is further corroborated by investigations performed by the Austrian authority AGES (T. Coja et al., presented e.g. at the 10th International Fresenius Conference held in Mainz in February 2012).

Table 6.9-1: Toxicological endpoints - pyraclostrobin

Endpoint	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.03 mg/kg bw/d	2-year study, rat	100	SANCO/1420/2001-Final (08.09.2004)
Acute Reference Dose (ARfD)	0.03 mg/kg bw/d	developmental toxicity, rabbit (maternal effects)	100	SANCO/1420/2001-Final (08.09.2004)

The ADI and ARfD for the metabolite 500M04 (synonym: BF 500-5) are summarized in the table below.

Table 6.9-2: Toxicological endpoints – 500M04

Endpoint	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.52 mg/kg bw/d	90 day study, rat	200	M-CA 5.8
Acute Reference Dose (ARfD)	none	n.a.	n.a.	M-CA 5.8

The ADI and ARfD for the metabolites 500M24, 500M49, 500M51 and 500M76 are summarized in the table below. For the metabolites, genotoxicity tests have been performed. They are summarized in M-CA 5.8. As none of them showed any genotoxicity potential, TTC values (Cramer class III) can be used for an indicative dietary exposure assessment.

Table 6.9-3: Toxicological endpoints – TTC approach

Endpoint	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.0015 mg/kg bw/d	none	not relevant	TTC
Acute Reference Dose (ARfD)	0.005 mg/kg bw/d	none	not relevant	TTC

For pyraclostrobin, an *in-vitro* comparison study was performed in human, rat, rabbit and dog hepatocytes / microsomes (see M-CA 5.1). Based on the study results, it cannot be excluded that the metabolites 500M02, 500M106 and 500M107 (glucuronic acid conjugate of 500M106) might be also formed in humans under *in-vivo* conditions. These metabolites were identified in rabbit cell cultures, but not detected in significant amounts in rat. For the metabolite 500M106 a 28-day rat study (see M-CA 5.8) was performed which also covers metabolites 500M02 and 500M107.

Table 6.9-4: Toxicological endpoints – 500M02 and 500M106 / 500M107

Endpoint	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.5 mg/kg bw/d	28 day study, rat	600	M-CA 5.8
Acute Reference Dose (ARfD)	none	n.a.	n.a.	M-CA 5.8

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

Pyraclostrobin

TMDI calculation

A revised chronic exposure assessment was performed, for which all crops and maximum residue levels (either the established MRL value, or if different the proposed MRL) used are summarized in Table 6.9-5. * indicates the lower limit of analytical determination.
The re-evaluation of the existing MRLs according to Reg. 396/2005 Art. 12 is complete. Some MRLs were changed as an outcome of this re-evaluation. As a result of EFSA Journal 2013;11(2):3109 (Reasoned opinion on the modification of the existing MRLs for pyraclostrobin in cucumbers and Jerusalem artichokes) - see also EU regulation 51/2014 - and EFSA Journal 2014;12(5):3685 (Reasoned opinion on the modification of the existing MRL for pyraclostrobin in chicory roots), the MRLs for Jerusalem artichokes and chicory roots were changed.
Beyond that, since the beginning of April 2013 there were further changes of MRLs caused by the adoption of CODEX MRLs into EU law (see Reg. No. 293/2013).
The summary of the calculation using the EFSA model rev 2.0 is presented in Table 6.9-5. For the assessment, an ADI of 0.03 mg/kg bw/day was used. According to the EFSA model the TMDI has been simultaneously calculated for adults, children, toddlers and infants (different age groups), vegetarian and elderly in different EU countries.

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
100000	11. FRUIT FRESH OR FROZEN NUTS	
110000	(i) Citrus fruit	
110010	Grapefruit (Shaddocks, pomelos, sweeties, tangelo (except mineola), ugli and other hybrids)	1
110020	Oranges (Bergamot, bitter orange, chinotto and other hybrids)	2
110030	Lemons (Citron, lemon, Buddha's hand (Citrus medica var. sarcodactylis))	1
110040	Limes	1
110050	Mandarins (Clementine, tangerine, mineola and other hybrids tangor (Citrus reticulata x sinensis))	1
110990	Others	1

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
120000	(ii) Tree nuts	
120010	Almonds	0.02*
120020	Brazil nuts	0.02*
120030	Cashew nuts	0.02*
120040	Chestnuts	0.02*
120050	Coconuts	0.02*
120060	Hazelnuts (Filbert)	0.02*
120070	Macadamia	0.02*
120080	Pecans	0.02*
120090	Pine nuts	0.02*
120100	Pistachios	1
120110	Walnuts	0.02*
120990	Others	0.02*
130000	(iii) Pome fruit	0.5
130010	Apples (Crab apple)	0.5
130020	Pears (Oriental pear)	0.5
130030	Quinces	0.5
130040	Medlar	0.5
130050	Loquat	0.5
130990	Others	0.5
140000	(iv) Stone fruit	
140010	Apricots	1
140020	Cherries (Sweet cherries, sour cherries)	3
140030	Peaches (Nectarines and similar hybrids)	0.3
140040	Plums (Damson, greengage, mirabelle, sloe, red date/Chinese date/Chinese jujube (Ziziphus zizyphus))	0.8
140990	Others	0.02*

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
150000	(v) Berries & small fruit	
151000	(a) Table and wine grapes	
151010	Table grapes	1
151020	Wine grapes	2
152000	(b) Strawberries	1.5
153000	(c) Cane fruit	
153010	Blackberries	3
153020	Dewberries (Loganberries, tayberries, boysenberries, cloudberries and other Rubus hybrids)	2
153030	Raspberries (Wineberries, arctic bramble/raspberry, (Rubus arcticus), nectar raspberries (Rubus arcticus x Rubus idaeus))	3
153990	Others	2
154000	(d) Other small fruit & berries	
154010	Blueberries (Bilberries)	4
154020	Cranberries (Cowberries/red bilberries (V. vitis-idaea))	3
154030	Currants (red, black and white)	3
154040	Gooseberries (Including hybrids with other Ribes species)	3
154050	Rose hips	3
154060	Mulberries (Arbutus berry)	3
154070	Azarole (mediteranean medlar) (Kiwiberry (Actinidia arguta))	3
154080	Elderberries (Black chokeberry/appleberry, mountain ash, buckthorn/sea sallowthorn, hawthorn, serviceberries, and other treeberries)	3
154990	Others	3

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
160000	(vi) Miscellaneous fruit	
161000	(a) Edible peel	0.02*
161010	Dates	0.02*
161020	Figs	0.02*
161030	Table olives	0.02*
161040	Kumquats (Marumi kumquats, nagami kumquats, limequats (Citrus aurantifolia x Fortunella spp.))	0.02*
161050	Carambola (Bilimbi)	0.02*
161060	Persimmon	0.02*
161070	Jambolan (java plum) (Java apple/water apple, pomerac, rose apple, Brazilean cherry, Surinam cherry/grumichama (Eugenia uniflora))	0.02*
161990	Others	0.02*
162000	(b) Inedible peel, small	0.02*
162010	Kiwi	0.02*
162020	Lychee (Litchi) (Pulasan, rambutan/hairy litchi, longan, mangosteen, langsat, salak)	0.02*
162030	Passion fruit	0.02*
162040	Prickly pear (cactus fruit)	0.02*
162050	Star apple	0.02*
162060	American persimmon (Virginia kaki) (Black sapote, white sapote, green sapote, canistel/yellow sapote, mammey sapote)	0.02*
162990	Others	0.02*
163000	(c) Inedible peel, large	
163010	Avocados	0.02*
163020	Bananas (Dwarf banana, plantain, apple banana)	0.02*
163030	Mangoes	0.05
163040	Papaya	0.07
163050	Pomegranate	0.02*
163060	Cherimoya (Custard apple, sugar apple/sweetsop, ilama (Annona diversifolia) and other medium sized Annonaceae fruits)	0.02*
163070	Guava (Red pitaya/dragon fruit (Hylocereus undatus))	0.02*
163080	Pineapples	0.02*
163090	Bread fruit (Jackfruit)	0.02*
163100	Durian	0.02*
163110	Soursop (guanabana)	0.02*
163990	Others	0.02*

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
200000	12. VEGETABLES FRESH OR FROZEN	
210000	(i) Root and tuber vegetables	
211000	(a) Potatoes	0.02*
212000	(b) Tropical root and tuber vegetables	0.02*
212010	Cassava (Dasheen, eddoe/Japanese taro, tannia)	0.02*
212020	Sweet potatoes	0.02*
212030	Yams (Potato bean/yam bean, Mexican yam bean)	0.02*
212040	Arrowroot	0.02*
212990	Others	0.02*
213000	(c) Other root and tuber vegetables except sugar beet	
213010	Beetroot	0.1
213020	Carrots	0.5
213030	Celeriac	0.3
213040	Horseradish (Angelica roots, lovage roots, gentiana roots)	0.3
213050	Jerusalem artichokes (Crosne)	0.06
213060	Parsnips	0.3
213070	Parsley root	0.1
213080	Radishes (Black radish, Japanese radish, small radish and similar varieties, tiger nut (Cyperus esculentus))	0.5
213090	Salsify (Scorzonera, Spanish salsify/Spanish oysterplant, edible burdock)	0.1
213100	Swedes	0.02*
213110	Turnips	0.02*
213990	Others	0.02*
220000	(ii) Bulb vegetables	
220010	(a) Garlic	0.3
220020	(b) Onions (Other bulb onions, silverskin onions)	1.5
220030	(c) Shallots	0.3
220040	(d) Spring onions and welsh onions (Other green onions and similar varieties)	1.5
220990	(e) Others	0.02*

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
230000	(iii) Fruiting vegetables	
231000	(a) Solanacea	
231010	Tomatoes (Cherry tomatoes, Physalis spp., gojiberry, wolfberry (Lycium barbarum and L. chinense), tree tomato)	0.3
231020	Peppers (Chilli peppers)	0.5
231030	Aubergines (egg plants) (Pepino, antroewa/white eggplant (S. macrocarpon))	0.3
231040	Okra (lady's fingers)	0.02*
231990	Others	0.02*
232000	(b) Cucurbits — edible peel	0.5
232010	Cucumbers	0.5
232020	Gherkins	0.5
232030	Courgettes (Summer squash, marrow (patisson), lauki (Lagenaria siceraria), chayote, sopropo/bitter melon, snake gourd, angled luffa/teroi)	0.5
232990	Others	0.5
233000	(c) Cucurbits-inedible peel	0.5
233010	Melons (Kiwano)	0.5
233020	Pumpkins (Winter squash, marrow (late variety))	0.5
233030	Watermelons	0.5
233990	Others	0.5
234000	(d) Sweet corn (Baby corn)	**0.07**
239000	(e) Other fruiting vegetables	0.02*
240000	(iv) Brassica vegetables	
241000	(a) Flowering brassica	0.1
241010	Broccoli (Calabrese, Broccoli raab, Chinese broccoli)	0.1
241020	Cauliflower	0.1
241990	Others	0.1
242000	(b) Head brassica	
242010	Brussels sprouts	0.3
242020	Head cabbage (Pointed head cabbage, red cabbage, savoy cabbage, white cabbage)	0.2
242990	Others	0.02*

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
243000	(c) Leafy brassica	1.5
243010	Chinese cabbage (Indian or Chinese) mustard, pak choi, Chinese flat cabbage/ai goo choi), choi sum, Peking cabbage/pe-tsai)	1.5
243020	Kale (Borecole/curly kale, collards, Portuguese Kale, Portuguese cabbage, cow cabbage)	1.5
243990	Others	1.5
244000	(d) Kohlrabi	0.02*
250000	(v) Leaf vegetables & fresh herbs	
251000	(a) Lettuce and other salad plants including Brassicacea	
251010	Lamb's lettuce (Italian corn salad)	10
251020	Lettuce (Head lettuce, lollo rosso (cutting lettuce), iceberg lettuce, romaine (cos) lettuce)	2
251030	Scarole (broad-leaf endive) (Wild chicory, red-leaved chicory, radicchio, curly leaf endive, sugar loaf (C. endivia var. crispum/C. intybus var. foliosum), dandelion greens)	0.4
251040	Cress (Mung bean sprouts, alfalfa sprouts)	10
251050	Land cress	10
251060	Rocket, Rucola (Wild rocket (Diplotaxis spp.))	10
251070	Red mustard	10
251080	Leaves and sprouts of Brassica spp, including turnip greens (Mizuna, leaves of peas and radish and other babyleaf crops, including brassica crops (crops harvested up to 8 true leaf stage), kohlrabi leaves)	10
251990	Others	10
252000	(b) Spinach & similar (leaves)	
252010	Spinach (New Zealand spinach, amaranthus spinach (pak-khom, tampara), tajer leaves, bitterblad/bitawiri)	0.5
252020	Purslane (Winter purslane/miner's lettuce, garden purslane, common purslane, sorrel, glassworth, agretti (Salsola soda))	0.02*
252030	Beet leaves (chard) (Leaves of beetroot)	0.5
252990	Others	0.02*
253000	(c) Vine leaves (grape leaves) (Malabar nightshade, banana leaves, climbing wattle (Acacia pennata))	0.02*

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
254000	(d) Water cress (Morning glory/Chinese convolvulus/water convolvulus/water spinach/kangkung (Ipomea aquatica), water clover, water mimosa)	0.02*
255000	(e) Witloof	0.02*
256000	(f) Herbs	2
256010	Chervil	2
256020	Chives	2
256030	Celery leaves (Fennel leaves, coriander leaves, dill leaves, caraway leaves, lovage, angelica, sweet cisely and other Apiacea leaves, culantro/stinking/long coriander/stink weed (Eryngium foetidum))	2
256040	Parsley (leaves of root parsley)	2
256050	Sage (Winter savory, summer savory, Borago officinalis leaves)	2
256060	Rosemary	2
256070	Thyme (Marjoram, oregano)	2
256080	Basil (Balm leaves, mint, peppermint, holy basil, sweet basil, hairy basil, edible flowers (marigold flower and others), pennywort, wild betel leaf, curry leaves)	2
256090	Bay leaves (laurel) (Lemon grass)	2
256100	Tarragon (Hyssop)	2
256990	Others	2
260000	(vi) Legume vegetables (fresh)	0.02*
260010	Beans (with pods) (Green bean/French beans/snap beans, scarlet runner bean, slicing bean, yard long beans, guar beans, soya beans)	0.02*
260020	Beans (without pods) (Broad beans, flageolets, jack bean, lima bean, cowpea)	0.02*
260030	Peas (with pods) (Mangetout/sugar peas/snow peas)	0.02*
260040	Peas (without pods) (Garden pea, green pea, chickpea)	0.02*
260050	Lentils	0.02*
260990	Others	0.02*

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
270000	(vii) Stem vegetables (fresh)	
270010	Asparagus	0.02*
270020	Cardoons (Borago officinalis stems)	0.02*
270030	Celery	0.02*
270040	Fennel	0.02*
270050	Globe artichokes (Banana flower)	2
270060	Leek	0.7
270070	Rhubarb	0.02*
270080	Bamboo shoots	0.02*
270090	Palm hearts	0.02*
270990	Others	0.02*
280000	(viii) Fungi	0.02*
280010	Cultivated fungi (Common mushroom, oyster mushroom, shiitake, fungus mycelium (vegetative parts))	0.02*
280020	Wild fungi (Chanterelle, truffle, morel, cep)	0.02*
280990	Others	0.02*
290000	(ix) Sea weeds	0.02*
300000	13. PULSES, DRY	
300010	Beans (Broad beans, navy beans, flageolets, jack beans, lima beans, field beans, cowpeas)	0.3
300020	Lentils	0.5
300030	Peas (Chickpeas, field peas, chickling vetch)	0.3
300040	Lupins	0.05
300990	Others	0.3

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
400000	14. OILSEEDS AND OILFRUITS	
401000	(i) Oilseeds	
401010	Linseed	0.2
401020	Peanuts	0.04
401030	Poppy seed	0.2
401040	Sesame seed	0.2
401050	Sunflower seed	0.3
401060	Rape seed (Bird rapeseed, turnip rape)	0.2
401070	Soya bean	0.05
401080	Mustard seed	0.2
401090	Cotton seed	0.3
401100	Pumpkin seeds (Other seeds of Cucurbitaceae)	0.02*
401110	Safflower	0.2
401120	Borage (Purple viper's bugloss/Canary flower (Echium plantagineum), Corn Gromwell (Buglossoides arvensis))	0.2
401130	Gold of pleasure	0.2
401140	Hempseed	0.02*
401150	Castor bean	0.2
401990	Others	0.02*
402000	(ii) Oilfruits	0.02*
402010	Olives for oil production	0.02*
402020	Palm nuts (palmoil kernels)	0.02*
402030	Palmfruit	0.02*
402040	Kapok	0.02*
402990	Others	0.02*

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
500000	15. CEREALS	
500010	Barley	1
500020	Buckwheat (Amaranthus, quinoa)	0.02*
500030	Maize	0.02*
500040	Millet (Foxtail millet, teff, finger millet, pearl millet)	0.02*
500050	Oats	1
500060	Rice (Indian/wild rice (Zizania aquatica))	0.02*
500070	Rye	0.2
500080	Sorghum	0.5
500090	Wheat (Spelt, triticale)	0.2
500990	Others (Canary grass seeds (Phalaris canariensis))	0.02*
600000	16. TEA, COFFEE, HERBAL INFUSIONS AND COCOA	
610000	(i) Tea	0.1*
620000	(ii) Coffee beans	0.3
630000	(iii) Herbal infusions (dried)	0.1*
631000	(a) Flowers	0.1*
631010	Camomille flowers	0.1*
631020	Hybiscus flowers	0.1*
631030	Rose petals	0.1*
631040	Jasmine flowers (Elderflowers (Sambucus nigra))	0.1*
631050	Lime (linden)	0.1*
631990	Others	0.1*
632000	(b) Leaves	0.1*
632010	Strawberry leaves	0.1*
632020	Rooibos leaves (Ginkgo leaves)	0.1*
632030	Maté	0.1*
632990	Others	0.1*
633000	(c) Roots	0.1*
633010	Valerian root	0.1*
633020	Ginseng root	0.1*
633990	Others	0.1*
639000	(d) Other herbal infusions	0.1*
640000	(iv) Cocoabeans (fermented or dried)	0.1*
650000	(v) Carob (st johns bread)	0.1*
700000	17. HOPS (dried)	15

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
800000	18. SPICES	
810000	(i) Seeds	0.1*
810010	Anise	0.1*
810020	Black caraway	0.1*
810030	Celery seed (Lovage seed)	0.1*
810040	Coriander seed	0.1*
810050	Cumin seed	0.1*
810060	Dill seed	0.1*
810070	Fennel seed	0.1*
810080	Fenugreek	0.1*
810090	Nutmeg	0.1*
810990	Others	0.1*
820000	(ii) Fruits and berries	0.1*
820010	Allspice	0.1*
820020	Sichuan pepper (Anise pepper, Japan pepper)	0.1*
820030	Caraway	0.1*
820040	Cardamom	0.1*
820050	Juniper berries	0.1*
820060	Pepper, black, green and white (Long pepper, pink pepper)	0.1*
820070	Vanilla pods	0.1*
820080	Tamarind	0.1*
820990	Others	0.1*
830000	(iii) Bark	0.1*
830010	Cinnamon (Cassia)	0.1*
830990	Others	0.1*
840000	(iv) Roots or rhizome	
840010	Liquorice	0.1*
840020	Ginger	0.1*
840030	Turmeric (Curcuma)	0.1*
840040	Horseradish	0.1*
840990	Others	0.1*
850000	(v) Buds	0.1*
850010	Cloves	0.1*
850020	Capers	0.1*
850990	Others	0.1*

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
860000	(vi) Flower stigma	0.1*
860010	Saffron	0.1*
860990	Others	0.1*
870000	(vii) Aril	0.1*
870010	Mace	0.1*
870990	Others	0.1*
900000	19. SUGAR PLANTS	
900010	Sugar beet (root)	0.2
900020	Sugar cane	0.02*
900030	Chicory roots	0.02*
900990	Others	0.02*
1000000	20. PRODUCTS OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS	
1010000	(i) Tissue	0.05*
1011000	(a) Swine	0.05*
1011010	Muscle	0.05*
1011020	Fat	0.05*
1011030	Liver	0.2 (incl. CF = 4)
1011040	Kidney	0.05*
1011050	Edible offal	0.05*
1011990	Others	0.05*
1012000	(b) Bovine	0.05*
1012010	Muscle	0.05*
1012020	Fat	0.05*
1012030	Liver	0.2 (incl. CF = 4)
1012040	Kidney	0.05*
1012050	Edible offal	0.05*
1012990	Others	0.05*
1013000	(c) Sheep	0.05*
1013010	Muscle	0.05*
1013020	Fat	0.05*
1013030	Liver	0.2 (incl. CF = 4)
1013040	Kidney	0.05*
1013050	Edible offal	0.05*
1013990	Others	0.05*

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
1014000	(d) Goat	0.05*
1014010	Muscle	0.05*
1014020	Fat	0.05*
1014030	Liver	0.2 (incl. CF = 4)
1014040	Kidney	0.05*
1014050	Edible offal	0.05*
1014990	Others	0.05*
1015000	(e) Horses, asses, mules or hinnies	0.05*
1015010	Muscle	0.05*
1015020	Fat	0.05*
1015030	Liver	0.2 (incl. CF = 4)
1015040	Kidney	0.05*
1015050	Edible offal	0.05*
1015990	Others	0.05*
1016000	(f) Poultry chicken, geese, duck, turkey and Guinea fowl, ostrich, pigeon	0.05*
1016010	Muscle	0.05*
1016020	Fat	0.05*
1016030	Liver	0.05*
1016040	Kidney	0.05*
1016050	Edible offal	0.05*
1016990	Others	0.05*
1017000	(g) Other farm animals (Rabbit, kangaroo, deer)	0.05*
1017010	Muscle	0.05*
1017020	Fat	0.05*
1017030	Liver	0.05*
1017040	Kidney	0.05*
1017050	Edible offal	0.05*
1017990	Others	0.05*
1020000	(ii) Milk	0.01*
1020010	Cattle	0.01*
1020020	Sheep	0.01*
1020030	Goat	0.01*
1020040	Horse	0.01*
1020990	Others	0.01*

Table 6.9-5: Pyraclostrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply (a)	Pyraclostrobin (F) EU MRL
1030000	(iii) Bird eggs	0.05*
1030010	Chicken	0.05*
1030020	Duck	0.05*
1030030	Goose	0.05*
1030040	Quail	0.05*
1030990	Others	0.05*
1040000	(iv) Honey (Royal jelly, pollen, honey comb with honey (comb honey))	0.05*
1050000	(v) Amphibians and reptiles (Frog legs, crocodiles)	0.05*
1060000	(vi) Snails	0.05*
1070000	(vii) Other terrestrial animal products (Wild game)	0.05*

With the current EFSA model the chronic risk assessment ranges from 10 to 77% of ADI (see Table 6.9-6). The diet with the highest TMDI is "DE child" with 77% of ADI. For this diet, the highest contributors are oranges with 25.4% of ADI. The diet with the second highest TMDI is “NL child” with 60.1% of ADI, in which also oranges are the major contributor with 20.8% of ADI.

According to the presented TMDI calculation a chronic intake of pyraclostrobin residues is unlikely to present a public health concern.

Table 6.9-6: Pyraclostrobin (BAS 500 F): TMDI calculation based on input values listed in Table 6.9-1

Pyraclostrobin			
Status of the active substance:		Code no.	
LOQ (mg/kg bw):	0.02	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	0.03	ARfD (mg/kg bw):	0.03
Source of ADI:	04/30/EC	Source of ARfD:	04/30/EC
Year of evaluation:	2004	Year of evaluation:	2004

Prepare workbook for refined calculations

Undo refined calculations

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum	
10	77
No of diets exceeding ADI:	—

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
77.0	DE child	25.4	Oranges	21.2	Pome fruit	4.2	Table grapes	0.9
60.1	NL child	20.8	Oranges	11.3	Pome fruit	3.2	Wheat	1.6
55.6	WHO Cluster diet B	11.9	Wine grapes	5.7	Wheat	5.7	Oranges	0.9
49.6	IE adult	8.3	Wine grapes	7.0	Oranges	4.1	Barley	1.0
46.0	UK Toddler	15.2	Sugar beet (root)	13.2	Oranges	3.1	Pome fruit	1.1
45.9	FR all population	26.7	Wine grapes	6.3	Other lettuce and other salad plants	2.2	Wheat	0.2
37.7	FR toddler	13.3	Oranges	4.8	Pome fruit	4.1	Carrots	1.9
35.2	WHO cluster diet E	10.7	Wine grapes	3.0	Oranges	2.7	Barley	0.6
33.8	PT General population	16.6	Wine grapes	4.1	Oranges	2.6	Wheat	0.5
31.7	ES child	14.4	Oranges	3.0	Wheat	2.8	Lettuce	0.7
31.2	UK infant	8.7	Oranges	6.7	Sugar beet (root)	3.0	Pome fruit	1.8
29.4	NL general	9.9	Oranges	4.2	Wine grapes	2.3	Pome fruit	0.5
27.7	WHO Cluster diet F	5.8	Oranges	4.0	Wine grapes	2.4	Wheat	0.5
26.9	ES adult	8.6	Oranges	3.6	Lettuce	2.8	Wine grapes	0.4
26.4	WHO cluster diet D	4.3	Wheat	2.5	Onions	2.4	Wine grapes	0.6
26.2	DK child	5.0	Pome fruit	3.7	Wheat	2.9	Rye	0.7
24.5	WHO regional European diet	3.3	Oranges	2.5	Lettuce	2.2	Onions	0.6
24.5	FR infant	6.1	Oranges	4.7	Pome fruit	4.4	Carrots	1.3
24.0	SE general population 90th percentile	5.0	Oranges	2.4	Pome fruit	2.3	Onions	0.9
23.4	UK vegetarian	5.8	Oranges	5.4	Wine grapes	2.5	Sugar beet (root)	0.3
23.3	IT kids/toddler	4.4	Wheat	3.7	Other lettuce and other salad plants	3.2	Oranges	0.3
22.1	IT adult	5.3	Other lettuce and other salad	2.6	Wheat	2.5	Lettuce	0.2
20.8	UK Adult	7.2	Wine grapes	3.7	Oranges	2.7	Sugar beet (root)	0.3
18.8	DK adult	9.3	Wine grapes	1.7	Pome fruit	1.3	Wheat	0.3
17.1	FI adult	6.5	Oranges	2.0	Wine grapes	0.8	Currants (red, black and white)	0.3
12.0	PL general population	3.9	Pome fruit	1.6	Onions	1.1	Table grapes	0.3
10.0	LT adult	3.4	Pome fruit	0.7	Rye	0.7	Wheat	0.4

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of Pyraclostrobin is unlikely to present a public health concern.

NEDI calculation

In order to check the contribution of fish, a dietary exposure assessment was performed using the German consumption data as published in 2012. Besides the child, the revised spreadsheet contains two additional consumer groups (total population: age group 14 – 80 years, females in child bearing age: 14 – 50 years).

Three assessments are performed; in accordance with the EFSA PRIMo model, the % ADI utilizations are based on mean body weights.

- In the first assessment, the same input values as for PRIMo were entered into the calculation.
- In the second assessment, the contribution of fish (at 0.05 mg/kg) was investigated.
- In the third assessment, the overall assessment was performed.

The NEDI calculation showed the low contribution of fish commodities to the chronic dietary risk for pyraclostrobin. The data clearly demonstrates that the presence of pyraclostrobin in fish does not result in any consumer risk. Compared to all other commodities, the contribution of fish to the chronic exposure is even under worst case assumptions rather small.

Table 6.9-7: NEDI calculations using the German model (NVS2_V_0-9_DE1)

Subpopulation group	% ADI utilization based on current MRLs	% ADI utilization fish commodities	% ADI utilization (total)
German child (VELS)	77	0.1	77.1
General population	36.8	0.1	36.9
Women in child bearing age	39.2	0.1	39.3

Metabolites

Metabolites identified in crop and livestock metabolism studies

The main purpose of the information presented below is to support the establishment of a robust residue definition for risk assessment purposes. Therefore, the data being summarized are going far beyond the scope of this dossier which supports the uses in potatoes, cereals and maize. In the assessment the contributions to the chronic dietary risk of all crops that are currently registered in the EU (including import tolerances) were taken into account.

For facilitating the evaluation of the exposure assessment performed, the relevant exposure data for the metabolites are derived in a separate report.

Report:	CA 6.9/1 Bross M., Mackenroth C., 2014a Pyraclostrobin (BAS 500 F): Refinement of the dietary exposure assessment of Pyraclostrobin metabolites in plant and animal commodities 2014/1001541
Guidelines:	<none>
GLP:	no

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	BAS 500 F (pyraclostrobin, Reg No. 304428)
	Description:	not relevant
	Lot/Batch #:	not relevant
	Purity:	not relevant
	CAS#:	175013-18-0
	Development code:	not applicable
	Spiking levels:	not relevant

3.	**Test Commodity:**	
	Crop:	not relevant
	Type:	not relevant
	Variety:	not relevant
	Botanical name:	not relevant
	Crop parts(s) or processed commodity:	not relevant
	Sample size:	not relevant

B. STUDY DESIGN AND METHODS

In order to assess the contribution of the metabolites to the chronic dietary risk, the metabolites being present in the plant and livestock metabolism studies were grouped into six groups. The grouping was performed based on sub structural elements.

- Group 1: Desmethoxy metabolite 500M07 (synonym: BF 500-3)
- Group 2: Chlorphenyl pyrazole derivatives
- Group 3: Anthranilic acid derivatives
- Group 4: Hydroxylated metabolites (chlorphenyl pyrazole moiety)
- Group 5: Hydroxylated metabolites (tolyl moiety)
- Group 6: Photo metabolite 500M76

Depending on the availability of exposure data, the input data for the exposure assessment are derived differently for the individual groups.

Desmethoxy metabolite 500M07:
The residue levels of the metabolite 500M07 were quantified in the big majority of the samples generated in the course of the supervised field trials and processing studies. Consequently, the actual values measured were taken into account. In case of food of animal origin, the input values were derived from the metabolism studies.

Chlorophenyl pyrazole derivatives (500M04 and conjugates, 500M85):
The residue levels of the metabolite 500M04 and its glycoside were quantified in selected samples from supervised field trials. For the selection of appropriate samples, the metabolism studies had been re-evaluated before. The metabolite 500M04 and its conjugates were mainly found in the green parts (forage or green matter samplings) of the plants. Consequently, the actual values measured were taken into account. In case of food of animal origin, the input values were derived from the metabolism and the feeding studies. The metabolites 500M04 and 500M85 are covered by the common moiety method used during the analysis of the cow feeding study.

Anthranilic acid derivative 500M24:
The metabolite 500M24 was exclusively identified in grain within the wheat metabolism. The exposure estimate for cereal grains (w/o rice) is based on the calculation of the metabolite / parent ratio from the ^{14}C study.

Anthranilic acid derivative 500M49:
The metabolite 500M49 was detected in the hydrolysis study at exaggerated temperatures (simulating oil deodorization) and in the hen metabolism study. As it was not measured in any residue / feeding study, the potential exposure has been estimated based on the ^{14}C studies.

Anthranilic acid derivative 500M51:
The metabolite 500M51 was exclusively found in the goat metabolism study in kidney. For estimating the amounts being present at a realistic feed burden, the mg/kg values which are expressed in parent equivalents were converted into metabolite levels and adjusted by the overdosing factor.

Hydroxylated metabolites (chlorphenyl pyrazole moiety):
Group 4 type metabolites were found in plant and livestock metabolism studies. As they were not measured in any residue / feeding study, the potential exposure has been estimated based on the ^{14}C studies. For avoiding a considerable overestimation of the exposure, the relevant metabolism studies were carefully re-evaluated. Hydroxylated metabolites were found in the grape metabolism study (fruits) and in green matter samples. The exposure estimate is based on the calculation of the metabolite / parent ratios from the ^{14}C studies. The individual values were finally summed up and compared with the parent ADI.

Hydroxylated metabolites (tolyl moiety):
For group 5 type metabolites the same approach was selected as for group 4.

Photo metabolite 500M76:
The metabolite 500M76 was identified in forage and straw samples within the wheat metabolism. The exposure estimate for leafy crops is based on the calculation of the metabolite / parent ratio.

II. RESULTS AND DISCUSSION

Desmethoxy metabolite 500M07:
With the current EFSA model the chronic risk assessment ranges from 0.3 to 1.7% of ADI (see chapter 5.1.1 of DocID 2014/1001541). The diet with the highest TMDI is "WHO Cluster Diet B" with 1.7% of ADI. For this diet, the highest contributor is wheat with 0.6% of ADI. The diet with the second highest TMDI is “DE child” with 1.7% of ADI, in which pome fruits are the major contributor with 0.8% of ADI.

Chlorophenyl pyrazole derivatives (500M04 and conjugates, 500M85):
With the current EFSA model the chronic risk leads to no values above 0.0% of ADI (see chapter 5.1.2 of DocID 2014/1001541).

Anthranilic acid derivative 500M24:
With the current EFSA model the chronic risk assessment ranges from 0 to 8.9% of ADI (see chapter 5.1.3.1 of DocID 2014/1001541). The diet with the highest TMDI is "IE Adult" with 8.9% of ADI. For this diet, the highest contributor is barley with 8.3% of ADI. The diet with the second highest TMDI is “WHO Cluster Diet E” with 6.3% of ADI, in which also barley is the major contributor with 5.4% of ADI.

Anthranilic acid derivative 500M49:
With the current EFSA model the chronic risk assessment ranges from 0.0 to 0.1% of ADI (see chapter 5.1.3.2 of DocID 2014/1001541). The diet with the highest TMDI is "WHO Cluster Diet E" with 0.1% of ADI. For this diet, the highest contributor is rape seed with 0.1% of ADI. The diet with the second highest TMDI is “WHO Cluster Diet B” with 0.1% of ADI, in which sunflower seed is the major contributor with 0.1% of ADI.

Anthranilic acid derivative 500M51:
The estimation of the metabolite in food of animal origin resulted in median residue levels far below 0.001 mg/kg. Due to negligible exposure, no chronic exposure assessment was performed.

Hydroxylated metabolites (chlorphenyl pyrazole moiety):
With the current EFSA model the chronic risk assessment ranges from 0.1 to 0.6% of ADI (see chapter 5.1.4 of DocID 2014/1001541). The diet with the highest TMDI is "DE child" with 0.6% of ADI. For this diet, the highest contributors are pome fruits with 0.3% of ADI. The diet with the second highest TMDI is “NL child” with 0.5% of ADI, in which also pome fruits are the major contributor with 0.2% of ADI.

Hydroxylated metabolites (tolyl moiety):
With the current EFSA model the chronic risk assessment ranges from 0.1 to 0.6% of ADI (see chapter 5.1.5 of DocID 2014/1001541). The diet with the highest TMDI is "DE child" with 0.6% of ADI. For this diet, the highest contributors are pome fruits with 0.3% of ADI. The diet with the second highest TMDI is “WHO Cluster Diet B” with 0.4% of ADI, in which wine grapes are the major contributor with 0.2% of ADI.

Photo metabolite 500M76:
With the current EFSA model the chronic risk assessment ranges from 0 to 0.2% of ADI (see chapter 5.1.6 of DocID 2014/1001541). The diet with the highest TMDI is "WHO Cluster Diet B" with 0.2% of ADI. For this diet, the highest contributor is lettuce with 0.1% of ADI. The diet with the second highest TMDI is “FR toddler” with 0.2% of ADI, in which leek is the major contributor with 0.1% of ADI.

III. CONCLUSION

Pyraclostrobin follows a common pathway in crops and livestock. In general, the following metabolic key steps were found in all commodities investigated.

- Desmethoxylation of the side chain resulting in the metabolite 500M07
- Cleavage between the ring systems resulting in chlorophenyl pyrazole derivatives
- Cleavage between the ring systems resulting in anthranilic acid derivatives
- Hydroxylation of the chlorophenyl pyrazole moiety (followed by conjugation)
- Hydroxylation of the tolyl moiety (followed by conjugation)
- Photolytic rearrangement reaction resulting in the metabolite 500M76.

For performing indicative assessments, the metabolites were grouped according to sub-structures. In a second step, the exposure was estimated based on all available data (combination of metabolism information and data from residue field trials / feeding studies). Subsequently, chronic dietary exposure assessments were performed for identifying the contributions of the metabolites to the total dietary risk. The exposure estimates applying worst case assumptions (for vegetables: no consideration of processing) did not indicate any dietary concern. The calculation of the % ADI utilizations resulted in values far below 100% and – even more important - clearly below the ones of the parent molecule. No significant contribution to the chronic exposure can be expected from any plant or livestock metabolite.

Metabolites identified in the *in-vitro* comparison study

TMDI calculation

Based on the study results of an *in-vitro* comparison study with cell cultures from different species, it cannot be excluded that humans might be exposed to metabolites 500M02, 500M106 and 500M107 (glucuronic acid conjugate of 500M106) after consumption of food containing pyraclostrobin residues. The "disproportionate" metabolites under consideration are not quantitatively formed from parent. In the *in-vitro* study, further metabolites were found. Metabolites as 500M04, 500M73, 500M108, 500M103, 500M104 and 500M88 are common to all test species and do not require any separate assessment.

For the time being no guidance document exists how to perform a dietary risk assessment for such metabolites. However, as metabolite 500M106 (covering 500M02 and 500M107) showed a considerable lower toxicity in the 28-day rat study compared to parent, no separate dietary risk assessment should be needed. The assessment is covered by the chronic exposure estimate provided for the parent molecule.

The following publication was added as supplemental information to this dossier. The metabolic behaviour of pyraclostrobin and the metabolite BF 500-3 (synonym: 500M07) in plants was investigated over 10 days. It shows the metabolization of the parent molecule, the formation of the metabolite BF 500-3 at intermediate sampling periods and its fast degradation. It supports BASF's proposal not to include the metabolite BF 500-3 into the residue definition for MRL setting / risk assessment purposes.

Report:	CA 6.9/2 Xiangwei Y. et al., 2012a Dissipation of Pyraclostrobin and its metabolite BF-500-3 in maize under field conditions 2012/1366722
Guidelines:	none
GLP:	no

Executive Summary

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Pyraclostrobin, BF 500-3

Description:	250 g/L, EC formulation, not further specified
Lot/Batch #:	not applicable
Purity:	Pyraclostrobin: 99.6% BF 500-3: 99.7%
CAS#:	Pyraclostrobin: 175013-18-0 BF 500-3: 512165-96-7
Development code:	not applicable
Spiking levels:	0.01-1.0 mg/kg

2. Test Commodity: Cereals

Crop:	Maize
Type:	not relevant
Variety:	not applicable
Botanical name:	*Zea mays L.*
Crop parts(s) or processed commodity:	maize plant and maize grain
Sample size:	not reported

B. STUDY DESIGN AND METHODS

1. Test procedure

Two experiments were conducted in China (Beijing and Shandong) in the year 2010. In the first experiment each treatment consisted of three replicate plots and a control plot. In order to investigate the dissipation of pyraclostrobin in maize plant, a pyraclostrobin containing formulation (250 g/L EC) was sprayed at a dosage of 0.422 kg a.s./ha in the experiment plots when the maize plant was at 8-10 leaf stage (BBCH 18-19).
The terminal residues of pyraclostrobin in maize grain were investigated in a second experiment. The recommended dosage (0.282 kg a.s./ha) was sprayed 3 times in the first treatment and 4 times in the second treatment. Each treatment consisted one plot. There was a 7 days interval between two applications.
Representative samples were collected at random from each plot at different time intervals. To investigate the dissipation of pyraclostrobin, the maize plant samples were collected 2h, 12h, 1, 3, 5, 7 days after spraying. To determine the terminal residue of pyraclostrobin, both maize grain and maize plant samples were collected at the harvest time (7 and 10 days after the last spraying). All samples were homogenized and stored at -20°C prior to analysis.

2. Description of analytical procedures

The specimens were analyzed for residues of pyraclostrobin and its metabolite BF 500-3. Homogenized samples were extracted with acetonitrile. An aliquot of the extract was centrifuged and clean-up was achieved with C_{18}. The determination of the analytes was performed by HPLC-MS/MS. In context of analysis, a Zorbax XDB C18 column was used. Isocratic elution was performed using an eluent system consisting of methanol and water (90:10, v:v).
The method has a limit of quantification (LOQ) of 0.002 mg/kg for both analytes in maize grain. In maize plant the LOQ was 0.02 mg/kg for pyraclostrobin and 0.01 mg/kg for BF 500-3.
The average recoveries of pyraclostrobin and BF 500-3 were found in the range of 83.6 to 104.9% with relative standard deviations (RSDs) of 2.3 to 10.0%. Further details are given in Table 6.9-8.

Table 6.9-8: Summary of recoveries

Matrix	Fortification Level (mg/kg)	Summary Recoveries							
		n	Mean (%)	SD (+/-)	RSD (%)	n	Mean (%)	SD (+/-)	RSD (%)
		Pyraclostrobin				BF 500-3			
Maize plant	0.01-0.02	5	87.3	n r.	10.0	5	90.4	n.r.	2.5
	0.2	5	95.1	n r.	5.7	5	94.8	n.r.	4.3
	1.0	5	94.8	n r.	4.0	5	98.0	n.r.	4.7
Maize grain	0.002	5	89.4	n r.	3.5	5	83.6	n.r.	3.7
	0.02	5	104.9	n r.	2.3	5	96.5	n.r.	2.5
	0.1	5	99.2	n r.	3.2	5	97.7	n.r.	6.4

n r. not reported

II. RESULTS AND DISCUSSION

The initial concentrations of total pyraclostrobin (sum of parent pyraclostrobin and its desmethoxy metabolite BF 500-3) in maize plant were 6.43 and 6.54 mg/kg in Shandong and Beijing with half-lives of 1.7 days in both plots. The initial concentrations of parent pyraclostrobin in maize plant were 6.32 and 6.42 mg/kg in Shandong and Beijing with half-lives of 1.7 days and 1.6 days, respectively. Concentrations of both, total pyraclostrobin and parent pyraclostrobin, were reduced more than 90% on day 7 in both Shandong and Beijing. The initial residues of metabolite BF 500-3 in maize plant were 0.012 mg/kg in Shandong and 0.11 mg/kg and Beijing. However, the maximum concentrations emerged at day 1 and day 3 in Beijing and Shandong, respectively.
The terminal residues of pyraclostrobin at harvest time in the maize grain samples were below LOQ and no BF 500-3 residues were detected. The terminal residues of pyraclostrobin and BF 500-3 in maize plant were 0.47-4.91 and 0.063-0.73 mg/kg.

III. CONCLUSION

The results showed, that parent pyraclostrobin dissipated rapidly in maize plant, while the metabolite BF 500-3 (synonym: 500M07) increased first and then decreased continuously.
The terminal residues of pyraclostrobin at harvest time in the maize grain samples were below LOQ and no BF 500-3 residues were detected. The terminal residues of pyraclostrobin and BF 500-3 in maize plant were 0.47-4.91 and 0.063-0.73 mg/kg. These results are indicating that the application of pyraclostrobin at the dosage recommended in China is safe.
The terminal residue results indicated that the residue of pyraclostrobin in maize grain was much lower than the MRL set by USA and Canada. This work will give a reference for the establishment of MRLs in China, and also provide guidance for the safe and reasonable use of pyraclostrobin.

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

Pyraclostrobin

For the assessment, an ARfD of 0.03 mg/kg bw/day was used. The calculation was done with the default variability factor. The following MRLs for the representative crops of the active substance pyraclostrobin were considered as reported in Table 6.9-5:

Potatoes: 0.02 mg/kg
Sweet corn: 0.07 mg/kg
Barley: 1.0 mg/kg
Maize: 0.02 mg/kg
Wheat: 0.2 mg/kg

The evaluation resulted in ARfD utilizations well below 100% for all representative crops, see Table 6.9-9.

Furthermore, potatoes and cereal grains are traded as bulk commodity and are always consumed after processing. Both steps result in a significant decrease of the residue levels. No acute risk can be expected from the consumption of potato and cereal processed fractions.

In context of this dossier, MRLs for fish are proposed for the first time. In order to check the contribution of fish to acute exposure, an indicative assessment has been performed. The proposed MRL of 0.05 mg/kg was entered into the German model as it includes the relevant consumption data. The maximum % ARfD utilizations are comparable for all subpopulation groups (child, total population, women in child bearing age); maximums of about 4.5% are reached for shell fish and freshwater fish, respectively.

Table 6.9-9: NESTI calculation of pyraclostrobin (EFSA model rev. 2), based on established EU-MRLs and default variability factors

Acute risk assessment /children						Acute risk assessment / adults / general population					
The acute risk assessment is based on the ARfD.											
For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the ESTI calculation.											
In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used. In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3.											
Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.											
No of commodities for which ARfD/ADI is exceeded (IESTI 1)		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2)**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 1)**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2)**		---
IESTI 1	*)	**)	**IESTI 2**	*)	**)	**IESTI 1**	*)	**)	**IESTI 2**	*)	**)
Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
17.1	Sweet corn	0.07 / -	12.2	Sweet corn	0.07 / -	24.1	Barley	1 / -	24.1	Barley	1 / -
10 3	Potatoes	0.02 / -	9.6	Wheat	0.2 / -	5.2	Wheat	0.2 / -	5 2	Wheat	0.2 / -
9.6	Wheat	0.2 / -	7.3	Potatoes	0.02 / -	5.1	Sweet corn	0.07 / -	3.6	Sweet corn	0.07 / -
5.9	Barley	1 / -	5.9	Barley	1 / -	2.0	Potatoes	0.02 / -	1 6	Potatoes	0.02 / -
0.4	Maize	0.02 / -	0.4	Maize	0.02 / -	0.1	Maize	0.02 / -	0.1	Maize	0.02 / -
No of critical MRLs (IESTI 1)			—			**No of critical MRLs (IESTI 2)**			—		

Metabolites

Metabolites identified in crop and livestock metabolism studies

The main purpose of the information presented below is to support the establishment of a robust residue definition for risk assessment purposes. Therefore, the data being summarized are going far beyond the scope of this dossier which supports the uses in potatoes, cereals and maize. The safety of pyraclostrobin parent residues in various crops has been already assessed by Germany / EFSA during the establishment of MRLs. In the re-evaluation according to Regulation 396/2005, Art. 12, the previous assessments have been re-confirmed (Review of the existing maximum residue levels (MRLs) for pyraclostrobin according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2011;9(8):2344. [92 pp.]doi:10.2903/j.efsa.2011.2344).

In the assessment performed the contribution of the metabolites to the acute dietary risk of all crops being currently registered in Europe (including import tolerances) was taken into account.

In order to assess the contribution of the metabolites to the acute dietary risk, the metabolites being present in the plant and livestock metabolism studies were grouped into six groups. The grouping was performed based on sub structural elements; in general, they are identical for chronic and for acute assessment.

- Group 1: Desmethoxy metabolite 500M07 (synonym: BF 500-3)
- Group 2: Chlorphenyl pyrazole derivatives
- Group 3: Anthranilic acid derivatives
- Group 4: Hydroxylated metabolites (chlorphenyl pyrazole moiety)
- Group 5: Hydroxylated metabolites (tolyl moiety)
- Group 6: Photo metabolite 500M76

In deviation to the chronic assessment, acute assessments are not required for all groups. They are provided for group 1 and selected metabolites from group 3, 4, 5 and 6. The input values to be used have been derived as described above.

The exposure estimates applying worst case assumptions (for vegetables: no consideration of processing) did not indicate any acute dietary concern. The calculation of the % ARfD utilizations resulted in values far below 100% and – even more important clearly below the % ARfD of the parent molecule. No significant contribution to the acute exposure can be expected from any plant or livestock metabolite.

Desmethoxy metabolite 500M07:
For the assessment, an ARfD of 0.03 mg/kg bw/day was used. The calculation was done with the default variability factors, except of pome fruits and table grapes in the IESTI2 calculation, for which a variability factor of 3 was applied (already accepted by EFSA, see EFSA Journal 2011;9(8):2344).
The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for celery (29.1%), followed by onions (18.1%) and cucumbers (17.5%).

Anthranilic acid derivative 500M49:
For the assessment, an ARfD of 0.005 mg/kg bw/day was used. The calculation was done with the default variability factors. As a result, no ARfD utilization above 0.0% was determined.

Anthranilic acid derivative 500M51:
For the assessment, an ARfD of 0.005 mg/kg bw/day was used. The calculation was done with the default variability factors.
The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for bovine kidney (0.2%), followed by swine kidney (0.1%).

Hydroxylated metabolites (chlorphenyl pyrazole moiety):
For the assessment, an ARfD of 0.03 mg/kg bw/day was used. The calculation was done with the default variability factors, except of pome fruits and table grapes in the IESTI2 calculation, for which a variability factor of 3 was applied (already accepted by EFSA, see EFSA Journal 2011;9(8):2344).
The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization (IESTI2) was observed for melons (8.1%), followed by watermelons (6.5%) and table grapes (5.2%).

Hydroxylated metabolites (tolyl moiety):
For the assessment, an ARfD of 0.03 mg/kg bw/day was used. The calculation was done with the default variability factors, except of pome fruits and table grapes in the IESTI2 calculation, for which a variability factor of 3 was applied (already accepted by EFSA, see EFSA Journal 2011;9(8):2344).
The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization (IESTI2) was observed for melons (8.1%), followed by kale (7.9%) and watermelons (6.5%).

Photo metabolite 500M76:
For the assessment, an ARfD of 0.005 mg/kg bw/day was used. The calculation was done with the default variability factors.
The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization (IESTI2) was observed for kale (10.6%), followed by Chinese cabbage (8.2%) and globe artichokes (6.3%).

Metabolites identified in the *in-vitro* comparison study

Based on the study results from an *in-vitro* comparison study with cell cultures from different species, it cannot be excluded that humans might be exposed to the metabolites 500M02, 500M106 and 500M107 (glucuronic acid conjugate of 500M106) after consumption of food containing pyraclostrobin residues. The "disproportionate" metabolites under consideration are not quantitatively formed from parent. In the *in-vitro* study, further metabolites were found. Metabolites as 500M04, 500M73, 500M108, 500M103, 500M104 and 500M88 are common to all test species and do not require any separate assessment.
For the time being, no guidance document exists how to perform a dietary risk assessment for such metabolites. However, as metabolite 500M106 (covering 500M02 and 500M107) showed a considerable lower acute toxicity compared to parent, no separate dietary risk assessment should be needed. The assessment is covered by the exposure estimates provided for the parent molecule.

CA 6.10 Other studies

No other/special studies are deemed necessary. The studies and information provided in the previous sections are considered adequate and sufficient.

CA 6.10.1 Effect on the residue level in pollen and bee products

Residues in honey are expected when plant protection products have been applied according to Good Agricultural Practice (GAP) in the following cases:

- during the blossom stage of the crop or non-target plants that may be affected by the use of the plant protection product, or
- in cases where the active substance of the plant protection product has systemic properties and can be translocated into pollen and/or nectar and application occurs prior to the flowering stage (before BBCH 59) of the target or non-target plant, or
- via honeydew collected on plant-sucking insects.

The representative uses of this dossier are potatoes, cereals and maize incl. sweet corn. Due to the lack of an appropriate accepted guidance document, but also driven by expected low consumer exposure from pyraclostrobin residues via honey, no study has been performed.

Honey is considered as only relevant matrix in context of dietary exposure and of potential MRL setting. The consumer risk in honey resulting from the uses of pyraclostrobin is considered as negligible due to the following reasons.

- Honey is defined as the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants, from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which bees collect, transform by combining with specific substances on their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature.
- The draft guidance document for MRL setting in honey (SANCO 11105/2009 rev. 01) includes a decision tree which allows to evaluate by when residues in honey could be expected.
- According to the document, as first step the attractiveness of a crop for bees should be assessed. A given crop is more or less attractive to bees according to availability, quantity, quality of pollen and/or nectar (as well as that of honeydew).
- In Appendix III of the document, a table is provided which gives an indication of the possibility of producing honey from available nectar and/or honeydew in crops.
- Neither nectar nor honeydew are present in potatoes or cereals (including corn) No specific MRL is needed for these cases.
- Pyraclostrobin is a non-systemic and low water soluble fungicide. These properties have been confirmed in metabolism but also in residue studies.
- Furthermore, pollination of cereals (including corn) occurs by wind and not by bees.
- Due to both reasons, it is unlikely that there is any direct transfer of pyraclostrobin residues from maize pollen into honey.

Tier 1 Summaries of the Supervised Field Residue Trials for the Representative Crops

Potatoes

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Potatoes	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 510 F
Content of active substance (g/kg or g/L)	67 g/kg	(common name and content)	Boscalid 267 g/kg
Formulation (e.g. WP)	BAS 516 00 F (WG)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
160753	VR 0589	1. 17.04.03	Agrotop	0.0134	150	0.0201	4	48	tuber	<0.02	<0.02	<0.04	0	BASF Method
2004/1015948	Astate	2. 23.06.-20.07.03	biocycle				05.09.03		tuber	<0.02	<0.02	<0.04	3	No. 445/0
16833 Brunne		3. 29.09.-02.10.03	mounted						tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
Germany			boom						tuber	<0.02	<0.02	<0.04	13	
ACK/07/03			sprayer											
160753	VR 0589	1. 15.04.03	portable	0.0134	150	0.0201	4	48	tuber	<0.02	<0.02	<0.04	0	BASF Method
2004/1015948	Gloria	2. 10.05.-20.05.03	VCR				26.06.03		tuber	<0.02	<0.02	<0.04	4	No. 445/0
6595 MS Ottersum		3. 07.07.-13.07.03	knapsack						tuber	<0.02	<0.02	<0.04	8	LOQ 0.02 mg/kg
The Netherlands			boom						tuber	<0.02	<0.02	<0.04	14	
AGR/04/03			sprayer											
160753	VR 0589	1. 09.05.03	AZO	0.0134	150	0.0201	4	97	tuber	<0.02	<0.02	<0.04	0	BASF Method
2004/1015948	Save	2. 17.07.-29.07.03	knapsack				28.08.03		tuber	<0.02	<0.02	<0.04	4	No. 445/0
5500 Middelfart		3. 01.09.-30.09.03	precision						tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
Denmark			plot						tuber	<0.02	<0.02	<0.04	14	
ALB/09/03			boom											

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Potatoes	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 510 F
Content of active substance (g/kg or g/L)	67 g/kg	(common name and content)	Boscalid 267 g/kg
Formulation (e.g. WP)	BAS 516 00 F (WG)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
160753	VR 0589	1. 17.04.03	ATH boom	0.0134	150	0.0201	4	47	tuber	<0.02	<0.02	<0.04	0	BASF Method
2004/1015948	Europa	2. 01.07.-15.07.03	sprayer 3m				31.07.03		tuber	<0.02	<0.02	<0.04	3	No. 445/0
67116 Handschuheim		3. 05.08.-18.08.03							tuber	<0.02	<0.02	<0.04	6	LOQ 0.02 mg/kg
France (N)									tuber	<0.02	<0.02	<0.04	12	
FAN/01/03														
160753	VR 0589	1. 30.04.03	Azo small	0.0134	150	0.0201	4	81	tuber	<0.02	<0.02	<0.04	0	
2004/1015948	Victoria	2. 01.07.-14.07.03	plot sprayer				05.08.03		tuber	<0.02	<0.02	<0.04	3	
OX27 9AS		3. 08.08.-09.08.03							tuber	<0.02	<0.02	<0.04	7	
Bichester									tuber	<0.02	<0.02	<0.04	14	
United Kingdom														
OAT/13/03														

1) at treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.02" is set 0.02

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Potatoes	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 510 F
Content of active substance (g/kg or g/L)	67 g/kg	(common name and content)	Boscalid 267 g/kg
Formulation (e.g. WP)	BAS 516 00 F (WG)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
160753	VR 0589	1. 27.03.03	ATH boom	0.0134	150	0.0201	4	49	tuber	<0.02	<0.02	<0.04	0	BASF Method
2004/1015948	Agatha	2. 29.05.-05.06.03	sprayer 3m				25.07.03		tuber	<0.02	<0.02	<0.04	3	No. 445/0
31330 midi-Pyrenées France (S) FTL/08/03		3. 28.07.-01.08.03							tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
									tuber	<0.02	<0.02	<0.04	14	

1) at treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.02" is set 0.02

Northern Europe

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Potatoes	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
210253	VR 0589	1. 18.04.2005	Agrotop	0.0333	300	0.1000	3	71	tuber	<0.02	<0.02	<0.04	0	BASF Method
2006/1000581	Kuras	2. 25.07.-11.08.05	Airmis						tuber	<0.02	<0.02	<0.04	3	No. 445/0
99867 Warza		3. 03.09.2005	PSP						tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
Germany			Agrartest						tuber	<0.02	<0.02	<0.04	15	
AT-05/006-1														
210253	VR 0589	1. 10.05.2005	Pulvexper	0.0333	300	0.1000	3	45	tuber	<0.02	<0.02	<0.04	0	BASF Method
2006/1000581	Agata	2. 08.07.-02.08.05	spray				22.07.2005		tuber	<0.02	<0.02	<0.04	3	No. 445/0
51240 Marson		3. 12.09.2005	boom with						tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
France (N)			nozzles						tuber	<0.02	<0.02	<0.04	14	
05 F PT FR P21														
210253	VR 0589	1. 29.04.2005	Pulvexper	0.0333	300	0.1000	3	47	tuber	<0.02	<0.02	<0.04	0	BASF Method
2006/1000581	Cesar	2. 16.08.-05.09.05	spray				07.09.2005		tuber	<0.02	<0.02	<0.04	2	No. 445/0
51320 Faux		3. 06.10.2005	boom with						tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
France (N)			nozzles						tuber	<0.02	<0.02	<0.04	15	
05 F PT FR P22														

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Potatoes	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
210253	VR 0589	1. 13.05.2005	RAM 3-10	0.0333	300	0.1000	3	47-48	tuber	<0.02	<0.02	<0.04	0	BASF Method
2006/1000581	Bintje	2. 15.07.-25.07.05					24.08.2005		tuber	<0.02	<0.02	<0.04	2	No. 445/0
1470 Baisy-Thy		3. 30.09.2005							tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
Belgium									tuber	<0.02	<0.02	<0.04	14	
G018-05 F-B														
210253	VR 0589	1. 17.05.2005	Spray boom	0.0333	300	0.1000	3	89	tuber	<0.02	<0.02	<0.04	0	BASF Method
2006/1000581	Hamlet	2. 15.07.-27.07.05	112 / air				30.08.2005		tuber	<0.02	<0.02	<0.04	3	No. 445/0
5500 Middelfart		3. 25.08.-30.09.05	assisted						tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
Denmark			Knapsack						tuber	<0.02	<0.02	<0.04	14	
ALB/190506-01			sprayer with 3m boom											

1) at treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.02" is set 0.02

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Potatoes	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
210253	VR 0589	1. 02.04.2005	Azo	0.0333	300	0.1000	3	47	tuber	<0.02	<0.02	<0.04	0	BASF Method
2006/1000581	Dalifa	2. 15.06.-10.07.05	pressurized				21.07.05		tuber	<0.02	<0.02	<0.04	3	No. 445/0
GR 57007		3. 10.08.-25.08.05	gas sprayer						tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
Chalkidona			with lance						tuber	<0.02	<0.02	<0.04	14	
Greece														
05RF048														
210253	VR 0589	1. 31.08.2005	Schachtner	0.0333	300	0.1000	3	47	tuber	<0.02	<0.02	<0.04	0	BASF Method
2006/1000581	Gliceta	2. n.a.	air				05.12.05		tuber	<0.02	<0.02	<0.04	4	No. 445/0
E-41808 Villanueva		3. 20.12.-28.12.05	compressed						tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
Spain			boom sprayer						tuber	<0.02	<0.02	<0.04	14	
05ES083R														

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Potatoes	Producer of commercial product	67056 Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
210253	VR 0589	1. 01.04.2005	Echo 4100	0.0333	300	0.1000	3	85-87	tuber	<0.02	<0.02	<0.04	0	BASF Method
2006/1000581	Primura	2. 17.05.-03.06.05	SHR				07.07.05		tuber	<0.02	<0.02	<0.04	4	No. 445/0
40054 Bagnarola di Budrio		3. 14.07.-20.07.05							tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
Italy									tuber	<0.02	<0.02	<0.04	14	
0538R														
210253	VR 0589	1. 01.03.2005	Pulver spray	0.0333	300	0.1000	3	47	tuber	<0.02	<0.02	<0.04	0	BASF Method
2006/1000581	Vitesse	2. 20.05.-06.06.05	boom with				20.06.05		tuber	<0.02	<0.02	<0.04	3	No. 445/0
84740 Velleron		3. 07.07.2005	nozzles						tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
France (S)									tuber	<0.02	<0.02	<0.04	14	
05 F PT FR P23														
210253	VR 0589	1. 17.08.2005	Pulvexper	0.0333	300	0.1000	3	47	tuber	<0.02	<0.02	<0.04	1	BASF Method
2006/1000581	Chérie	2. 20.09.-30.09.05	spray				01.11.05		tuber	<0.02	<0.02	<0.04	3	No. 445/0
64510 Boeil-Bezing		3. 15.11.2005	boom with						tuber	<0.02	<0.02	<0.04	7	LOQ 0.02 mg/kg
France (S)			nozzles						tuber	<0.02	<0.02	<0.04	14	
05 F PT FR P24														

1) at treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.02" is set 0.02
n.a. not applicable

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Potatoes / root and tuber vegetables	Producer of commercial product	BASF SE Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 510 F
Content of active substance (g/kg or g/L)	67 g/kg	(common name and content)	Boscalid 267 g/kg
Formulation (e.g. WP)	BAS 516 07 F (WG)	Residues calculated as:	BAS 500 F (pyraclostrobin) and its metabolite 500M07 calculated as parent equivalent

1	2	3	4	5			6	7	8	9			10	11
Study-No. Trial No. BASF DocID Location (region)	Commodity/ Variety	1. Date of: 2. Sowing/Planting 3. Flowering Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	g a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
773801 L150224 2016/1000547 France Vaucluse Velleron	VR 0589 Actrice	1. 12.03.2015 2. - 3. 18.07.2015	Experimental compressed air sprayer Pulexper	0.0056	300	16.75	4 03.07.2015	48	tuber tuber tuber tuber	<0.010 <0.010 <0.010 <0.010	<0.011 <0.011 <0.011 <0.011	<0.021 <0.021 <0.021 <0.021	0 3 7 14	BASF Method No. 535/01 LOQ 0.01 mg/kg
773801 L150225 2016/1000547 Greece Central Macedonia Thessaloniki	VR 0589 Jearla	1. 10.03.2015 2. - 3. 01.06.2015	Knapsack AZO sprayer with boom	0.0056	300	16.75	4 26.05.2015	48	tuber tuber tuber tuber	<0.010 <0.010 <0.010 <0.010	<0.011 <0.011 <0.011 <0.011	<0.021 <0.021 <0.021 <0.021	0 3 7 14	BASF Method No. 535/01 LOQ 0.01 mg/kg
773801 L150226 2016/1000547 Italy Bari Altamura	VR 0589 Spunta	1. 18.03.2015 2. - 3. 06.07.2015	Aluminium boom sprayer + compressed air pump	0.0056	300	16.75	4 03.07.2015	48	tuber tuber tuber tuber	<0.010 <0.010 <0.010 <0.010	<0.011 <0.011 <0.011 <0.011	<0.021 <0.021 <0.021 <0.021	0 3 7 13	BASF Method No. 535/01 LOQ 0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Potatoes / root and tuber vegetables	Producer of commercial product	BASF SE Ludwigshafen, Germany
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 510 F
Content of active substance (g/kg or g/L)	67 g/kg	(common name and content)	Boscalid 267 g/kg
Formulation (e.g. WP)	BAS 516 07 F (WG)	Residues calculated as:	BAS 500 F (pyraclostrobin) and its metabolite 500M07 calculated as parent equivalent

1	2	3	4	5			6	7	8	9			10	11
Study-No. Trial No. BASF DocID Location (region)	Commodity/ Variety	1. Date of: Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	g a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
773801	VR 0589	1. 07.05.2015	Boomsprayer	0.0056	300	16.75	4	48	tuber	<0.010	<0.011	<0.021	0	BASF Method
L150227	El Mundo	2. -					26.05.2015		tuber	<0.010	<0.011	<0.021	3	No. 535/01
2016/1000547		3. 08.06.2015							tuber	<0.010	<0.011	<0.021	7	LOQ 0.01 mg/kg
Spain									tuber	<0.010	<0.011	<0.021	13	
Seville														
Alcala del Rio														

1) at last treatment
2) expressed as parent equivalent, conversion factor for calculation of 500M07 to parent is 1.08
3) for residues < 0.01 mg/kg (for 500M07 < 0.011 mg/kg), value was set to 0.01 mg/kg (for 500M07 to 0.011 mg/kg) for calculation of sum

Wheat

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371232 2011/1135915 04668 Motterwitz Germany L100085	GC 0654 Cubus	1. 10.10.2009 2. 16.06.-21.06.2010 3. 03.08.2010	foliar application	0.125	200	0.25	2 21.06.10	69	whole plant[4] ears rest of plant[4] ears rest of plant[4] grain straw	5.55 0.22 1.98 0.38 2.66 <0.01 1.74	0.37 0.07 0.49 0.11 0.63 <0.01 0.76	5.92 0.29 2.47 0.49 3.29 <0.02 2.50	0 28 28 35 35 43 43	BASF Method L0076/01 LOQ=0.01 mg/kg
371232 2011/1135915 CO11 2NF Manningtree UK L100086	GC 0654 Solstice	1. 30.09.2010 2. 10.06.-24.06.2010 3. 05.08.2010	foliar application	0.125	200	0.25	2 24.06.10	69	whole plant[4] ears rest of plant[4] grain straw grain straw	2.41 0.63 4.31 0.02 5.76 0.02 4.02	0.23 0.15 1.20 <0.01 1.72 <0.01 1.37	2.64 0.78 5.51 0.03 7.48 0.03 5.39	0 28 28 35 35 42 42	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371232 2011/1135915 37230 Fondettes France (N) L100087	GC 0654 Mercato	1. 16.10.2009 2. 29.05.-07.06.2010 3. 19.07.2010	foliar application	0.125	200	0.25	2 07.06.10	69	whole plant[4] ears rest of plant[4] ears rest of plant[4] grain straw	2.65 0.34 3.88 0.36 3.27 0.02 3.01	0.26 0.08 0.80 0.10 0.83 <0.01 0.94	2.91 0.42 4.68 0.46 4.10 0.03 3.95	0 29 29 36 36 42 42	
371232 2011/1135915 4930 Maribo Denmark L100088	GC 0654 Frument	1. 26.09.2009 2. 28.06.-07.07.2010 3. 19.08.2010	foliar application	0.125	200	0.25	2 07.07.10	69	whole plant[4] ears rest of plant[4] ears rest of plant[4] grain straw	2.97 1.06 6.18 0.49 5.59 0.02 5.43	0.24 0.37 1.91 0.13 1.90 <0.01 2.22	3.21 1.43 8.09 0.62 7.49 0.03 7.65	0 29 29 36 36 43 43	
371236 2012/1067588 74193 Stetten a. H. Germany L110158	GC 0654 Asano	1. 01.11.2010 2. 24.05.-31.05.2011 3. 26.07.2011	foliar application	0.125	200	0.25	2 31.05.2011	69	whole plant[4] ears rest of plant[4] ears rest of plant[4] grain straw grain straw	4.90 0.04 0.15 0.05 0.23 <0.01 0.23 <0.01 0.30	0.19 0.02 0.07 0.02 0.11 <0.01 0.13 <0.01 0.20	5.09 0.06 0.22 0.07 0.34 <0.02 0.36 <0.02 0.50	0 29 29 36 36 42 42 56 56	BASF method L0076/01 LOQ=0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371236 2012/1067588 04668 Motterwitz Germany L110159	GC 0654 Asano	1. 11.11.2010 2. 01.06.-10.06.2011 3. 22.07.2011	foliar application	0.125	200	0.25	2 10.06.2011	69	whole plant[4] ears rest of plant[4] grain straw grain straw	5.86 0.12 1.57 0.14 1.98 <0.01 1.96	0.20 0.04 0.55 0.05 0.82 <0.01 0.86	6.06 0.16 2.12 0.19 2.80 <0.02 2.82	0 27 27 35 35 42 42	
371236 2012/1067588 CO11 2NF Manningtree UK L110160	GC 0654 Lomerit	1. 13.10.2010 2. 23.05.-09.06.2011 3. 27.07.2011	foliar application	0.125	200	0.25	2 09.06.2011	69	whole plant[4] ears rest of plant[4] ears rest of plant[4] ears rest of plant[4] grain straw	2.68 0.07 0.53 0.07 0.73 0.09 0.45 <0.01 0.51	0.15 0.03 0.17 0.03 0.26 0.05 0.20 <0.01 0.29	2.83 0.10 0.70 0.10 0.99 0.14 0.65 <0.02 0.80	0 27 27 34 34 41 41 48 48	
371236 2012/1067588 6595 ME, Ottersum The Netherlands L110161	GC 0654 Tabasco	1. 05.11.2010 2. 30.05.-10.06.2011 3. 02.08.2011	foliar application	0.125	200	0.25	2 09.06.2011	69	whole plant[4] ears rest of plant[4] ears rest of plant[4] grain straw grain straw	5.32 0.21 0.73 0.15 0.85 0.03 0.81 <0.01 1.30	0.15 0.08 0.28 0.05 0.28 0.01 0.38 <0.01 0.66	5.47 0.29 1.01 0.20 1.31 0.04 1.19 <0.02 1.96	0 27 27 35 35 41 41 54 54	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
401831 2012/1194991 2014/1090810 21614 Germany L110188	GC 0654 Julius	1. 24.09.10 2. 27.05.-07.06.11 3. 03.08.11	foliar application with boom sprayer	0.125	200	0.25	2 07.06.2011	69	whole plant[4]	5.4	0.28	5.7	0	BASF method L0076/01 LOQ=0.01 mg/kg
									ears	0.36	0.12	0.48	29	
									rest of plant[4]	1.3	0.38	1.7	29	
									ears	0.35	0.12	0.47	36	
									rest of plant[4]	1.4	0.61	2.0	36	
									ears	0.50	0.19	0.69	43	
									rest of plant[4]	1.9	0.82	2.7	43	
									grain	0.016	<0.01	0.026	57	
									straw	1.9	1.1	3.0	57	
401831 2012/1194991 2014/1090810 HU17 9SL UK L110189	GC 0654 Oakley	1. 05.09.10 2. n.a. 3. 16.08.11	foliar application with boom sprayer	0.125	200	0.25	2 20.06.11	69	whole plant[4]	3.9	0.14	4.1	0	
									ears	0.33	0.17	0.50	29	
									rest of plant[4]	1.7	0.63	2.3	29	
									grain	0.025	0.014	0.038	36	
									straw	2.1	0.95	3.1	36	
									grain	0.020	0.012	0.033	42	
									straw	5.5	1.97	7.4	42	
									grain	0.039	0.023	0.062	57	
									straw	2.5	1.2	3.7	57	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
419892	GC 0654	1. 05.11.2011	Boom-	0.125	200	0.25	2	69	whole plant[4]	3.7	0.21	3.9	0	BASF method
2013/1336790	Asano	2. 24.05.-05.06.2012	sprayer				05.06.2012		ears	0.068	0.028	0.095	28	L0076/01
74193		3. 01.08.2012							rest of plant[4]	0.27	0.10	0.37	28	LOQ=0.01 mg/kg
Stetten a. H.									ears	0.050	0.021	0.071	34	
Germany									rest of plant[4]	0.13	0.04	0.17	34	
L120232									grain	<0.01	<0.01	<0.02	42	
									straw	0.29	0.12	0.41	42	
									grain	<0.01	<0.01	<0.02	50	
									straw	0.41	0.16	0.57	50	
419892	GC 0654	1. 18.10.2011	Boom-	0.125	200	0.25	2	69	whole plant[4]	3.0	0.03	3.0	0	
2013/1336790	JB Diego	2. 05.07.-25.07.2012	sprayer				05.07.2012		grain	0.012	<0.01	0.022	28	
OX15 6EP		3. 16.08.-20.08.2012							straw	0.74	0.15	0.89	28	
Banbury									grain	<0.01	<0.01	<0.02	36	
UK									straw	0.84	0.21	1.0	36	
L120233									grain	<0.01	<0.01	<0.02	42	
									straw	1.3	0.30	1.6	42	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots
n.a. = not applicable

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371232 2011/1135915 17120 Floirac France (S) L100089	GC 0654 Euclide	1. 28.10.2009 2. 20.05.-30.05.2010 3. 08.07.2010	foliar application	0.125	200	0.25	2 28.05.10	69	whole plant[4]	2.74	0.18	2.92	0	BASF Method L0076/01 LOQ=0.01 mg/kg
									ears	0.11	0.04	0.15	28	
									rest of plant[4]	1.71	0.48	2.19	28	
									ears	0.18	0.06	0.24	35	
									rest of plant[4]	2.39	0.72	3.11	35	
									grain	<0.01	<0.01	<0.02	41	
									straw	2.26	1.11	3.37	41	
371232 2011/1135915 58300 Galatades Greece L100090	GC 0654 Svevo	1. 25.11.2009 2. 20.04.-30.04.2010 3. 07.06.2010	foliar application	0.125	200	0.25	2 27.04.10	69	whole plant[4]	4.99	0.51	5.50	0	
									ears	0.34	0.36	0.70	28	
									rest of plant[4]	1.46	0.77	2.23	28	
									ears	0.39	0.37	0.76	34	
									rest of plant[4]	1.89	0.98	2.87	34	
									grain	<0.01	<0.01	<0.02	41	
									straw	1.55	1.00	2.55	41	
371232 2011/1135915 20060 San Martino Olearo Italy L100091	GC 0654 Aubusson	1. 13.11.20009 2. 20.05.-28.05.2010 3. 30.06.2010	foliar application	0.125	200	0.25	2 26.05.10	69	whole plant[4]	4.98	0.17	5.15	0	
									ears	0.93	0.44	1.37	28	
									rest of plant[4]	3.48	1.17	4.65	28	
									grain	0.01	<0.01	0.02	35	
									straw	4.17	1.93	6.10	35	
									grain	0.01	<0.01	0.02	42	
									straw	2.83	1.44	4.27	42	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371232	GC 0654	1. 13.12.2009	foliar	0.125	200	0.25	2	69	whole plant[4]	6.10	0.73	6.83	0	
2011/1135915	Prospero	2. 15.04.-25.04.2010	application				21.04.10		ears	0.46	0.21	0.67	28	
41710 Utrera		3. 26.05.2010							rest of plant[4]	2.91	1.00	3.91	28	
Spain									grain	<0.01	<0.01	<0.02	35	
L100092									straw	4.08	1.81	5.89	35	
									grain	<0.01	<0.01	<0.02	42	
									straw	2.27	1.32	3.59	42	
371236	GC 0654	1. 07.11.2010	foliar	0.125	200	0.25	2	69	whole plant[4]	6.98	0.33	7.31	0	BASF Method
2012/1067588	Quality	2. 02.05.-09.05.2011	application				09.05.2011		ears	0.32	0.17	0.49	28	L0076/01
32600		3. 21.06.2011							rest of plant[4]	4.66	1.25	5.91	28	LOQ=0.01 mg/kg
L'Isle Jourdain									ears	0.54	0.29	0.83	36	
France (S)									rest of plant[4]	5.32	1.66	6.98	36	
L110162									grain	<0.01	<0.01	<0.02	43	
									straw	6.96	2.38	9.34	43	
371236	GC 0654	1. 25.11.2010	foliar	0.125	200	0.25	2	69	whole plant[4]	7.02	0.24	7.26	0	
2012/1067588	Svevo	2. 23.04.-30.04.2011	application				02.05.2011		ears	0.27	0.22	0.49	28	
58300		3. 20.06.2011							rest of plant[4]	0.60	0.22	0.82	28	
Galatades									ears	0.28	0.22	0.50	35	
Greece									rest of plant[4]	0.87	0.40	1.27	35	
L110163									ears	0.29	0.21	0.50	42	
									rest of plant[4]	0.48	0.19	0.67	42	
									grain	<0.01	<0.01	<0.02	49	
									straw	0.85	0.36	1.21	49	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371236 2012/1067588 20060 San Martino Olearo Italy L110164	GC 0654 Aubusson	1. 04.11.2010 2. 20.05.-28.05.2011 3. 23.06.2011	foliar application	0.125	200	0.25	2 11.05.2011	69	whole plant[4] ears rest of plant[4] grain straw grain straw	7.50 0.19 2.84 0.01 4.18 0.02 3.66	0.51 0.07 0.93 <0.01 1.53 <0.01 1.35	8.01 0.26 3.77 <0.02 5.71 0.03 5.01	0 27 27 35 35 43 43	
371236 2012/1067588 41710 Utrera Spain L110165	GC 0654 Califa Sur	1. 25.01.2011 2. 25.04.-06.05.2011 3. 15.06.2011	foliar application	0.125	200	0.25	2 03.05.2011	69	whole plant[4] grain straw grain straw grain straw	8.20 0.02 4.18 0.02 4.40 0.02 3.78	0.36 <0.01 1.63 <0.01 1.79 <0.01 1.61	8.56 0.03 5.81 0.03 6.19 0.03 5.39	0 28 28 36 36 43 43	
401831 2012/1194991 2014/1090810 82290 France L110190	GC 0654 PR22 R58	1. 02.11.10 2. n.a. 3. 15.06.11	foliar application with boom sprayer	0.125	200	0.25	2 04.05.2011	69	whole plant[4] ears rest of plant[4] ears rest of plant[4] grain straw	5.7 0.25 6.1 0.31 5.4 0.011 0.95	0.46 0.14 1.6 0.20 1.8 <0.01 0.42	6.1 0.40 7.6 0.52 7.1 0.021 1.4	0 28 28 35 35 42 42	BASF Method L0076/01 LOQ=0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
401831	GC 0654	1. 20.10.10	foliar	0.125	200	0.25	2	69	whole plant[4]	10	0.41	11	0	
2012/1194991	Bologna	2. n.a.	application				13.05.2011		ears	0.19	0.078	0.27	27	
		3. 21.06.2011	with boom						rest of plant[4]	1.5	0.47	1.9	27	
			sprayer						ears	0.16	0.081	0.24	34	
Italy									rest of plant[4]	1.2	0.45	1.6	34	
L110191									grain	<0.01	<0.01	<0.01	39	
									straw	0.95	0.42	1.4	39	
419892	GC 0654	1. 10.11.2011	Boom	0.125	200	0.25	2	69	whole plant[4]	6.7	0.072	6.8	0	BASF method
2013/1336790	Gallareta	2. 02.05.-11.05.2012	sprayer				17.05.12		ears	0.25	0.10	0.35	28	L0076/01
29200		3. 28.06.2012							rest of plant[4]	0.48	0.22	0.71	28	LOQ=0.01 mg/kg
Antaquera									grain	<0.01	<0.01	<0.02	36	
Spain									straw	0.34	0.15	0.49	36	
L120234									grain	0.010	<0.01	0.02	42	
									straw	0.46	0.22	0.69	42	
419892	GC 0654	1. 29.11.2011	Boom	0.125	200	0.25	2	69	whole plant[4]	3.5	0.051	3.5	0	
2013/1336790	Ingenio	2. 01.06.-10.06.2012	sprayer				08.06.12		ears	0.51	0.23	0.74	28	
47120		3. 20.07.2012							rest of plant[4]	4.3	1.1	5.4	28	
Duras									ears	0.52	0.26	0.78	35	
France (S)									rest of plant[4]	3.9	1.2	5.1	35	
L120235									grain	0.017	<0.01	0.027	42	
									straw	1.5	0.45	1.9	42	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots
n.a. = not applicable

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE, 67056 Ludwighafen
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	Metconazole 80 g/L
Content of active substance (g/kg or g/L)	130 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 556 03 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
401831	GC 0654	1. 24.09.2010	foliar	0.072	200	0.143	2	69	whole plant[4]	3.2	0.22	3.4	0	BASF method
2012/1194991	Julius	2. 27.05.-07.06.2011	application				07.06.2011		ears	0.19	0.064	0.26	29	L0076/01
2014/1090810		3. 03.08.2011	with boom						rest of plant[4]	1.2	0.39	1.6	29	LOQ=0.01 mg/kg
21614			sprayer						ears	0.29	0.104	0.39	36	
Germany									rest of plant[4]	1.5	0.56	2.0	36	
L110188									ears	0.36	0.15	0.51	43	
									rest of plant[4]	1.5	0.67	2.2	43	
									grain	<0.01	<0.01	<0.01	57	
									straw	1.9	0.97	2.9	57	
401831	GC 0654	1. 05.09.2010	foliar	0.072	200	0.143	2	69	whole plant[4]	2.0	0.069	2.1	0	
2012/1194991	Oakley	2. n.a.	application				20.06.2011		ears	0.31	0.12	0.43	29	
2014/1090810		3. 16.08.2011	with boom						rest of plant[4]	0.82	0.36	1.2	29	
HU179SL			sprayer						grain	0.025	0.011	0.037	36	
UK									straw	2.2	1.0	3.3	36	
L110189									grain	0.013	<0.01	0.023	42	
									straw	1.5	0.78	2.3	42	
									grain	0.020	<0.01	0.030	57	
									straw	1.3	0.76	2.0	57	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots
n.a. = not applicable

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE, 67056 Ludwighafen
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	Metconazole 80 g/L
Content of active substance (g/kg or g/L)	130 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 556 03 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
401831	GC 0654	1. 02.11.10	foliar	0.072	200	0.143	2	69	whole plant[4]	3.0	0.24	3.2	0	BASF Method
2012/1194991	PR22 R58	2. n.a.	application				04.05.2011		ears	0.19	0.10	0.29	28	L0076/01
2014/1090810		3. 15.06.11	with boom						rest of plant[4]	3.0	1.1	4.1	28	LOQ=0.01 mg/kg
82290			sprayer						ears	0.29	0.15	0.44	35	
France									rest of plant[4]	1.9	0.75	2.6	35	
L110190									grain	<0.01	<0.01	<0.01	42	
									straw	0.49	0.30	0.79	42	
401831	GC 0654	1. 20.10.10	foliar	0.072	200	0.143	2	69	whole plant[4]	3.8	0.20	4.0	0	
2012/1194991	Bologna	2. n.a.	application				13.05.2011		ears	0.11	0.044	0.15	27	
2014/1090810		3. 21.06.2011	with boom						rest of plant[4]	0.57	0.22	0.79	27	
40057			sprayer						ears	0.061	0.031	0.092	34	
Italy									rest of plant[4]	0.88	0.28	1.2	34	
L110191									grain	<0.01	<0.01	<0.01	39	
									straw	1.2	0.61	1.8	39	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots
n.a. = not applicable

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 700 F (fluxapyroxad)
Content of active substance (g/kg or g/L)	150 g/L	(common name and content)	75 g/L
Formulation (e.g. WP)	BAS 703 04 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
419892	GC 0654	1. 05.11.2011	Boom-	0.11	200	0.225	2	69	whole plant[4]	4.0	0.19	4.1	0	BASF method
2013/1336790	Asano	2. 24.05.-05.06.2012	sprayer				05.06.2012		ears	0.045	0.018	0.063	28	L0076/01
74193		3. 01.08.2012							rest of plant[4]	0.30	0.11	0.41	28	LOQ=0.01 mg/kg
Stetten a. H.									ears	<0.01	<0.01	<0.02	34	
Germany									rest of plant[4]	<0.01	<0.01	<0.02	34	
L120232									grain	<0.01	<0.01	<0.02	42	
									straw	0.29	0.13	0.42	42	
									grain	<0.01	<0.01	<0.02	50	
									straw	0.37	0.15	0.53	50	
419892	GC 0654	1. 18.10.2011	Boom-	0.11	200	0.225	2	69	whole plant[4]	3.7	0.042	3.8	0	
2013/1336790	JB Diego	2. 05.07.-25.07.2012	sprayer				05.07.2012		grain	<0.01	<0.01	<0.02	28	
OX15 6EP		3. 16.08.-20.08.2012							straw	1.1	0.21	1.3	28	
Banbury									grain	<0.01	<0.01	<0.02	36	
UK									straw	1.1	0.24	1.3	36	
L120233									grain	<0.01	<0.01	<0.02	42	
									straw	1.1	0.25	1.4	42	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Wheat	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 700 F (fluxapyroxad)
Content of active substance (g/kg or g/L)	150 g/L	(common name and content)	75 g/L
Formulation (e.g. WP)	BAS 703 04 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
419892	GC 0654	1. 10.11.2011	Boom	0.11	200	0.225	2	69	whole plant[4]	4.2	0.045	4.2	0	BASF Method
2013/1336790	Gallareta	2. 02.05.-11.05.2012	sprayer				17.05.2012		ears	0.35	0.075	0.42	28	L0076/01
29200		3. 28.06.2012							rest of plant[4]	0.26	0.047	0.30	28	LOQ=0.01 mg/kg
Antequera									grain	<0.01	<0.01	<0.02	36	
Spain									straw	0.30	0.10	0.41	36	
L120234									grain	<0.01	<0.01	<0.02	42	
									straw	0.45	0.13	0.58	42	
419892	GC 0654	1. 29.11.2011	Boom	0.11	200	0.225	2	69	whole plant[4]	3.8	0.025	3.8	0	
2013/1336790	Ingenio	2. 01.06.-10.06.2012	sprayer				08.06.2012		ears	0.48	0.19	0.67	28	
47120		3. 20.07.2012							rest of plant[4]	1.6	0.41	2.0	28	
Duras									ears	0.35	0.14	0.49	35	
France (S)									rest of plant[4]	1.3	0.38	1.7	35	
L120235									grain	0.015	<0.01	0.025	42	
									straw	1.6	0.56	2.2	42	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots

Barley

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371238 2012/1067587 67294 Mauchenheim Germany L110150	GC 0640 Marthe	1. 06.03.2011 2. 27.05.-03.06.11 3. 29.07.-04.08.11	foliar application	0.125	200	0.25	2 03.06.11	69	whole plant[4]	4.92	0.17	5.09	0	BASF Method L0076/01 LOQ=0.01 mg/kg
									ears	0.05	0.04	0.09	27	
									rest of plant[4]	0.37	0.16	0.53	27	
									grain	0.02	0.01	0.03	35	
									straw	0.50	0.26	0.76	35	
									grain	0.01	0.01	0.02	42	
									straw	0.42	0.26	0.68	42	
									grain	<0.01	<0.01	<0.02	52	
									straw	0.29	0.19	0.48	52	
371238 2012/1067587 04668 Motterwitz Germany L110151	GC 0640 Laverda	1. 23.09.2010 2. 16.05.-26.05.11 3. 07.07.2011	foliar application	0.125	200	0.25	2 26.05.11	69	whole plant[4]	8.16	0.32	8.48	0	
									ears	0.86	0.49	1.35	28	
									rest of plant[4]	6.10	2.16	8.26	28	
									grain	0.22	0.09	0.31	34	
									straw	7.68	2.89	10.57	34	
									grain	0.22	0.09	0.31	42	
									straw	8.73	3.93	12.66	42	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371238 2012/1067587 6598 MG Heijen Gennep The Netherlands L110152	GC 0640 Lomerit	1. 10.10.2010 2. 10.05.-20.05.11 3. 05.07.2011	foliar application	0.125	200	0.25	2 18.05.11	69	whole plant[4]	5.76	0.15	5.91	0	
									ears	0.20	0.11	0.31	28	
									rest of plant[4]	2.72	0.59	3.31	28	
									ears	0.15	0.09	0.24	34	
									rest of plant[4]	2.24	0.62	2.86	34	
									grain	0.09	0.03	0.12	41	
									straw	2.61	0.73	3.34	41	
									grain	0.08	0.03	0.11	48	
									straw	2.23	0.80	3.03	48	
371238 2012/1067587 37230 Le Grand Barré France (N) L110153	GC 0640 Cervoise	1. 16.10.2010 2. 01.05.-10.05.2011 3. 20.06.2011	foliar application	0.125	200	0.25	2 10.05.11	69	whole plant[4]	4.64	0.12	4.76	0	
									ears	0.22	0.19	0.41	27	
									rest of plant[4]	3.02	0.81	3.83	27	
									ears	0.21	0.20	0.41	35	
									rest of plant[4]	3.04	0.88	3.92	35	
									grain	0.07	0.05	0.12	41	
									straw	3.98	1.15	5.13	41	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371234 2011/1135916 04668 Motterwitz Germany L100093	GC 0640 Zoom	1. 07.10.2009 2. 05.06.-11.06.2010 3. 22.07.2010	foliar application	0.125	200	0.25	2 11.06.2010	69	whole plant[4]	5.80	0.10	5.90	0	BASF method L0076/01 LOQ=0.01 mg/kg
									ears	0.26	0.16	0.42	28	
									rest of plant[4]	1.67	0.40	2.07	28	
									ears	0.44	0.25	0.69	35	
									rest of plant[4]	2.17	0.54	2.71	35	
									grain	0.10	0.06	0.16	41	
									straw	2.75	0.71	3.46	41	
371234 2011/1135916 CO112NF Manningtree UK L100094	GC 0640 Saffron	1. 30.09.2009 2. 25.05.-01.06.10 3. 20.07.2010	foliar application	0.125	200	0.25	2 01.06.2010	69	whole plant[4]	3.60	0.16	3.76	0	
									ears	0.16	0.08	0.24	28	
									rest of plant[4]	2.26	0.48	2.74	28	
									ears	0.27	0.18	0.45	35	
									rest of plant[4]	1.06	0.24	1.30	35	
									ears	0.20	0.14	0.34	42	
									rest of plant[4]	1.99	0.54	2.53	42	
									grain	0.08	0.04	0.12	49	
									straw	1.28	0.42	1.70	49	
371234 2011/1135916 4910 Maribo Denmark (N) L100095	GC 0640 Quench	1. 05.04.2010 2. 02.07.-15.07.10 3. 25.08.2010	foliar application	0.125	200	0.25	2 14.07.2010	69	whole plant[4]	7.34	0.31	7.65	0	
									ears	3.93	1.22	5.15	29	
									rest of plant[4]	7.92	1.70	9.62	29	
									ears	2.66	0.98	3.64	36	
									rest of plant[4]	7.13	1.69	8.82	36	
									grain	0.82	0.13	0.95	42	
									straw	6.30	1.83	8.13	42	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371234 2011/1135916 37380 Crotelles France (N) L100096	GC 0640 Abondance	1. 09.10.2009 2. 11.05.-23.05.10 3. 02.07.2010	foliar application	0.125	200	0.25	2 21.05.2010	69	whole plant[4]	5.44	0.34	5.78	0	
									ears	0.13	0.08	0.21	28	
									rest of plant[4]	2.35	0.66	3.01	28	
									ears	0.22	0.15	0.37	35	
									rest of plant[4]	2.32	0.74	3.06	35	
									grain	0.11	0.06	0.17	42	
									straw	2.42	0.83	3.25	42	
316160 2010/1006342 67294 Mauchenheim Germany L090026	GC 0640 Marthe	1. 20.03.2009 2. 06.06.-17.06.2009 3. 25.07.2009	foliar application	0.125	200	0.250	2 17.06.2009	69	whole plant[4]	4.59	0.20	4.79	0	BASF method L0076/01 LOQ=0.01 mg/kg
									grain	0.08	0.08	0.16	29	
									straw	2.23	0.89	3.12	29	
									grain	0.05	0.07	0.12	34	
									straw	2.50	0.99	3.49	34	
									grain	0.06	0.07	0.13	41	
									straw	2.05	1.04	3.09	41	
316160 2010/1006342 08190 Avaux France (N) L090027	GC 0640 Prestige	1. 03.10.2008 2. 11.05.-16.05.2009 3. 02.07.2009	foliar application	0.125	200	0.250	2 27.05.2009	71	whole plant[4]	3.54	0.12	3.66	0	
									ears	1.01	0.38	1.39	27	
									rest of plant[4]	0.88	0.20	1.08	27	
									grain	0.09	0.05	0.14	35	
									straw	1.17	0.22	1.39	35	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
401831_1	GC 0640	1. 22.09.10	foliar	0.125	200	0.25	2	69-73	whole plant[4]	9.2	0.56	9.8	0	BASF method
2012/1194990	Souleyka	2. 19.-29.05.11	application				30.05.11		ears	0.39	0.25	0.64	28	L0076/01
21717		3. 11.07.11	with boom						rest of plant[4]	3.5	1.3	4.9	28	LOQ=0.01 mg/kg
Germany			sprayer						ears	0.34	0.23	0.57	36	
L110192									rest of plant[4]	3.2	1.5	4.7	36	
									grain	0.065	0.042	0.11	42	
									straw	4.1	2.4	6.5	42	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
401831_1 2012/1194990 HU178TB UK L110193	GC 0640 Cavia	1. 16.09.10 2. n.a. 3. 03.08.11	foliar application with boom sprayer	0.125	200	0.25	2 10.06.11	69	whole plant[4]	1.9	0.078	2.0	0	
									ears	0.082	0.048	0.13	28	
									rest of plant[4]	0.39	0.17	0.56	28	
									ears	0.049	0.040	0.089	34	
									rest of plant[4]	0.48	0.19	0.67	34	
									grain	0.039	0.027	0.066	41	
									straw	0.93	0.47	1.4	41	
									grain	0.030	0.026	0.056	54	
									straw	0.46	0.25	0.71	54	
419891 2013/1282605 67294 Mauchenheim Germany L120228	GC 0640 Propino	1. 03.03.2012 2. 05.06.-18.06.2012 3. 23.07.2012	Boom sprayer	0.125	200	0.25	2 15.06.2012	69	whole plant[4]	5.2	0.13	5.3	0	BASF method L0076/01 LOQ=0.01 mg/kg
									ears	0.11	0.068	0.17	27	
									rest of plant[4]	1.9	0.37	2.2	27	
									grain	0.056	0.030	0.086	34	
									straw	2.4	0.59	3.0	34	
									grain	0.052	0.031	0.083	41	
									straw	2.9	0.78	3.6	41	
419891 2013/1282605 41310 Villeporcher France (N) L120229	GC 0640 Cervoise	1. 17.10.2011 2. 25.05.-31.05.2012 3. 10.07.-18.07.2012	Boom sprayer	0.125	200	0.25	2 31.05.2012	69	whole plant[4]	4.4	0.32	4.7	0	
									ears	0.34	0.22	0.56	28	
									rest of plant[4]	0.67	0.21	0.88	28	
									grain	0.33	0.16	0.49	36	
									straw	2.8	0.99	3.8	36	
									grain	0.35	0.15	0.49	47	
									straw	2.3	0.85	3.2	47	

1) at last treatment, 2) as BAS 500 F equivalent (conversion factor is 1.08) , 3) for calculation purposes, "< 0.01" is set 0.01, 4) no roots
n.a. = not applicable

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371238 2012/1067587 32130 Villamot France (S) L110154	GC 0640 Franzi	1. 25.10.2010 2. 29.04.-04.05.11 3. 15.06.2011	foliar application	0.125	200	0.25	2 04.05.11	69	whole plant[4]	6.40	0.36	6.76	0	BASF Method L0076/01 LOQ=0.01 mg/kg
									ears	0.24	0.18	0.42	28	
									rest of plant[4]	2.68	0.73	3.41	28	
									ears	0.24	0.21	0.45	35	
									rest of plant[4]	3.08	0.92	4.00	35	
									grain	0.03	0.02	0.05	42	
									straw	3.36	1.08	4.44	42	
371238 2012/1067587 58300 Galatades Greece L110155	GC 0640 Moutso	1. 25.11.2010 2. 20.04.-30.04.11 3. 10.06.2011	foliar application	0.125	200	0.25	2 15.04.11	69	whole plant[4]	7.04	0.32	7.36	0	
									ears	0.10	0.09	0.19	28	
									rest of plant[4]	1.29	0.15	1.44	28	
									ears	0.06	0.05	0.11	35	
									rest of plant[4]	1.02	0.14	1.16	35	
									ears	0.06	0.05	0.11	41	
									rest of plant[4]	0.87	0.14	1.01	41	
									grain	<0.01	<0.01	<0.02	56	
									straw	1.04	0.23	1.27	56	
371238 2012/1067587 20090 Premenugo di Settala Italy L110156	GC 0640 Mattina	1. 31.10.2010 2. 30.04.-08.05.11 3. 17.06.2011	foliar application	0.125	200	0.25	2 07.05.11	69	whole plant[4]	5.82	0.28	6.10	0	
									ears	0.57	0.31	0.88	27	
									rest of plant[4]	2.04	0.52	2.56	27	
									grain	0.13	0.09	0.22	34	
									straw	3.33	0.80	4.13	34	
									grain	0.14	0.11	0.25	41	
									straw	3.53	1.08	4.61	41	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371238 2012/1067587 41710 Utrera Spain L110157	GC 0640 Caballar	1. 25.01.2011 2. 28.04.-08.05.11 3. 16.06.2011	foliar application	0.125	200	0.25	2 06.05.11	69	whole plant[4]	5.64	0.08	5.72	0	
									ears	0.70	0.35	1.05	27	
									rest of plant[4]	3.28	0.86	4.14	27	
									grain	0.09	0.05	0.14	34	
									straw	2.77	0.82	3.59	34	
									grain	0.07	0.04	0.11	41	
									straw	3.05	1.06	4.11	41	
371234 2011/1135916 32600 L'Isle Jourdain France (S) L100097	GC 0640 Yoole	1. 26.10.2009 2. 02.05.-10.05.2010 3. 30.06.2010	foliar application	0.125	200	0.25	2 10.05.2010	69	whole plant[4]	4.32	0.11	4.43	0	BASF Method L0076/01 LOQ=0.01 mg/kg
									ears	0.14	0.03	0.17	28	
									rest of plant[4]	0.70	0.09	0.79	28	
									ears	0.10	0.02	0.12	36	
									rest of plant[4]	0.59	0.10	0.69	36	
									grain	0.11	0.02	0.13	44	
									straw	0.77	0.14	0.91	44	
									grain	0.09	0.02	0.11	51	
									straw	0.75	0.15	0.90	51	
371234 2011/1135916 58300 Galatades Greece L100098	GC 0640 Moutso	1. 10.11.2009 2. 10.04.-20.04.2010 3. 11.06.2010	foliar application	0.125	200	0.25	2 20.04.2010	69	whole plant[4]	6.54	0.35	6.89	0	
									ears	0.32	0.22	0.54	28	
									rest of plant[4]	1.25	0.19	1.44	28	
									ears	0.18	0.17	0.35	35	
									rest of plant[4]	0.76	0.13	0.89	35	
									ears	0.26	0.26	0.52	42	
									rest of plant[4]	0.71	0.13	0.84	42	
									grain	0.06	0.04	0.10	52	
									straw	0.98	0.18	1.16	52	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
371234 2011/1135916 20060 Liscate Italy L100099	GC 0640 Mattina	1. 22.11.2009 2. 05.05.-15.05.2010 3. 24.06.2010	foliar application	0.125	200	0.25	2 14.05.2010	69	whole plant[4] grain straw grain straw grain straw	5.14 0.65 3.33 0.54 3.79 0.44 3.53	0.08 0.41 0.91 0.35 1.09 0.31 0.88	5.22 1.06 4.24 0.89 4.88 0.75 4.41	0 27 27 36 36 41 41	
371234 2011/1135916 11500 Puerto Santa Maria Spain L100100	GC 0640 Henley	1. 04.12.2009 2. 15.04.-25.04.10 3. 03.06.2010	foliar application	0.125	200	0.25	2 23.04.2010	69	whole plant[4] grain straw grain straw grain straw	7.46 0.13 2.45 0.13 4.24 0.08 2.30	0.31 0.07 0.79 0.09 1.59 0.06 0.80	7.77 0.20 3.24 0.22 5.83 0.14 3.10	0 27 27 34 34 41 41	
316160 2010/1006342 41710 Utrera Spain L090028	GC 0640 Cecilia	1. 16.12.2008 2. 10.04.-25.04.09 3. 04.06.2009	foliar application	0.125	200	0.25	2 23.04.2009	69	whole plant[4] ears rest of plant[4] grain straw grain straw	6.60 0.59 4.17 0.03 1.77 0.03 2.87	0.36 0.32 1.34 0.03 0.70 0.03 1.08	6.96 0.91 5.51 0.06 2.47 0.06 3.95	0 29 29 35 35 42 42	BASF Method L0076/01 LOQ=0.01 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
316160 2010/1006342 40057 Viadagola Italy L090029	GC 0640 Otis	1. 24.02.2009 2. 16.05.-23.05.09 3. 03.07.2009	foliar application	0.125	200	0.25	2 23.05.2009	69	whole plant[4]	6.60	0.15	6.75	0	
									ears	0.17	0.10	0.27	27	
									rest of plant[4]	2.46	0.79	3.25	27	
									grain	0.01	0.02	0.03	34	
									straw	0.79	0.28	1.07	34	
									grain	0.02	0.02	0.04	41	
									straw	0.46	0.27	0.73	41	
401831_1 2012/1194990 82130 France (S) L110194	GC 0640 Azurel	1. 05.10.10 2. End of April 3. 10.06.11	foliar application with boom sprayer	0.125	200	0.25	2 29.04.11	69	whole plant[4]	6.3	0.21	6.5	0	BASF method L0076/01 LOQ=0.01 mg/kg
									ears	0.066	0.064	0.13	28	
									rest of plant[4]	1.2	0.35	1.5	28	
									ears	0.11	0.16	0.27	35	
									rest of plant[4]	0.63	0.30	0.93	35	
									grain	0.029	0.025	0.054	42	
									straw	1.3	0.52	1.8	42	
401831_1 2012/1194990 40055 Italy L110195	GC 0640 Amorosa	1. 15.12.10 2. May 3. 20.06.11	foliar application with boom sprayer	0.125	200	0.25	2 16.05.11	69-71	whole plant[4]	7.3	0.33	7.7	0	
									ears	0.18	0.16	0.35	29	
									rest of plant[4]	1.9	0.70	2.6	29	
									ears	0.21	0.23	0.44	35	
									rest of plant[4]	1.2	0.46	1.6	35	
									grain	0.049	0.033	0.081	35	
									straw	2.1	0.69	2.8	35	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
419891	GC 0640	1. 28.12.2011	Boom	0.125	200	0.250	2	69	whole plant[4]	6.0	0.040	6.0	0	BASF method
2013/1282605	Pewter	2. 02.05.-11.05.2012	sprayer				17.05.2012		ears	0.51	0.26	0.77	28	L0076/01
29200 Antequera		3. 28.06.2012							rest of plant[4]	0.58	0.20	0.78	28	LOQ=0.01 mg/kg
Spain									grain	0.15	0.085	0.23	36	
L120230									straw	1.2	0.44	1.6	36	
									grain	0.12	0.069	0.19	42	
									straw	1.2	0.39	1.6	42	
419891	GC 0640	1. 25.11.2011	Boom	0.125	200	0.250	2	69	whole plant[4]	4.5	0.16	4.7	0	
2013/1282605	Arta	2. 15.04.-25.04.2012	sprayer				24.04.2012		ears	0.020	0.019	0.039	29	
58300 Galatades		3. 01.06.-10.06.2012	Knapsack						rest of plant[4]	0.68	0.079	0.76	29	
Greece									ears	<0.01	<0.01	<0.02	35	
L120231									rest of plant[4]	0.60	0.074	0.67	35	
									grain	<0.01	<0.01	<0.02	42	
									straw	0.56	0.22	0.78	42	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 480 F (42 g/L)
Content of active substance (g/kg or g/L)	67 g/L	(common name and content)	BAS 700 F (42 g/L)
Formulation (e.g. WP)	BAS 702 01 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
316160	GC 0640	1. 20.03.2009	foliar	0.100	200	0.201	2	69	whole plant[4]	3.89	0.10	3.99	0	BASF method
2010/1006342	Marthe	2. 06.06.-17.06.2009	application				17.06.2009		grain	0.06	0.05	0.11	29	L0076/01
67294		3. 25.07.2009							straw	2.02	0.59	2.61	29	LOQ=0.01 mg/kg
Mauchenheim									grain	0.05	0.04	0.09	34	
Germany									straw	1.66	0.60	2.26	34	
L090026									grain	0.07	0.05	0.12	41	
									straw	2.09	0.81	2.90	41	
316160	GC 0640	1. 03.10.2008	foliar	0.100	200	0.201	2	71	whole plant[4]	4.34	0.10	4.44	0	
2010/1006342	Prestige	2. 11.05.-16.05.2009	application				27.05.2009		ears	0.99	0.29	1.28	27	
08190 Avaux		3. 02.07.2009							rest of plant w/o roots	1.26	0.10	1.36	27	
France (N)									grain	0.21	0.09	0.30	35	
L090027									straw	2.10	0.26	2.36	35	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 480 F (42 g/L)
Content of active substance (g/kg or g/L)	67 g/L	(common name and content)	BAS 700 F (42 g/L)
Formulation (e.g. WP)	BAS 702 01 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
316160	GC 0640	1. 16.12.2008	foliar	0.100	200	0.201	2	69	whole plant[4]	3.44	0.15	3.59	0	BASF Method
2010/1006342	Cecilia	2. 10.04.-25.04.09	application				23.04.2009		ears	0.21	0.09	0.30	29	L0076/01
41710 Utrera		3. 04.06.2009							rest of plant w/o roots	2.63	0.48	3.11	29	LOQ=0.01 mg/kg
Spain									grain	0.02	0.02	0.04	35	
L090028									straw	1.63	0.42	2.05	35	
									grain	0.02	0.02	0.04	42	
									straw	1.94	0.59	2.53	42	
316160	GC 0640	1. 24.02.2009	foliar	0.100	200	0.201	2	69	whole plant[4]	6.40	0.21	6.61	0	
2010/1006342	Otis	2. 16.05.-23.05.09	application				23.05.2009		ears	0.26	0.12	0.38	27	
40057		3. 03.07.2009							rest of plant w/o roots	2.84	0.73	3.57	27	
Viadagola									grain	0.03	0.03	0.06	34	
Italy									straw	2.42	0.70	3.12	34	
L090029									grain	0.03	0.02	0.05	41	
									straw	1.19	0.53	1.72	41	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE, 67056 Ludwighafen
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 555 F
Content of active substance (g/kg or g/L)	130 g/L	(common name and content)	Metconazole 80 g/L
Formulation (e.g. WP)	BAS 556 03 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
401831_1 2012/1194990 21717 Germany L090026 L110192	GC 0640 Souleyka	1. 22.09.10 2. 19.-29.05.11 3. 11.07.11	foliar application with boom sprayer	0.072	200	0.143	2 30.05.2011	69-73	whole plant[4]	5.1	0.37	5.4	0	BASF method L0076/01 LOQ=0.01 mg/kg
									ears	0.26	0.14	0.39	28	
									rest of plant[4]	2.0	0.92	2.9	28	
									ears	0.23	0.18	0.42	36	
									rest of plant[4]	1.7	0.82	2.5	36	
									grain	0.059	0.036	0.095	42	
									straw	3.2	1.9	5.1	42	
401831_1 2012/1194990 HU178TB UK L110193	GC 0640 Calvia	1. 15.09.2010 2. not applicable 3. 03.08.2011	foliar application with boom sprayer	0.072	200	0.143	2 10.06.2011	69	whole plant[4]	2.3	0.086	2.4	0	
									ears	0.082	0.062	0.14	28	
									rest of plant[4]	0.25	0.13	0.39	28	
									ears	0.078	0.060	0.14	34	
									rest of plant[4]	0.27	0.15	0.42	34	
									grain	0.035	0.022	0.057	41	
									straw	0.65	0.39	1.0	41	
									grain	0.030	0.022	0.052	54	
									straw	0.42	0.25	0.67	54	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE, 67056 Ludwighafen
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 555 F
Content of active substance (g/kg or g/L)	130 g/L	(common name and content)	Metconazole 80 g/L
Formulation (e.g. WP)	BAS 556 03 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
401831_1 2012/1194990 82130 France L110194	GC 0640 Azurel	1. 05.10.10 2. End of April 3. 10.06.11	foliar application with boom sprayer	0.072	200	0.143	2 29.04.2011	69	whole plant[4]	3.7	0.21	3.9	0	BASF Method L0076/01 LOQ=0.01 mg/kg
									ears	0.049	0.082	0.13	28	
									rest of plant[4]	0.60	0.23	0.83	28	
									ears	0.042	0.072	0.11	35	
									rest of plant[4]	0.41	0.24	0.66	35	
									grain	0.019	0.020	0.038	42	
									straw	0.55	0.30	0.85	42	
401831_1 2012/1194990 40055 Italy L110195	GC 0640 Amorosa	1. 15.12.10 2. May 3. 20.06.11	foliar application with boom sprayer	0.072	200	0.143	2 16.05.2011	69-71	whole plant[4]	4.2	0.18	4.3	0	
									ears	0.11	0.071	0.18	29	
									rest of plant[4]	1.5	0.43	1.9	29	
									ears	0.11	0.11	0.22	35	
									rest of plant[4]	1.3	0.42	1.7	35	
									grain	0.038	0.020	0.058	35	
									straw	1.5	0.48	1.9	35	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 700 F
Content of active substance (g/kg or g/L)	150 g/L	(common name and content)	Fluxapyroxad 75 g/L
Formulation (e.g. WP)	BAS 703 04 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
419891 2013/1282605 67294 Mauchenheim Germany L120228	GC 0640 Propino	1. 03.03.2012 2. 05.06.-18.06.2012 3. 23.07.2012	Boom-sprayer	0.11	200	0.225	2 15.06.2012	69	whole plant[4]	4.6	0.13	4.8	0	BASF method L0076/01 LOQ=0.01 mg/kg
									ears	0.11	0.060	0.17	27	
									rest of plant[4]	1.4	0.29	1.7	27	
									grain	0.040	0.020	0.060	34	
									straw	1.9	0.46	2.3	34	
									grain	0.043	0.023	0.066	41	
									straw	1.7	0.50	2.2	41	
419891 2013/1282605 41310 Villeporcher France (N) L120229	GC 0640 Cervoise	1. 17.10.2011 2. 25.05.-31.05.2012 3. 10.07.-18.07.2012	Boom-sprayer	0.11	200	0.225	2 31.05.2012	69	whole plant[4]	3.6	0.16	3.7	0	
									ears	0.78	0.36	1.1	28	
									rest of plant[4]	1.4	0.33	1.7	28	
									grain	0.25	0.089	0.34	36	
									straw	1.2	0.30	1.5	36	
									grain	0.22	0.072	0.29	47	
									straw	1.4	0.39	1.7	47	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Barley	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 700 F
Content of active substance (g/kg or g/L)	150 g/L	(common name and content)	Fluxapyroxad 75 g/L
Formulation (e.g. WP)	BAS 703 04 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin) 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remark
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
419891	GC 0640	1. 28.12.2011	Boom	0.11	200	0.225	2	69	whole plant[3]	5.1	0.028	5.1	0	BASF Method
2013/1282605	Pewter	2. 02.05.-11.05.2012	sprayer				17.05.2012		ears	0.69	0.25	0.94	28	L0076/01
29200 Antequera		3. 28.06.2012							rest of plant[3]	0.52	0.14	0.66	28	LOQ=0.01 mg/kg
Spain									grain	0.21	0.066	0.28	36	
L120230									straw	0.80	0.20	1.0	36	
									grain	0.27	0.082	0.35	42	
									straw	0.81	0.23	1.0	42	
419891	GC 0640	1. 25.11.2011	Boom	0.11	200	0.225	2	69	whole plant[3]	4.4	0.11	4.5	0	
2013/1282605	Arta	2. 15.04.-25.04.2012	sprayer				24.04.2012		ears	0.011	0.010	0.021	29	
58300		3. 01.06.-10.06.2012							rest of plant[3]	0.36	0.038	0.40	29	
Galatades									ears	0.27	0.25	0.51	35	
Greece									rest of plant[3]	0.55	0.12	0.67	35	
L120231									grain	<0.01	<0.01	<0.02	42	
									straw	<0.01	<0.01	<0.02	42	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots

Maize

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
422769 2013/1308888 47574 Goch-Nierswalde Germany L120357	GC0645 Athlet	1. 28.04.2012 2. n.a. 3. 19.10.2012	foliar application	0.048	400	0.191	1 02.08.2012	65	Whole plant[4]	2.0	<0.010	2.00	0	BASF method L0076/01 LOQ=0.01 mg/kg
									Cobs w/o husks	0.013	<0.010	0.023	36	
									Cobs with husks	0.012	<0.010	0.022	36	
									Rest of plant[4]	0.27	0.038	0.31	36	
									Cobs w/o husks	0.015	<0.010	0.025	56	
									Cobs with husks	<0.010	<0.010	<0.020	56	
									Rest of plant[4]	0.36	0.032	0.39	56	
									Maize grain	<0.010	<0.010	<0.020	78	
									Maize straw	0.08	0.016	0.096	78	
422769 2013/1308888 NR231NY Wighton; Norfolk UK L120358	GC0645 NK Bull	1. 11.-16.04.2012 2. n.a. 3. 26.09.2012	foliar application	0.05	400	0.200	1 23.08.2012	65	Whole plant[4]	4.2	<0.010	4.20	0	
									Cobs w/o husks	<0.010	<0.010	<0.020	19	
									Cobs with husks	0.075	<0.010	0.085	19	
									Rest of plant[4]	0.25	0.052	0.30	19	
									Cobs w/o husks	<0.010	<0.010	<0.020	26	
									Cobs with husks	0.036	<0.010	0.046	26	
									Rest of plant[4]	0.22	0.037	0.26	26	
									Maize grain	<0.010	<0.010	<0.020	34	
									Maize straw	0.14	0.026	0.16	34	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
422769 2013/1308888 6599 Limburg The Netherlands L120359	GC0645 Pionerr P8000	1. 02.05.2012 2. n.a. 3. 19.10.2012	foliar application	0.053	400	0.211	1 09.08.2012	65	Whole plant[4]	3.7	0.11	3.8	0	
									Cobs w/o husks	0.018	<0.010	0.028	29	
									Cobs with husks	0.017	<0.010	0.027	29	
									Rest of plant[4]	0.71	0.21	0.92	29	
									Cobs w/o husks	0.011	<0.010	0.021	43	
									Cobs with husks	0.022	<0.010	0.032	43	
									Rest of plant[4]	0.47	0.17	0.64	43	
									Maize grain	<0.010	<0.010	<0.020	71	
									Maize straw	<0.010	<0.010	<0.020	71	
422769 2013/1308888 7540 Velaines, Hainaut Belgium L120360	GC0645 LG 30222	1. 24.05.2012 2. n.a. 3. 08.11.2012	foliar application	0.051	400	0.205	1 22.08.2012	65	Whole plant[4]	2.7	<0.010	2.7	0	
									Cobs w/o husks	0.021	<0.010	0.031	29	
									Cobs with husks	0.067	<0.010	0.077	29	
									Rest of plant[4]	0.42	0.07	0.50	29	
									Cobs w/o husks	0.039	<0.010	0.049	44	
									Cobs with husks	0.044	<0.010	0.054	44	
									Rest of plant[4]	0.33	0.06	0.39	44	
									Maize grain[5]	-	-	-	-	
									Maize straw[5]	-	-	-	-	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
422769_1 2014/1001741	GC0645 Pomeri	1. 21.04.2013 2. n. a. 3. 05.11.2013	foliar application	0.05	400	0.200	1 12.08.2013	65	Whole plant[4]	1.60	<0.010	1.60	0	
									Cobs w/o husks	<0.010	<0.010	<0.020	29	
									Cobs with husks	0.028	<0.010	0.038	29	
									Rest of plant[4]	0.26	0.066	0.33	29	
									Cobs w/o husks	<0.010	<0.010	<0.020	42	
									Cobs with husks	0.016	<0.010	0.026	42	
									Rest of plant[4]	0.44	0.071	0.51	42	
									Maize grain	<0.010	<0.010	<0.020	85	
									Maize straw	0.19	0.047	0.24	85	
306328 2010/1039144 49685 Emstek / Halen Germany L090168	GC0645 Nescio	1. 01.05.2009 2. 20.07.-04.08.2009 3. 29.10.2009	Plotsprayer (Agrotop)	0.05	400	0.200	1 28.07.2009	67	Whole plant[4]	1.4	< 0.01	1.4	0	BASF method 535/1 LOQ=0.01 mg/kg
									cob with husks	0.03	< 0.01	0.04	13	
									Rest of plant[4]	0.25	0.04	0.29	13	
									cob with husks	0.01	< 0.01	0.02	29	
									Rest of plant[4]	0.17	0.05	0.22	29	
									cob with husks	< 0.01	< 0.01	< 0.02	57	
									Rest of plant[4]	0.12	0.039	0.16	57	
									grain	< 0.01	< 0.01	< 0.02	91	
									Rest of plant[4]	0.09	0.036	0.13	91	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
306328	GC0645	1. 18.04.2009	Pulvexper	0.05	400	0.200	1	65	Whole plant[4]	1.6	0.02	1.7	0	BASF method
2010/1039144	Ohio	2. 23.07.-06.08.2009	boom				30.07.2009		cob with husks	0.05	< 0.01	0.06	12	535/1
Banbury		3. 15.10.2009	sprayer						Rest of plant[4]	0.43	0.11	0.54	12	LOQ=0.01 mg/kg
UK									cob with husks	0.10	< 0.01	0.11	19	
L090170									Rest of plant[4]	0.22	0.04	0.26	19	
									cob with husks	0.01	< 0.01	0.02	46	
									Rest of plant[4]	0.16	0.04	0.20	46	
									grain	< 0.01	< 0.01	< 0.02	71	
									Rest of plant[4]	0.08	0.02	0.10	71	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots
5) In error the farmer harvested the trial plots before the final sampling could be conducted. Therefore, no harvest grain and straw samples were available for analysis.
n.a. = not applicable

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
422769	GC0645	1. 07.05.2012	foliar	0.052	400	0.207	1	65	Whole plant[4]	1.8	<0.010	1.8	0	BASF method
2013/1308888	Octet	2. n.a.	application				26.07.2012		Cobs w/o husks	<0.010	<0.010	<0.020	24	L0076/01
01990 Sandrans		3. 16.10.2012							Cobs with husks	0.068	<0.010	0.078	24	LOQ=0.01 mg/kg
Rhone Alpes									Rest of plant[4]	0.27	0.049	0.31	24	
France (S)									Cobs w/o husks	<0.010	<0.010	<0.020	45	
L120361									Cobs with husks	0.052	<0.010	0.062	45	
									Rest of plant[4]	0.20	0.05	0.25	45	
									Maize grain	<0.010	<0.010	<0.020	82	
									Maize straw	0.30	0.079	0.38	82	
422769	GC0645	1. 29.04.2012	foliar	0.05	400	0.200	1	65	Whole plant[4]	1.5	0.088	1.6	0	
2013/1308888	AS 72	2. n.a.	application				05.07.2012		Cobs w/o husks	<0.010	<0.010	<0.020	27	
GR-59032		3. 29.08.2012							Cobs with husks	<0.010	<0.010	<0.020	27	
Platanos, Imathia									Rest of plant[4]	0.27	0.078	0.35	27	
Greece									Cobs w/o husks	<0.010	<0.010	<0.020	42	
L120362									Cobs with husks	<0.010	<0.010	<0.020	42	
									Rest of plant[4]	0.76	0.13	0.89	42	
									Maize grain	<0.010	<0.010	<0.020	55	
									Maize straw	0.23	0.058	0.29	55	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
422769	GC0645	1. 25.03.2012	foliar	0.05	400	0.199	1	65	Whole plant[4]	1.7	<0.010	1.7	0	
2013/1308888	Antiss	2. n.a.	application				09.07.2012		Cobs w/o husks	<0.010	<0.010	<0.020	30	
26813, Graffignana		3. 14.09.2012							Cobs with husks	<0.010	<0.010	<0.020	30	
Lombardia									Rest of plant[4]	0.15	0.074	0.22	30	
Italy									Cobs w/o husks	<0.010	<0.010	<0.020	44	
L120363									Cobs with husks	<0.010	<0.010	<0.020	44	
									Rest of plant[4]	0.21	0.078	0.28	44	
									Maize grain	<0.010	<0.010	<0.020	67	
									Maize straw	0.29	0.074	0.36	67	
422769	GC0645	1. 20.04.2012	foliar	0.05	400	0.200	1	65	Whole plant[4]	2.2	<0.010	2.2	0	
2013/1308888	PR33Y-7U	2. n.a.	application				16.07.2012		Cobs w/o husks	<0.010	<0.010	<0.020	15	
25670 Termens,		3. 02.10.2012							Cobs with husks	0.030	<0.010	0.040	15	
Catalunya									Rest of plant[4]	0.60	0.23	0.82	15	
Spain									Cobs w/o husks	<0.010	<0.010	<0.020	32	
L120364									Cobs with husks	0.018	<0.010	0.028	32	
									Rest of plant[4]	0.22	0.13	0.34	32	
									Maize grain	<0.010	<0.010	<0.020	78	
									Maize straw	0.25	0.15	0.40	78	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	-
Content of active substance (g/kg or g/L)	200 g/L	(common name and content)	
Formulation (e.g. WP)	BAS 500 06 F (EC)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
306328	GC0645	1. 16.03.2009	Echo SHR	0.05	400	0.200	1	65	Whole plant[4]	1.9	0.04	1.9	0	BASF method
2010/1039144	Dekalb 66-77	2. 28.06.-06.07.2009	150 SI				01.07.2009		cob with husks	0.05	< 0.01	0.06	9	L0076/01
40054 Dugliolo di Budrio Italy		3. 27.08.2009							Rest of plant[4]	0.29	0.07	0.35	9	LOQ=0.01 mg/kg
L090172									cob with husks	0.05	< 0.01	0.06	20	
									Rest of plant[4]	0.15	0.06	0.21	20	
									cob with husks	0.02	< 0.01	0.03	30	
									Rest of plant[4]	0.13	0.05	0.18	30	
									grain	< 0.01	< 0.01	< 0.02	57	
									Rest of plant[4]	0.13	0.04	0.17	57	
306328	GC0645	1. 01.04.2009	Sprayer	0.05	400	0.200	1	65	Whole plant[4]	4.3	0.05	4.3	0	
2010/1039144	Anadon	2. 15.07.-25.07.2009	with booms				15.07.2009		cob with husks	0.05	< 0.01	0.06	16	
47120 Duras France (S)		3. 05.10.2009	(Agrotop)						Rest of plant[4]	0.89	0.09	0.98	16	
L090174									cob with husks	0.02	< 0.01	0.03	27	
									Rest of plant[4]	0.35	0.04	0.39	27	
									cob with husks	0.02	< 0.01	0.03	49	
									Rest of plant[4]	0.28	0.03	0.31	49	
									grain	< 0.01	< 0.01	< 0.02	77	
									Rest of plant[4]	0.50	0.05	0.54	77	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots
n.a. = not applicable

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 480 F
Content of active substance (g/kg or g/L)	133 g/L	(common name and content)	Epoxiconazole 50 g/L
Formulation (e.g. WP)	BAS 512 04 F (SE)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
306326	GC0645	1. 03.05.2008	Plotsprayer	0.0499	400	0.1995	1	65	whole plant[4]	2.26	0.02	2.28	0	BASF method
2010/1025690	Delitop	2. 11.07.-21.07.2008					15.07.2008		cob with husks	0.07	< 0.01	0.08	13	535/1
49685		3. 27.10.2008							rest of plant[4]	0.33	0.05	0.38	13	LOQ=0.01 mg/kg
Emstek									cob with husks	0.04	< 0.01	0.05	24	
Germany									rest of plant[4]	0.16	0.02	0.18	24	
L080323									cob with husks	0.02	< 0.01	0.03	56	
									rest of plant[4]	0.09	0.02	0.11	56	
									grain	< 0.01	< 0.01	0.02	99	
									rest of plant[4]	0.16	0.03	0.19	99	
306326	GC0645	1. 20.04.2008	Sprayer	0.0499	400	0.1995	1	65	whole plant[4]	2.86	0.03	2.89	0	BASF method
2010/1025690	PR 39T13	2. 18.07.-01.08.2008	with booms				23.07.2008		cob with husks	0.04	< 0.01	0.05	12	535/1
02190		3. 05.10.2008							rest of plant[4]	0.84	0.18	1.02	12	LOQ=0.01 mg/kg
Amifontaine									cob with husks	< 0.01	< 0.01	0.02	42	
France (N)									rest of plant[4]	0.14	0.05	0.19	42	
L080324									cob with husks	< 0.01	< 0.01	0.02	55	
									rest of plant[4]	0.13	0.04	0.17	55	
									grain	< 0.01	< 0.01	0.02	71	
									rest of plant[4]	0.05	0.02	0.07	71	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 480 F
Content of active substance (g/kg or g/L)	133 g/L	(common name and content)	Epoxiconazole 50 g/L
Formulation (e.g. WP)	BAS 512 04 F (SE)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
306326	GC0645	1. 17.04.2008	Pulvexper	0.0499	400	0.1995	1	65	whole plant[4]	2.13	0.01	2.14	0	
2010/1025690	Justina	2. 23.07.-18.08.08	boom				01.08.2008		cob with husks	0.09	0.02	0.11	18	
OX15 5HE		3. 27.10.2008	sprayer						rest of plant[4]	0.22	0.04	0.26	18	
Oxon									cob with husks	0.06	< 0.01	0.07	34	
United Kingdom									rest of plant[4]	0.17	0.02	0.19	34	
L080325									cob with husks	0.03	< 0.01	0.04	70	
									rest of plant[4]	0.1	0.02	0.12	70	
									grain	< 0.01	< 0.01	0.02	83	
									rest of plant[4]	0.17	0.03	0.20	83	
306326	GC0645	1. 28.04.2008	Plotsprayer	0.0499	400	0.1995	1	65	whole plant[4]	3.04	0.05	3.09	0	
2010/1025690	Adenzo	2. 12.07.-24.07.2008					16.07.2008		cob with husks	0.02	< 0.01	0.03	12	
9541 XH		3. 23.09.2008							rest of plant[4]	0.28	0.08	0.36	12	
Vlagtwedde									cob with husks	< 0.01	< 0.01	0.02	23	
The Netherlands									rest of plant[4]	0.09	0.03	0.12	23	
L080326									cob with husks	< 0.01	< 0.01	0.02	55	
									rest of plant[4]	0.05	0.01	0.06	55	
									grain	< 0.01	< 0.01	0.02	82	
									rest of plant[4]	0.03	0.01	0.04	82	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 480 F
Content of active substance (g/kg or g/L)	133 g/L	(common name and content)	Epoxiconazole 50 g/L
Formulation (e.g. WP)	BAS 512 04 F (SE)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
306328	GC0645	1. 01.05.2009	Plotsprayer	0.0499	400	0.1995	1	67	whole plant[4]	2.2	0.02	2.3	0	BASF method
2010/1039144	Nescio	2. 20.07.-04.08.2009	(Agrotop)				28.07.2009		cob with husks	0.04	< 0.01	0.05	13	535/1
49685 Emstek / Halen		3. 29.10.2009							rest of plant[4]	0.30	0.07	0.37	13	LOQ=0.01 mg/kg
Germany									cob with husks	0.02	< 0.01	0.03	29	
L090168									rest of plant[4]	0.20	0.06	0.26	29	
									cob with husks	0.02	< 0.01	0.03	57	
									rest of plant[4]	0.13	0.04	0.17	57	
									grain	< 0.01	< 0.01	< 0.02	91	
									rest of plant[4]	0.10	0.04	0.14	91	
306328	GC0645	1. 28.04.2009	Sprayer	0.0499	400	0.1995	1	65	whole plant[4]	2.9	0.03	2.9	0	BASF method
2010/1039144	Maxxis	2. 24.07.-31.07.2009	with booms				28.07.2009		cob with husks	< 0.01	< 0.01	< 0.02	15	535/1
37370 Chemille sur Deme	Duo	3. 17.10.2009	(Agrotop)						rest of plant[4]	0.09	0.03	0.12	15	LOQ=0.01 mg/kg
France (N)									cob with husks	< 0.01	< 0.01	< 0.02	36	
L090169									rest of plant[4]	< 0.01	< 0.01	< 0.02	36	
									cob with husks	< 0.01	< 0.01	< 0.02	43	
									rest of plant[4]	0.11	0.03	0.14	43	
									grain	< 0.01	< 0.01	< 0.02	79	
									rest of plant[4]	0.05	0.02	0.06	79	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 480 F
Content of active substance (g/kg or g/L)	133 g/L	(common name and content)	Epoxiconazole 50 g/L
Formulation (e.g. WP)	BAS 512 04 F (SE)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
306328	GC0645	1. 18.04.2009	Pulvexper	0.0499	400	0.1995	1	65	whole plant[4]	1.6	0.03	1.6	0	BASF method
2010/1039144	Ohio	2. 23.07.-06.08.2009	boom				30.07.2009		cob with husks	0.07	< 0.01	0.08	12	535/1
Banbury		3. 15.10.2009	sprayer						rest of plant[4]	0.66	0.17	0.83	12	LOQ=0.01 mg/kg
UK									cob with husks	0.02	< 0.01	0.03	19	
L090170									rest of plant[4]	0.32	0.08	0.40	19	
									cob with husks	< 0.01	< 0.01	< 0.02	46	
									rest of plant[4]	0.07	0.02	0.08	46	
									grain	< 0.01	< 0.01	< 0.02	71	
									rest of plant[4]	0.08	0.02	0.10	71	
306328	GC0645	1. 28.04.2009	Plotsprayer	0.0499	400	0.1995	1	65	whole plant[4]	2.2	< 0.01	2.2	0	BASF method
2010/1039144	LG 3218	2. 21.07.-05.08.2009	Agrotop						cob with husks	0.08	< 0.01	0.09	17	535/1
9541 XH		3. 30.09.2009							rest of plant[4]	0.21	0.02	0.23	17	LOQ=0.01 mg/kg
Vlagtwedde									cob with husks	0.10	< 0.01	0.11	28	
The Netherlands									rest of plant[4]	0.18	0.02	0.19	28	
L090171									cob with husks	0.051	< 0.01	0.06	64	
									rest of plant[4]	0.088	< 0.01	0.10	64	
									grain	< 0.01	< 0.01	< 0.02	86	
									rest of plant[4]	0.09	0.01	0.11	86	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 480 F
Content of active substance (g/kg or g/L)	133 g/L	(common name and content)	Epoxiconazole 50 g/L
Formulation (e.g. WP)	BAS 512 04 F (SE)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
NG-500-Mais	GC0645	1. 06.05.2011	Spray boom	0.05	400	0.200	1	71	Whole plant[4]	0.98	1.02	2.00	0	BASF method
2013/1065883	n.a.	2. 15.07.2011	mounted on				04.08.2011		Cob without husks	<0.01	<0.01	<0.02	7	L0076/01
67454 Haßloch		3. n.a.	a one						Cob with husks	0.03	<0.01	0.04	7	LOQ=0.01 mg/kg
Germany			wheeler						Husks	0.13	0.01	0.14	7	
L110466									Cob without husks	<0.01	<0.01	<0.02	22	
									Cob with husks	0.03	<0.01	0.04	22	
									Husks	0.04	<0.01	0.05	22	
									Cob without husks	<0.01	<0.01	<0.02	42	
									Cob with husks	0.03	<0.01	0.04	42	
									Husks	0.18	0.07	0.25	42	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots
n.a. = not applicable

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 480 F
Content of active substance (g/kg or g/L)	133 g/L	(common name and content)	Epoxiconazole 50 g/L
Formulation (e.g. WP)	BAS 512 04 F (SE)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
306326	GC0645	1. 15.03.2008	Echo SHR	0.0499	400	0.1995	1	65	whole plant[4]	1.93	0.03	1.96	0	BASF method
2010/1025690	66-77	2. 28.06.-02.07.2008	15051				30.06.2008		cob with husks	0.04	< 0.01	0.05	11	535/1
40054 Dugliolo	Dekalb	3. 05.09.-08.09.2008							rest of plant[4]	0.21	0.05	0.26	11	LOQ=0.01 mg/kg
Italy									cob with husks	0.02	< 0.01	0.03	16	
L080327									rest of plant[4]	0.17	0.04	0.21	16	
									cob with husks	0.03	< 0.01	0.04	30	
									rest of plant[4]	0.12	0.03	0.15	30	
									grain	< 0.01	< 0.01	0.02	67	
									rest of plant[4]	0.09	0.02	0.11	67	
306326	GC0645	1. 19.04.2008	Pressurized	0.0499	400	0.1995	1	65	whole plant[4]	3.49	0.06	3.55	0	BASF method
2010/1025690	3441	2. 05.07.-20.07.08	gas sprayer				08.07.2008		cob with husks	0.04	< 0.01	0.05	7	535/1
59032	Pioneer	3. 10.09.-30.09.08	AZO with						rest of plant[4]	0.57	0.22	0.79	7	LOQ=0.01 mg/kg
Imathia			6-nozzle						cob with husks	0.03	< 0.01	0.04	22	
Greece			boom						rest of plant[4]	0.21	0.11	0.32	22	
L080328									cob with husks	0.01	< 0.01	0.02	50	
									rest of plant[4]	0.10	0.05	0.15	50	
									grain	< 0.01	< 0.01	0.02	66	
									rest of plant[4]	0.27	0.16	0.43	66	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 480 F
Content of active substance (g/kg or g/L)	133 g/L	(common name and content)	Epoxiconazole 50 g/L
Formulation (e.g. WP)	BAS 512 04 F (SE)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
306326	GC0645	1. 05.05.2008	Sprayer with	0.0499	400	0.1995	1	65	whole plant[4]	2.65	0.14	2.79	0	BASF method
2010/1025690	Mitic	2. 22.07.-30.07.2008	booms				24.07.2008		cob with husks	0.05	< 0.01	0.06	26	535/1
47120 Duras		3. 20.10.2008	AN MESU						rest of plant[4]	0.19	0.03	0.22	26	LOQ=0.01 mg/kg
France (S)			06007						cob with husks	0.04	< 0.01	0.05	33	
L080329									rest of plant[4]	0.21	0.03	0.24	33	
									cob with husks	0.09	< 0.01	0.10	54	
									rest of plant[4]	0.16	0.02	0.18	54	
									grain	< 0.01	0.01	0.02	74	
									rest of plant[4]	0.26	0.02	0.28	74	
306326	GC0645	1. 10.05.2008	Agrotop	0.0499	400	0.1995	1	65	whole plant[4]	< 0.01	< 0.01	0.02	0	BASF method
2010/1025690	Tardio 130	2. 11.08.2008	boom				30.07.2008		cob with husks	< 0.01	< 0.01	0.02	28	535/1
Campillos		3. 28.09.-02.10.2008	sprayer						rest of plant[4]	0.08	0.02	0.10	28	LOQ=0.01 mg/kg
Spain									cob with husks	< 0.01	< 0.01	0.02	41	
L080330									rest of plant[4]	< 0.01	< 0.01	0.02	41	
									cob with husks	0.01	< 0.01	0.02	55	
									rest of plant[4]	< 0.01	0.02	0.03	55	
									grain	< 0.01	< 0.01	0.02	63	
									rest of plant[4]	0.15	0.02	0.17	63	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 480 F
Content of active substance (g/kg or g/L)	133 g/L	(common name and content)	Epoxiconazole 50 g/L
Formulation (e.g. WP)	BAS 512 04 F (SE)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
306328	GC0645	1. 16.03.2009	Echo SHR	0.0499	400	0.1995	1	65	whole plant[4]	2.4	0.06	2.5	0	BASF method
2010/1039144	Dekalb 66-77	2. 28.06.-06.07.2009	150 SI				01.07.2009		cob with husks	0.05	< 0.01	0.06	9	535/1
40054 Dugliolo di Budrio Italy		3. 27.08.2009							rest of plant[4]	0.18	0.05	0.23	9	LOQ=0.01 mg/kg
L090172									cob with husks	0.06	< 0.01	0.07	20	
									rest of plant[4]	0.11	0.04	0.16	20	
									cob with husks	0.05	< 0.01	0.06	30	
									rest of plant[4]	0.09	0.04	0.13	30	
									grain	< 0.01	< 0.01	< 0.02	57	
									rest of plant[4]	0.11	0.04	0.15	57	
306328	GC0645	1. 30.04.2009	Pressurized	0.0499	400	0.1995	1	65	whole plant[4]	2.6	0.03	2.6	0	BASF method
2010/1039144	PR-3345	2. 05.07.-20.07.2009	gas sprayer				09.07.2009		cob with husks	0.02	< 0.01	0.03	6	535/1
59032 Platanos Greece		3. 20.09.-30.09.2009	AZO with 6-nozzle boom						rest of plant[4]	0.72	0.15	0.87	6	LOQ=0.01 mg/kg
L090173									cob with husks	< 0.01	< 0.01	< 0.02	21	
									rest of plant[4]	0.74	0.20	0.94	21	
									cob with husks	< 0.01	< 0.01	< 0.02	49	
									rest of plant[4]	0.72	0.25	0.97	49	
									grain	< 0.01	< 0.01	< 0.02	69	
									rest of plant[4]	0.67	0.23	0.89	69	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Maize	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 480 F
Content of active substance (g/kg or g/L)	133 g/L	(common name and content)	Epoxiconazole 50 g/L
Formulation (e.g. WP)	BAS 512 04 F (SE)	Residues calculated as:	BAS 500 F (pyraclostrobin), 500M07 (BF 500-3)

1	2	3	4	5			6	7	8	9			10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)			PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				BAS 500 F	BF 500-3[2]	Total Pyraclost.[3]		
306328	GC0645	1. 01.04.2009	Sprayer	0.0499	400	0.1995	1	65	whole plant[4]	2.4	0.04	2.4	0	BASF method
2010/1039144	Anadon	2. 15.07.-25.07.2009	with booms				15.07.2009		cob with husks	0.03	< 0.01	0.04	16	535/1
47120		3. 05.10.2009	(Agrotop)						rest of plant[4]	0.49	0.08	0.57	16	LOQ=0.01 mg/kg
Duras									cob with husks	0.03	< 0.01	0.04	27	
France (S)									rest of plant[4]	0.46	0.04	0.50	27	
L090174									cob with husks	0.03	< 0.01	0.04	49	
									rest of plant[4]	0.36	0.04	0.40	49	
									grain	< 0.01	< 0.01	< 0.02	77	
									rest of plant[4]	0.50	0.06	0.56	77	
306328	GC0645	1. 10.06.2009	Boom	0.0499	400	0.1995	1	65	whole plant[4]	3.6	0.04	3.7	0	BASF method
2010/1039144	Castellano	2. 10.07.-23.07.2009	Sprayer				23.07.2009		cob with husks	0.01	< 0.01	0.02	14	535/1
29001 Màlaga		3. 10.09.2009	Agrartest						rest of plant[4]	0.34	0.21	0.54	14	LOQ=0.01 mg/kg
Spain									cob with husks	< 0.01	< 0.01	< 0.02	21	
L090175									rest of plant[4]	0.25	0.09	0.34	21	
									cob with husks	< 0.01	< 0.01	< 0.02	35	
									rest of plant[4]	0.34	0.15	0.48	35	
									grain	< 0.01	< 0.01	< 0.02	49	
									rest of plant[4]	0.27	0.07	0.34	49	

1) at last treatment
2) as BAS 500 F equivalent (conversion factor is 1.08)
3) for calculation purposes, "< 0.01" is set 0.01
4) no roots

Tier 1 Summaries of the Supervised Field Residue Trials for Supporting the Dietary Exposure Assessment for the Metabolite 500M04 (plus Conjugates)

Broccoli

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Broccoli	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
365214 2014/1001661 (2012/1157545) Altamura Italy L110141	VB 0400 Marathon	1. 01.06.2011 2. n. a. 3. 08.2011	Boom sprayer+ compressed air pump	0.025	400	0.100	3 02.08.11	49	Inflorescences Inflorescences	< 0.005 < 0.005	< 0.010 < 0.010	3 6	BASF Method L0220/01 500M04: LOQ= 0.005 mg/kg 500M79: LOQ=0.01 mg/kg
365214 2014/1001661 (2012/1157545) Thesalon ki Greece L110142	VB 0400 Marathon	1. 15.11.2010 2. n. a. 3. 10.04.2011	Knapsack pressurized sprayer with boom	0.025	400	0.100	3 12.04.11	49	Inflorescences Inflorescences	0.007 0.026	0.013 0.047	3 6	

1) at last treatment
n.a. = not applicable

Cauliflower

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Cauliflower	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
365214 2014/1001661 (2012/1157545) Mauguio France L110143	VB 0404 Fremont	1. 21.06.2011 2. n. a. 3. 19.09.2011	compressed air knapsack boom sprayer	0.025	400	0.100	3 15.09.11	47	Inflorescences Inflorescences	0.009 0.012	0.017 0.022	4 8	BASF Method L0220/01 500M04: LOQ= 0.005 mg/kg 500M79: LOQ=0.01 mg/kg
365214 2014/1001661 (2012/1157545) Granada Spain L110144	VB 0404 Equinox	1. 15.06.2011 2. n. a. 3. 09.2011	Back pack power sprayer	0.025	400	0.100	3 07.09.11	43	Inflorescences Inflorescences	< 0.005 < 0.005	< 0.010 < 0.010	3 7	

1) at last treatment
n.a. = not applicable

Lettuce

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Head Lettuce	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
365222 2014/1001661 (2012/1110543) Lambsheim Germany L110296	VL0482 Cavernet	1. 13.05.2011 2. n. a. 3. n. a.	foliar application	0.05	200	0.100	3 21.06.11	48	Head Head	0.024 0.009	0.044 0.016	7 14	BASF Method L0220/01 500M04: LOQ= 0.005 mg/kg 500M79: LOQ=0.01 mg/kg
365222 2014/1001661 (2012/1110543) Offenheim UK L110297	VL0482 Orille-Lollo Rosso	1. 21.06.2011 2. n. a. 3. 26.07.2011	foliar application	0.05	200	0.100	3 19.07.11	45-47	Head Head	0.018 0.019	0.034 0.035	7 14	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Head Lettuce	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
365222 2014/1001661 (2012/1110543) Villers-Perwin Belgium L110298	VL0482 Sansula	1. 01.04.11 22.04.11 (transpl.) 2. n. a. 3. n. a.	foliar application	0.05	200	0.100	3 20.05.11	44-46	Head Head	0.017 0.012	0.030 0.021	7 14	
365222 2014/1001661 (2012/1110543) Oberschaeffols-heim France (N) L110299	VL0482 Caipira	1. 10.05.2011 2. n. a. 3. 20.-23.06.2011	foliar application	0.05	200	0.100	3 10.06.11	39-45	Head Head	0.011 0.007	0.019 0.012	7 13	

1) at last treatment
n.a. = not applicable

Glasshouse

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Head Lettuce	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	Indoor/Glasshouse/Outdoor	Glasshouse
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
365220_1 2014/1001661 (2012/1195474) Tarn et Garonne France L100721 (Greenhouse)	VL0482 Augusta	1. 22.09.11 2. n. a. 3. 04.11.11	foliar application with boom sprayer	0.05 0.05 0.05	222 200 200	0.111 0.100 0.100	3 21.10.11	45-46	Head Head	< 0.005 0.009	< 0.010 0.017	14 20	BASF Method L0220/01 500M04: LOQ= 0.005 mg/kg 500M79: LOQ=0.01 mg/kg
365220_1 2014/1001661 (2012/1195474) Baden-Württemberg Germany L110493 (Greenhouse)	VL0482 Batavia Novelski	1. 21.09.11 2. n. a. 3. 11.11.11	foliar application with boom sprayer	0.05 0.05 0.05	200 200 200	0.100 0.100 0.100	3 28.10.11	46	Head Head	0.018 0.018	0.033 0.033	14 20	

1) at last treatment
n.a. = not applicable

Cabbage

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Cabbage white	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
365216 2014/1001661 2012/1157543 Le Mas Rillier France L110137	VL0041 Impala	1. 27.06.11 2. n. a. 3. 15.10.11	compressed air knapsack boom sprayer	0.0167	600	0.100	3 17.10.11	49	Head Head	0.019 < 0.005	0.034 < 0.010	3 7	BASF Method L0220/01 500M04: LOQ= 0.005 mg/kg 500M79: LOQ=0.01 mg/kg
365216 2014/1001661 (2012/1157543) Asti Italy L110138	VL0041 Farao F1	1. 04.04.11 2. n. a. 3. 06.2011	Boom sprayer + compressed air pump	0.0167	600	0.100	3 06.06.11	47	Head Head	< 0.005 < 0.005	< 0.010 < 0.010	3 7	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Cabbage white	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
365G216 2014/1001661 (2012/1157543) Thessaloniki Greece L110139	VL0041 Grandslam	1. 22.04.2011 2. n. a. 3. 25.06.2011	Knapsack pressurized sprayer with boom	0.0167	600	0.100	3 23.06.11	45-47	Head Head	< 0.005 < 0.005	< 0.010 < 0.010	4 8	
365216 2014/1001661 (2012/1157543) Granada Spain L110140	VL0041 Farnosa	1. 15.06.2011 2. n. a. 3. 09.2011	Backpack power sprayer	0.0167	600	0.100	3 07.09.11	49	Head Head	< 0.005 < 0.005	< 0.010 < 0.010	3 7	

1) at last treatment
n.a. = not applicable

Leek

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Leek	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 550 F
Content of active substance (g/kg or g/L)	40 g/L	(common name and content)	Dimethomorph 72 g/L
Formulation (e.g. WP)	BAS 536 01 F (EC)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
365219 2014/1001661 (2012/1157544) Thessalon ki Greece L110135	VA 0384 Kalamo praso	1. 16.06.11 2. n. a. 3. 20.08.11	Knapsack pressurized sprayer with boom	0.05	200	0.100	3 17.08.11	47/49	Whole Plant[2] Whole Plant[2]	0.020 0.018	0.037 0.032	2 7	BASF Method L0220/01 500M04: LOQ= 0.005 mg/kg 500M79: LOQ=0.01 mg/kg
365219 2014/1001661 (2012/1157544) Seville Spain L110136	VA 0384 Atal	1. 15.12.10 2. n. a. 3. 10.05.11	Boom-sprayer	0.05	200	0.100	3 25.04.11	47	Whole Plant[2] Whole Plant[2]	0.041 0.012	0.074 0.022	3 8	

1) at last treatment
2) w/o roots
n.a. = not applicable

Spinach

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Spinach	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 510 F
Content of active substance (g/kg or g/L)	67 g/kg	(common name and content)	Boscalid 267 g/kg
Formulation (e.g. WP)	BAS 516 07 F (WG)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
309344 2014/1001661 (2011/1125587) Maignelay-Montigny France (N) L100333	VL 0502 Aigle	1. 21.08.10 2. n. a. 3. 08.10.10	Agrotop Boom-sprayer	0.025	400	0.100	2 22.09.10	19	Leaves Leaves	0.039 0.030	0.070 0.055	14 21	BASF Method L0220/01 500M04: LOQ= 0.005 mg/kg 500M79: LOQ=0.01 mg/kg

1) at last treatment
n.a. = not applicable

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Spinach	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 510 F
Content of active substance (g/kg or g/L)	67 g/kg	(common name and content)	Boscalid 267 g/kg
Formulation (e.g. WP)	BAS 516 07 F (WG)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
309344 2014/1001661 (2011/1125587) Ravenna Italy L100334	VL 0502 Marabu	1. 02.03.2010 2. n. a. 3. 10.05.10	Echo SHR 150 SI	0.025	400	0.100	2 26.04.10	33	Leaves Leaves	0.026 0.020	0.048 0.036	14 21	BASF Method L0220/01 500M04: LOQ= 0.005 mg/kg 500M79: LOQ=0.01 mg/kg
309344 2014/1001661 (2011/1125587) Aiguillon France (S) L100335	VL 0502 Pelican	1. 20.09.2010 2. n. a. 3. 02.12.10	Pulvexper Boom- sprayer	0.025	400	0.100	2 05.11.10	19	Leaves Leaves	0.026 0.025	0.047 0.045	13 21	

1) at last treatment
n.a. = not applicable

Beans

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Green Beans	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 510 F
Content of active substance (g/kg or g/L)	67 g/L	(common name and content)	Boscalid 267 g/kg
Formulation (e.g. WP)	BAS 516 07 F (WG)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
309370 2014/1001661 (2011/1135971) Bougnon France (N) L100423	VP 0526 Pedra	1. 19.06.2010 2. 25.07.-10.08.2010 3. 29.09.2010	Pulvexper boom sprayer	0.025	400	0.100	2 17.09.10	81	Pods with Seeds Seeds	< 0.005 < 0.005	< 0.010 < 0.010	3 7	BASF Method L0220/01 500M04: LOQ= 0.005 mg/kg 500M79: LOQ=0.01 mg/kg
309370 2014/1001661 (2011/1135971) Lampertheim Germany L100591	VP 0526 Cadillac	1. 14.07.2010 2. mid Aug. 2010 3. 12.10.2010	Agrotop Plot Sprayer	0.25 0.26	150	0.378 0.396	2 29.09.10	80-81	Pods with Seeds Pods with Seeds Seeds	<0.005 0.007 <0.005	<0.010 0.013 <0.010	8 13 13	

1) at last treatment

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	Pyraclostrobin	Commercial Product (name)	-
Crop/crop group:	Green Beans	Producer of commercial product	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67056 Ludwigshafen	Indoor/Glasshouse/Outdoor	Outdoor
Country	Germany	Other active substance in the formulation	BAS 510 F
Content of active substance (g/kg or g/L)	67 g/L	(common name and content)	Boscalid 267 g/kg
Formulation (e.g. WP)	BAS 516 07 F (WG)	Residues calculated as:	500M04 and 500M79

1	2	3	4	5			6	7	8	9		10	11
Report-No. Location (Trial No.)	Commodity/ Variety	Date of: 1. Sowing/Planting 2. Flowering 3. Harvest	Method of Treatment	Application Rate per Treatment			No of Treatm. and Last Date	Growth Stage (BBCH)[1]	Portion Analysed	Residues (mg/kg)		PHI (days)	Remarks
				kg a.s./hL	Water (L/ha)	kg a.s./ha				500M04	500M79		
309370 2014/1001661 (2011/1135971) Thessalon ki Greece L100424	VP 0526 Etna	1. 11.05.2010 2. 18.06.-15.07.2010 3. 15.07.-10.08.2010	AZO boom sprayer	0.025	400	0.100	2 12.07.10	77	Pods with Seeds Pods with Seeds Seeds Seeds	0.008 < 0.005 < 0.005 < 0.005	0.014 < 0.010 < 0.010 < 0.010	7 15 7 15	BASF Method L0220/01 500M04: LOQ= 0.005 mg/kg 500M79: LOQ=0.01 mg/kg
309370 2014/1001661 (2011/1135971) Ganarolo dell'Emilia Italy L100425	VP 0526 Flavert	1. 28.04.2010 2. 23.06.-02.07.2010 3. 12.07.2010	Echo SHR 150-SI	0.025	400	0.1	2 05.07.10	73	Pods with Seeds Pods with Seeds Seeds Seeds	0.012 0.014 < 0.005 0.005	0.019 0.026 < 0.010 < 0.010	7 14 7 14	
309370 2014/1001661 (2011/1135971) Malaga Spain L100426	VP 0526 Marconi	1. 23.03.2010 2. 21.06.-05.07.2010 3. 12.07.2010	Stihl Atomizer	0.025	400	0.1	2 09.07.10	75	Pods with Seeds Pods with Seeds	0.005 < 0.005	< 0.010 < 0.010	7 14	

1) at last treatment

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Table of Contents

CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Pyraclostrobin (BAS 500 F), a fungicide for use in cereals, maize, potatoes, legumes, fruits, vegetables and various other crops, is registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive No 2004/30/EC and 2009/25/EC. Inclusion entered into force on 10 March 2004. The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011. Approval extension was granted until 31 January 2017 by Regulation No 823/2012/EU.

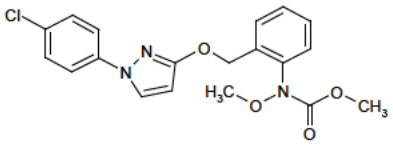
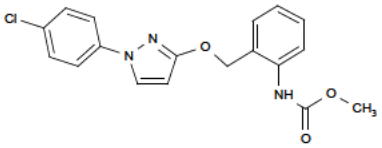
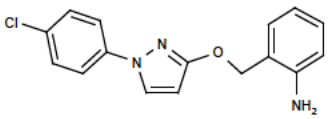
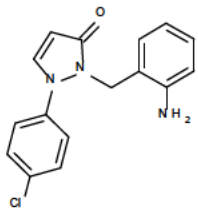
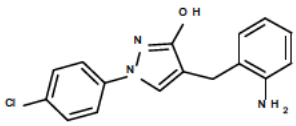
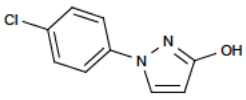
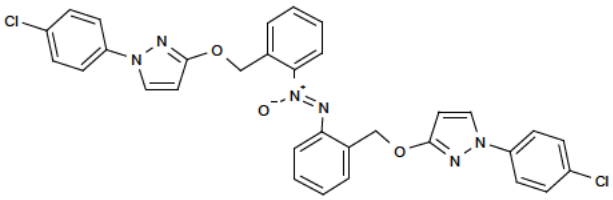
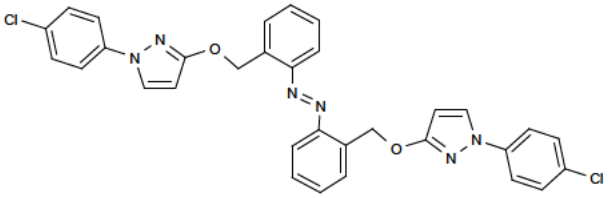
All relevant information on the first Annex I review and the endpoints used in environmental risk assessments can be found in the monograph of pyraclostrobin and in the SANCO/1420/2001-Final document (EU Review Report).

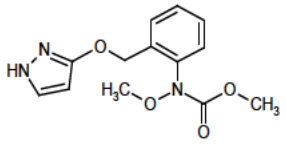
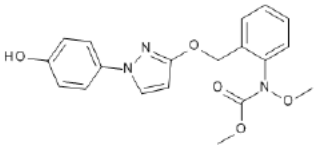
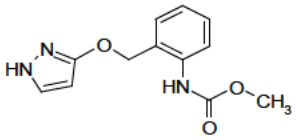
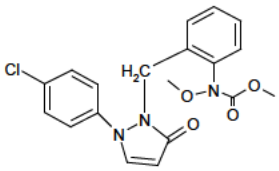
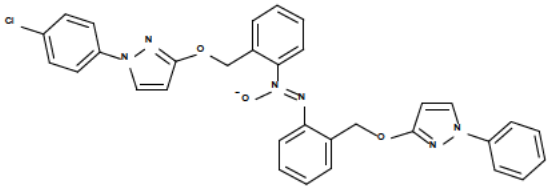
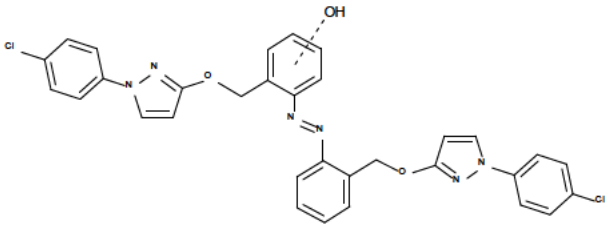
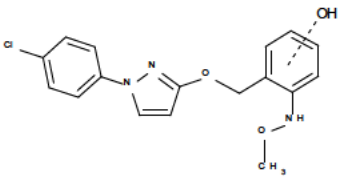
For the current registration renewal under Regulation 1107/2009, a data gap analysis according to new guidelines, new guidance documents and new procedures in kinetic evaluations and exposure assessments was performed and new studies or kinetic evaluations were initiated where considered necessary. All new data are provided in this section.

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. Although title and abstract of several publications indicated a potential connection to respective environmental fate chapters of this dossier, the detailed evaluation of these publications showed no endpoint of sufficient reliability which could be used for the required risk assessments. Consequently, for environmental fate no summaries of public literature data on pyraclostrobin are provided in this section. Further information on the literature assessment and respective justifications can be found in M-CA 9.

Studies on the route of degradation were usually performed using the tolyl-^{14}C or chlorophenyl-^{14}C labeled pyraclostrobin. For confirmation that no pyrazole-specific metabolite is formed, one new soil metabolism and one new soil photolysis study was performed with pyrazole-^{14}C-labeled active substance.

An overview of metabolites discussed in this section is given below. The table is including the different code numbers that are available for each metabolite. In the following chapters and study summaries synonym metabolite codes are given in brackets where deemed to be helpful.

Substance/ Meatbolite Code	Reference code (Reg.No.)	Synonyms	Structure
Pyraclostrobin BAS 500 F	304428	500M00	
BF 500-3	340266	500M07 "des-methoxy"	
BF 500-4	358672	500M73 "aniline"	
isomer of BF 500-4	not assigned	500M74	
isomer of BF 500-4	not assigned	500M75	
BF 500-5	298327	500M04 "OH-pyrazole"	
BF 500-6	364380	500M01 "azoxy"	 cis-trans isomerization possible (assignment of cis/trans refers to direction of phenyl rings at N=N double bond)
BF 500-7	369315	500M02 "azo"	 cis-trans isomerization possible

Substance/ Meatbolite Code	Reference code (Reg.No.)	Synonyms	Structure
BF 500-11	411847	500M60	
BF 500-12	412053	500M59	
BF 500-13	412785	500M62	
BF 500-14	413038	500M76	
500M96	not assigned		
500M97	not assigned		
500M98/ 500M99	not assigned		

Note: The order of the study summaries shown below is differing compared to the information given in the application submitted for renewal of approval. In case references are summarized that were not contained in the application or in case references listed in the application are not contained in this chapter, comments are made where appropriate.

CA 7.1 Fate and behaviour in soil

CA 7.1.1 Route of degradation in soil

The route of degradation in soil of pyraclostrobin is investigated with three different radio-labels (chlorophenyl-, pyrazole- and tolyl-label).

The degradation of pyraclostrobin in aerobic soil studies is characterized by a rather low mineralization rate (about 5% of applied radioactivity (AR) within 100 days) and a formation of high amounts of bound residues (about 55% AR within 100 days).

First step in degradation is the de-methoxylation of the carbamate group forming metabolite BF 500-3. Further degradation at the carbamate group leads to metabolite BF 500-4. Due to the anilinic structure of this metabolite, it reacts immediately with the organic matrix in soil or with another BF 500-4 molecule forming the dimeric structures BF 500-6 (max. 31% AR) or BF 500-7 (max. 14% AR).

A minor route of degradation can be the cleavage of the pyraclostrobin molecule forming metabolite BF 500-5. In aerobic soil, it never appeared in amounts higher than 2.8% AR, in anaerobic soil, it reached up to 7.7% AR. Some further minor reactions (hydroxylation at the tolyl-ring, de-chlorination of BF 500-6) are also reported, however, those metabolites never exceeded 4% AR.

The metabolites BF 500-3 and BF 500-4 are short-lived intermediates, which are detected in aerobic soil either only in very low amounts < 5% AR (BF 500-3) or not at all (BF 500-4). Only in strong anaerobic soil, where the oxygen demanding degradation reactions are considerably slowed down, these metabolites can be found in higher amounts.

Photolytical degradation leads to the same degradation products as observed during dark incubation. No special photoproduct was detected.

When analyzing the non-extractable residues, the major portion of radioactivity was usually associated with insoluble humins and high-molecular humic acids. A release of pyraclostrobin could never be observed, neither with harsh extraction methods (NaOH) nor by intensive digestion activity of soil-eating animals (earthworms).

Most of the information is derived from studies already peer-reviewed during the previous Annex I inclusion process. Nevertheless, some new studies are described below, which were conducted in order to confirm or complement the knowledge of pyraclostrobin degradation in soil.

CA 7.1.1.1 Aerobic degradation

Although results of the older soil metabolism studies with tolyl-labeled and chlorophenyl-labeled pyraclostrobin did not indicate the formation of a pyrazole-ring specific metabolite, for confirmatory purposes a soil metabolism and a soil photolysis study was performed with pyrazole-labeled active substance. In both cases, no metabolite consisting only of the pyrazole-moiety was detected.

Report:	CA 7.1.1.1/1 Kuhnke G.,Hassink J., 2014a Metabolism of (pyrazole-3-C14) BAS 500 F in soil under aerobic conditions 2013/1337273
Guidelines:	OECD 307, EPA 835.4100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This study was not correctly listed in the application submitted for renewal of approval. Authors and DocID have changed after submission of the application.

EXECUTIVE SUMMARY

The aerobic soil metabolism of pyrazole-3-^{14}C-labeled pyraclostrobin was investigated in LUFA 5M soil, a sandy loam (USDA). The nominal application rate was 0.67 mg kg^{-1} dry soil (corresponding to 250 g test item ha^{-1}). Soil aliquots of 100 g (dry weight basis) were weighed into test vessels and incubated at 20°C and 53% maximum water holding capacity (pF 2.5) in the dark for 120 days. A closed incubation system with continuous aeration was used with an attached trapping system for the determination of volatile compounds.

Soil samples were taken at 0, 1, 3, 7, 10, 14, 31, 58, 91 and 120 days after treatment (DAT).

The soil samples were extracted three times with acetonitrile and three times with acetonitrile/water (1:1, v:v). The individual extracts were measured for radioactivity by LSC. The combined acetonitrile as well as the combined acetonitrile/water extracts were analyzed by HPLC. The remaining soil after extraction was combusted to determine the amount of non-extractable soil bound residues. The non-extractable residues were further characterized by NaOH and water extraction and subsequent fractionation into fulvic acids, humic acids, and humins. The fulvic acid fraction was furthermore partitioned with ethyl acetate. A full material balance was provided for each sampling interval.

The amount of extractable radioactivity in soil decreased from 99.8% TAR at time 0 to 39.0% TAR at 120 DAT. The amount of test item pyraclostrobin decreased throughout the incubation from 92.0% TAR at day 0 after treatment to 11.2% TAR at the end of the study. The two metabolites BF 500-6 (500M01) and BF 500-7 (500M02) including their isomers were identified by mass spectrometry and by comparison of retention times to reference items in two chromatographic systems. The metabolite BF 500-6 increased to about 16.2% TAR at 91 DAT and still accounted for 11.9% at the end of the study (mean of two replicates). Metabolite BF 500-7 was detected with max. 4.7% TAR at 31 DAT and decreased to 3.6% TAR (mean) at the end of the study. The corresponding isomers of BF 500-6 and BF 500-7 were found only in low amounts of maximum 1.1% TAR.

Non-extractable radioactive residues (NER) were formed in considerable amounts during incubation. They increased from 0.2% TAR on day 0 to a maximum of 52.7% TAR after 120 days. Characterization of the NER at 120 DAT revealed that about half of it was tightly bound to humins, while the rest was distributed in a ratio of about 1:1 between the humic and the fulvic acid fraction. The fulvic acid fraction was further characterized by partitioning with ethyl acetate. Amounts of 9.7% TAR of the fulvic acid fraction were soluble in ethyl acetate, whereas 3.0% TAR remained in the water phase. The ethyl acetate soluble fractions from samples of 120 DAT were investigated by HPLC. The main peak found by HPLC (accounting for 8.4% TAR) was the metabolite BF 500-5 known to occur under alkaline conditions.

$^{14}CO_2$ was formed in low amounts up to 2.2% TAR. No other volatile compounds were detected in significant amounts. The material balance ranged from 93.8 to 110.3% TAR throughout the incubation period of 120 days, resulting in a mean value of 100.6% TAR.

Kinetic analysis and calculation of $DegT_{50}$ and $DegT_{90}$ values for pyraclostrobin and its metabolites BF 500-6 and BF 500-7 was performed following the recommendations of the FOCUS Kinetics workgroup. The analysis was done by non-linear regression methods using the software package KinGUI version 2.2012.320.1629.

A best-fit $DegT_{50}$ value of 22.3 days and $DegT_{90}$ value of 115.2 days were calculated for pyraclostrobin (FOMC). For BF 500-6 and BF 500-7, best-fit $DegT_{50}$ values of 95.9 and 81.5 days and $DegT_{90}$ values of 318.7 and 270.7 days were calculated (SFO), respectively.

Modeling endpoints were derived using SFO kinetics for all compounds. The $DegT_{50}$ of pyraclostrobin equals 26.2 days, the $DegT_{50}$ of BF 500-6 and BF 500-7 equal 68.0 and 59.0 days, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code:	BAS 500 F
Reg. No.:	304428
CAS-No.:	175013-18-0
Chemical name (IUPAC):	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3 yl]oxymethyl}phenyl)-(N-methoxy)carbamate
Molar mass:	387.8 g mol^{-1} (unlabeled)
Position of radiolabel:	pyrazol-3-^{14}C
Specific radioactivity of a.s.:	5.5 MBq mg^{-1} (330000 dpm μg^{-1})
Batch No.:	1073-1008
Radiochemical purity:	99.7%

2. Soil

The German soil LUFA 5M from LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer, Germany) was used in this study. It was sampled from 0-20 cm depth. The soil was passed through a 2 mm sieve, remoistened to approximately 8-12% soil moisture and stored for no longer than three months at 4°C before use. The soil characteristics are summarized in Table 7.1.1.1-1.

Table 7.1.1.1-1: Characteristics of soil LUFA 5M used for soil metabolism study with ^{14}C-pyraclostrobin

Soil designation	**LUFA 5 M** **13/1651/01** (Germany, Origin: LUFA Speyer)
DIN Particle size distribution [%] Sand 0.063 – 2 mm Silt 0.002 – 0.063 mm Clay < 0.002 mm Textural class	 53.6 33.3 13.1 loamy sand (Sl4)
USDA Particle size distribution [%] Sand 0.050 – 2 mm Silt 0.002 – 0.050 mm Clay < 0.002 mm Textural class	 57.0 29.9 13.1 sandy loam
Organic C [%] Organic matter [%] **	1.98 3.41
pH [H_2O] pH [$CaCl_2$]	7.9 7.4
Cation exchange capacity [$cmol^+ kg^{-1}$]	10.2
Max. water holding capacity [g/100g dry weight]	27.0
Water holding capacity at pF 2.0 [g/100g dry weight] Water holding capacity at pF 2.5 [g/100g dry weight]	0.174 0.147
Microbial biomass (start of study) [mg C/100g dry soil]	27.8 (certificate) 30.1*
Microbial biomass (after 58 days) [mg C/100g dry soil]	19.7*
Microbial biomass (end of study [121 days]) [mg C/100g dry soil]	16.3*

* determined at BASF test facility Limburgerhof

** organic matter = organic carbon x 1.724

B. STUDY DESIGN

1. Experimental conditions

The test substance was applied at a nominal concentration of 0.67 mg ^{14}C-pyraclostrobin/kg dry soil which corresponds to a field application rate of 250 g a.s. ha^{-1} (calculated on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}). Portions of 100 g soil (dry weight basis) were then filled into test vessels.

The mean value of the sum of extractable and non-extractable radioactive residues at day 0 in 100 g dry soil was defined as 100% TAR. The actual application rate was 0.6467 mg kg^{-1} (mean value of two samples immediately extracted after application).

For incubation, all test vessels were connected in line with aeration tubes in the soil metabolism apparatus. During the study, samples were continuously aerated with a slight stream of moistened synthetic air. For removing carbon dioxide, the air was passed through a bottle with NaOH before passing the test vessels. For trapping of volatiles possibly evolving from soil during the incubation, test vessels were connected to three gas washing flasks containing at least 50 mL ethylene glycol, 50 mL 0.5 M H_2SO_4, and 50 mL 0.5 M NaOH. The treated soils were incubated at 53% maximum water holding capacity and 20 ± 2°C in the dark.

To determine the microbial biomass at 0, 58 and 121 days after treatment, an additional soil portion (1500 g dry soil equivalents, 53% maximum water holding capacity) was treated only with 1 mL acetone (without test item). The samples for 58 and 121 DAT were incubated under the same conditions (dark, 20 ± 2°C) as the treated soil.

2. Sampling

Sampling dates were 0, 1, 3, 7, 10, 14, 31, 58, 91 and 120 days after treatment (DAT). On day 0, 58 and 120, two replicate soil samples were worked up.

3. Description of analytical procedures

For the determination of the extractable radioactive residues (ERR), the 100 g (dry weight basis) soil samples were consecutively extracted three times with 100 mL acetonitrile (ACN), and three times with 100 mL ACN/water (1:1; v:v). The ACN-extracts as well as the ACN/water-extracts were pooled and each solution was concentrated (rotary evaporator T≈ 35°C). Then the residues were re-dissolved in a well-defined volume solvent and analyzed by radio HPLC with two different HPLC systems.

The soil residues remaining after extraction were air-dried, homogenized by means of a small mill, and aliquots were combusted in a biological oxidizer. The evolved $^{14}CO_2$ from each combusted aliquot was trapped in Oxysolve C-400 scintillator and measured by LSC to determine the amount of the non-extractable residues (NER).

The non-extractable residues of the 120 DAT samples were further characterized by NaOH extraction. The samples were consecutively extracted with NaOH (three times) and water (twice). Finally, all extracts were pooled and acidified with concentrated hydrochloric acid to pH 1.5 to precipitate the humic acid fraction. After centrifugation, the supernatant (fulvic acids) was separated from the precipitate. The precipitate (humic acid fraction) was dissolved in 50 mL NaOH. The humic acid fraction and the fulvic acid fraction were measured for radioactivity. The remaining soil samples after NaOH and water extraction were dried at room temperature. Afterwards, aliquots were combusted. The released $^{14}CO_2$ was trapped and analyzed by LSC to determine the ^{14}C-residues in the humin fraction.

The fulvic acid fraction was partitioned with ethyl acetate. The organic phase was further analyzed by radio HPLC.

The microbial biomass in the soil samples treated with solvent only was determined at 0, 58 and 121 DAT. The method was based on the determination of oxygen consumption upon addition of glucose. The microbial biomass declined over the incubation phase. However, the results demonstrate that the soil was still viable and microbially active at days 58 and 121 (end) of the study.

4. Calculation of the degradation rate

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) as well as modeling endpoints. Kinetic analysis and calculation of $DegT_{50}$ and $DegT_{90}$ values was performed following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 (November 2011), 436 pp.*].

The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting [*SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. In: Del Re, A.A.M. et al. (Eds.): Proceedings of the XIII Symposium on Pesticide Chemistry, Piacenza, 2007, p. 916-923., SCHMITT, W., GAO, Z., MEYER, H. (2011) KinGUII, Version 2.2012.320.1629 Bayer CropScience AG*]. The error tolerance and the number of iterations of the optimization tool were set to 0.00001 and 100, respectively.

For each data set, the kinetic models proposed by the FOCUS Kinetics guidance document were tested in order to identify the best-fit model. The recommended kinetic models, i.e. the single first order kinetics (SFO) and the Gustafson-Holden model (FOMC), are already implemented in KinGUI. The Goodness-of-fit was evaluated by visual assessment, χ^2 minimum error, and type-I-error rate (t-test) [for details see Chapter 6.3 in FOCUS (2006)].

Where available, replicate measurements were considered for the parameter estimation. The initial concentration of the applied test item was set to the material balance recovered at day 0. The χ^2 value for the kinetic model was calculated as recommended by FOCUS, considering the average of replicate measurements. For the individual parameters, the t-test statistics were based on number of individual measurements.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The detailed results on the extractability of radioactive residues are presented in Table 7.1.1.1-2. The mass balance ranged from 93.8 to 110.3% TAR throughout the incubation period of 120 days, resulting in a mean value of 100.6% TAR.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in the soil is shown in Table 7.1.1.1-2. The total extractable radioactivity from the soils decreased from 99.8% TAR at time 0 to 39.0% TAR after 120 days. The non-extractable residues increased from 0.2% TAR immediately after treatment to 52.7% TAR after 120 days.

Table 7.1.1.1-2: Material balance and distribution of radioactivity after application of ^{14}C-pyraclostrobin to soil [% TAR]

DAT		ACN	ACN/water	Total extractable	Non-extractable	Volatiles*	Material balance
0	repl. I	97.5	1.3	98.9	0.2	n.a.	99.1
	repl. II	99.3	1.4	100.6	0.2	n.a.	100.9
	mean	98.4	1.4	99.8	0.2	n.a.	100.0
1		100.0	2.3	102.3	2.3	0.0	104.5
3		94.0	2.4	96.4	5.3	0.0	101.7
7		82.6	5.0	87.6	11.1	0.1	98.9
10		81.1	3.2	84.3	15.9	0.2	100.4
14		84.8	3.6	88.3	21.5	0.4	110.3
31		55.6	4.3	59.9	37.1	0.8	97.8
58	repl. I	48.8	4.2	53.0	49.1	1.3	103.4
	repl. II	47.7	4.1	51.8	48.9	1.3	102.0
	mean	48.2	4.1	52.4	49.0	1.3	102.7
91		38.1	4.5	42.6	51.4	1.7	95.7
120	repl. I	35.1	4.6	39.6	53.7	2.2	95.5
	repl. II	33.2	5.1	38.3	51.6	2.2	92.0
	mean	34.1	4.8	39.0	52.7	2.2	93.8

DAT = days after treatment
ACN = acetonitrile
n.a. = not analyzed
* no significant amounts of other volatiles than CO_2 were found

C. VOLATILISATION

Carbon dioxide was the only volatile degradation product reaching 2.2% TAR after 120 days of incubation. In sulfuric acid and ethylene glycol no significant radioactivity could be measured at any time point.

D. TRANSFORMATION OF PARENT COMPOUND

HPLC analysis of the soil extracts revealed degradation of pyraclostrobin in LUFA 5M soil. The extractable amount of pyraclostrobin decreased throughout the incubation period from 92.0% TAR at day 0 to 11.2% TAR at day 120 (Table 7.1.1.1-3).

Two metabolites (BF 500-6 and BF 500-7) were identified by mass spectrometry and by comparison of retention times with those of reference items in two chromatographic systems. The concentrations of pyraclostrobin and the identified metabolites BF 500-6 and BF 500-7 in the organic extracts are given in Table 7.1.1.1-3. Metabolite BF 500-6 increased to about 16.2% TAR at 91 DAT and still accounted for 11.9% TAR at the end of the study (mean of two replicates). Metabolite BF 500-7 was detected with max. 4.7% TAR at 31 DAT and decreased to 3.6% TAR (mean) at the end of the study. Both metabolites can appear in isomeric form (cis-trans isomers). The isomers occurred, if at all, only in very low amounts and never exceeded 1.1% TAR. No other metabolite exceeded 2.1% TAR at any sampling time. No pyrazole-specific metabolite could be identified.

Table 7.1.1.1-3: Radio HPLC analysis of extracts of soil LUFA 5M treated with ^{14}C-pyraclostrobin [%TAR]

days after treatment	BAS 500 F	BF 500-6	BF 500-6 isomer*	BF 500-6 sum	BF 500-7	BF 500-7 isomer*	BF 500-7 sum	Sum others**
0 repl. I	89.8	n.d.	n.d.	-	n.d.	n.d.	-	n.d.
repl. II	94.3	n.d.	n.d.	-	n.d.	n.d.	-	0.0
mean	92.0	n.d.	n.d.	-	n.d.	n.d.	-	0.0
1	97.3	n.d.	n.d.	-	n.d.	n.d.	-	0.6
3	93.6	2.0	n.d.	2.0	n.d.	n.d.	-	0.2
7	78.6	5.5	n.d.	5.5	2.0	n.d.	2.0	1.4
10	73.2	7.5	n.d.	7.5	1.7	n.d.	1.7	0.2
14	64.9	10.2	1.0	11.2	3.2	0.9	4.1	2.4
31	37.8	13.7	0.5	14.2	4.7	0.6	5.3	1.7
58 repl. I	23.0	16.8	n.d.	16.8	4.6	0.8	5.4	3.4
repl. II	22.6	15.1	n.d.	15.1	4.4	0.8	5.2	6.1
mean	22.8	16.0	n.d.	16.0	4.5	0.8	5.3	4.8
91	13.3	16.2	1.1	17.3	4.4	1.1	5.5	4.0
120 repl. I	10.8	8.6	0.8	9.4	3.1	0.4	3.5	12.1
repl. II	11.5	15.1	1.1	16.2	4.1	0.5	4.6	4.0
mean	11.2	11.9	1.0	12.9	3.6	0.5	4.1	8.1

TAR = total applied radioactivity

* concluded from mass spectrometric analysis and/or retention times of reference compounds

** Sum of unknown peaks, each individual peak ≤ 2.13% TAR

n.d. not detected

E. CHARACTERIZATION OF NON-EXTRACTABLE RESIDUES (NER)

For the 120 days samples, the non-extractable residues were extracted three times with NaOH (+ two water rinsings). After extraction with NaOH, about half (~26% TAR) of the ^{14}C-residues remained unextractable (humin fraction). The alkali-soluble radioactivity was further fractionated into fulvic and humic acids by precipitation with concentrated HCl. The results are given in Table 7.1.1.1-4 and Table 7.1.1.1-5. The radioactivity was almost evenly distributed between humic and fulvic acid fraction. The humic acid fraction was not further analyzed.

The fulvic acid fraction was partitioned with ethyl acetate. About three quarters of the fulvic acid fraction (9.7% TAR) was soluble in the ethyl acetate phase, whereas one quarter remained in the water phase (see Table 7.1.1.1-6).

HPLC analyses of the ethyl acetate phase of the fulvic acid fraction revealed the presence of one main and three small peaks. The main peak (amounting to 8.4% TAR) is presumably metabolite BF 500-5 known to occur under alkaline conditions. The possible assignment is supported by comparison of retention times in two chromatographic systems.

Table 7.1.1.1-4: Characterization of bound residues [% TAR]

days after treatment		total NER	total NaOH extractable			NER (Humins)	sum
			NaOH	Water	Total		
120	repl. I	53.7	25.5	3.1	28.7	26.4	55.1
	repl. II	51.6	22.1	2.4	24.5	25.5	50.0
	mean	52.7	23.8	2.8	26.6	26.0	52.5

TAR = total applied radioactivity
NER = non-extractable radioactive residues

Table 7.1.1.1-5: Fractionation of alkali-soluble residues [% TAR]

days after treatment		total NaOH extractable	Humic acids	Fulvic acids	Sum humic and fulvic acids
120	repl. I	28.7	11.8	13.2	25.0
	repl. II	24.5	10.7	12.7	23.4
	mean	26.6	11.2	13.0	24.2

TAR = total applied radioactivity

Table 7.1.1.1-6: Fractionation of residues from the fulvic acids [% TAR]

days after treatment		Fulvic acids	Ethyl acetate	Aqueous phase	Sum ethyl acetate and aqueous phase
120	repl. I	13.2	9.9	3.1	12.9
	repl. II	12.7	9.6	2.9	12.5
	mean	13.0	9.7	3.0	12.7

TAR = total applied radioactivity

F. KINETIC MODELING RESULTS

The estimated $DegT_{50}$ and $DegT_{90}$ values for pyraclostrobin as well as its metabolites BF 500-6 and BF 500-7 in soil were calculated according to the recommendation of FOCUS (2006).

$DegT_{50}$/$DegT_{90}$ values obtained are presented in Table 7.1.1.1-7 and Table 7.1.1.1-8.

Table 7.1.1.1-7: Trigger endpoints for pyraclostrobin, BF 500-6 and BF 500-7

Compound	Best-fit model	χ^2 error	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Pyraclostrobin	FOMC	2.20	22.3	115.2
BF 500-6	SFO	7.36	95.9	318.7
BF 500-7	SFO	10.55	81.5	270.7

Table 7.1.1.1-8: Modeling endpoints for pyraclostrobin, BF 500-6 and BF 500-7

Compound	Kinetic model	χ^2 error	$DegT_{50}$ [d]	k [d^{-1}]	Formation fraction [-]
Pyraclostrobin	SFO	5.05	26.2	0.0264	-
BF 500-6	SFO	8.42	68.0	0.0102	0.318
BF 500-7	SFO	10.60	59.0	0.0117	0.112

III. CONCLUSION

Pyrazole-3-^{14}C-pyraclostrobin was degraded rather fast in aerobic soil by formation of non-extractable residues, reaching 52.7% TAR at the end of the study after 120 days. $^{14}CO_2$ was formed in low amounts up to 2.1% TAR. No other volatile compounds were detected in significant amounts.

The two known metabolites BF 500-6 and BF 500-7 were confirmed with maximum concentrations of 17.3% TAR and 5.5% TAR, respectively (sum of both isomers). No pyrazole-specific metabolites could be identified.

A best-fit $DegT_{50}$ value of 22.3 days and $DegT_{90}$ value of 115.2 days were calculated for pyraclostrobin (FOMC). For BF 500-6 and BF 500-7, best-fit $DegT_{50}$ values of 95.9 and 81.5 days and $DegT_{90}$ values of 318.7 and 270.7 days were calculated (SFO), respectively.

Modeling endpoints were derived using SFO kinetics for all compounds. The $DegT_{50}$ of pyraclostrobin equals 26.2 days, the $DegT_{50}$ of BF 500-6 and BF 500-7 equal 68.0 and 59.0 days, respectively.

Normalization of degradation rates
Since for environmental fate modeling $DegT_{50}$ values at reference conditions (temperature of 20°C and soil moisture at field capacity, i.e. pF2) are required, the reported $DegT_{50}$ values for modeling were normalized following the recommendations of *FOCUS (2012):* [*Generic Guidance for Tier 1 FOCUS Ground Water Assessments. Version: 2.1*].

Since the study was performed at 20°C no temperature correction was necessary. The moisture normalization was performed using the moisture dependency equations by Walker as described in Equation 7.1.1.1-1.

Equation 7.1.1.1-1 Calculation of the moisture correction factor according to Walker

$$f_{moist} = \begin{cases} \left(\frac{\theta_{act}}{\theta_{ref}}\right)^{0.7} & if \quad \theta_{act} < \theta_{ref} \\ 1 & if \quad \theta_{act} \geq \theta_{ref} \end{cases}$$

with:			
	f_{moist}	moisture correction factor	[-]
	θ_{ref}	reference soil moisture at field capacity (pF2, 10 kPa)	[g / 100 g dry soil]
	θ_{act}	actual soil moisture during incubation	[g / 100 g dry soil]

The actual soil moisture was taken from the study report and the corresponding reference moisture at pF 2 was derived from FOCUS (2012). The normalized DT_{50} values were calculated by multiplying the DT_{50} values at study conditions by the correction factor f_{moist} as described in Equation 7.1.1.1-2.

Equation 7.1.1.1-2 Calculation of the DT_{50} at reference conditions (20°C, pF2)

$$DegT_{50,ref} = DegT_{50,act} \cdot f_{moist}$$

with:			
	$DegT_{50,ref}$	normalized $DegT_{50}$	[d]
	$DegT_{50,act}$	$DegT_{50}$ at study conditions	[d]
	f_{moist}	moisture correction factor	[-]

A summary of the normalized half-lives of pyraclostrobin and its aerobic soil metabolites BF 500-6 and BF 500-7 is given below:

Table 7.1.1.1-9: Normalization of $DegT_{50}$ values to reference conditions

Compound	Soil	pH ($CaCl_2$)	Kinetic model	θ_{act}	θ_{ref}	f_{moist}	$DegT_{50,act}$	$DegT_{50,ref}$
Pyraclostrobin	LUFA 5M	7.4	SFO	14.3	17.4	0.87	26.2	22.8
BF 500-6							68.0	59.2
BF 500-7							59.0	51.3

θ_{act}	actual soil moisture	[g / 100 g dry soil]
θ_{ref}	reference soil moisture at field capacity (pF 2) according to FOCUS (2012)	[g / 100 g dry soil]
f_{moist}	moisture correction factor	[-]
$DegT_{50,act}$	$DegT_{50}$ at study conditions	[d]
$DegT_{50,ref}$	$DegT_{50}$ at reference conditions	[d]

CA 7.1.1.2 Anaerobic degradation

No new anaerobic soil metabolism study with pyraclostrobin was performed. However, new information was obtained on the isomer structures (500M74, 500M75) of metabolite BF 500-4 (500M73), which were observed during the anaerobic soil metabolism studies with pyraclostrobin (already peer-reviewed during previous Annex I inclusion process). This information is provided in M-CA 7.1.2.1.4 *Anaerobic degradation of metabolites, breakdown and reaction products*.

CA 7.1.1.3 Soil photolysis

Report: CA 7.1.1.3/1
Hassink J.,Hermann M., 2014a
Soil photolysis of (pyrazole-3-C14) BAS 500 F
2013/1341955

Guidelines: EPA 161-3, EPA 835.2410, EEC 91/414 Annex II, OECD Draft Guideline Phototransformation of Chemicals on Soil Surfaces (January 2002), SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This study was not correctly listed in the application submitted for renewal of approval. Authors and DocID have changed after submission of the application.

EXECUTIVE SUMMARY

The soil photolysis of pyraclostrobin (BAS 500 F) was studied in a sandy loam soil (LUFA 5M, Speyer, Germany) for 15 days in a flow through system at 22°C ± 1°C under continuous irradiation and in the dark (control). Pyrazole-^{14}C-labeled pyraclostrobin was used for treatment at an application rate of 1.67 mg kg^{-1}, corresponding to a field application rate of 250 g ha^{-1}.

Soil samples were placed in a SUNTEST apparatus and were continuously exposed to a Xenon arc lamp emitting a light spectrum similar to sunlight (≥ 290 nm), simulating a clear sunny day around noon in Central Europe. The dark controls were stored in an incubator at 22°C ± 1°C.

Duplicate samples were taken at days 0, 2, 5, 8, 12 and 15 after treatment. Dark control samples were analyzed at the same sampling days. Volatiles were trapped in appropriate trapping solutions. Soil samples were extracted three times with acetonitrile (ACN) and three times with acetonitrile/water (1+1; v/v). The combined acetonitrile extracts as well as the combined acetonitrile/water extracts were analyzed by LSC and HPLC. Bound residues were quantified by combustion and subsequent LSC measurement. A full material balance is provided for each sampling interval.

The remaining soils after ACN and ACN/water extraction at 8, 12 and 15 days after treatment (DAT) were extracted three times with NaOH to determine the amount of alkali-soluble components. The dried soil was then combusted to determine the amount of residue in the humin fraction.

In the photolysis test, the total extractable radioactivity did not significantly differ from the dark control. After 15 days, about 7 and 10% of the total applied radioactivity (TAR) were not extractable from the irradiated and dark control samples, respectively. The alkali-soluble radioactivity amounted to 3-4% TAR in the period of 8-15 DAT in the photolysis, representing the fulvic acids and humic acids. The dark control revealed similar values (3-5% TAR).

The pyrazole-labeled pyraclostrobin declined in the photolysis experiment to 82.1% TAR after 15 days under continuous irradiation. A similar degradation was observed in dark control samples with 76.7% TAR pyraclostrobin present in the organic extracts at the end of the study.

Aliquots of all samples were analyzed by radio-HPLC. No degradation product exceeded 3% TAR at any sampling time with the exception of the known soil metabolite BF 500-6 which was confirmed by HPLC-MS/MS. It was detected with up to 3.5% TAR under the influence of light and up to 7.9% TAR in the dark control. A second metabolite identified as BF 500-7 accounted for up to 1.3% TAR in the photolysis experiment and up to 3.0% TAR in the dark control.

Kinetic analysis and calculation of $DegT_{50}$ and $DegT_{90}$ (best-fit and modeling) values for pyraclostrobin in soil was performed following the recommendations of the FOCUS Kinetics workgroup. The analysis was conducted by non-linear regression methods employing the software tool KinGUII. $DegT_{50}$ and $DegT_{90}$ values for pyraclostrobin accounted for 55.2 and 183.3 days in the photolysis experiment and 41.5 and 137.8 days for the dark control, respectively, using SFO kinetics.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Internal code	BAS 500 F
Reg. No.:	304428
CAS-No.:	175013-18-0
Chemical name (IUPAC):	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)-(N-methoxy)carbamate
Molar mass:	387.8 g mol^{-1} (unlabeled)
Position of radiolabel:	pyrazol-3-^{14}C
Specific radioactivity of a.s.:	5.5 MBq mg^{-1} (330000 dpm μg^{-1})
Batch No.:	1073-1008
Radiochemical purity:	99.7%

2. Soil

The soil used was classified as a sandy loam and originated from the Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, South Western Germany. The sampling depth was 0-20 cm. The soil was passed through a 2 mm sieve, remoistened to approximately 8-12% soil moisture and then stored at about 4°C in the dark no longer than three months before use. An overview of soil parameters is listed in Table 7.1.1.3-1.

Table 7.1.1.3-1: Characteristics of soil LUFA 5M used for soil photolysis study with ^{14}C-pyraclostrobin

Soil designation	**LUFA 5M** **13/1651/01** Germany (Origin LUFA Speyer)
DIN 4220 Particle size distribution [%] Sand 0.063 – 2 mm Silt 0.002 – 0.063 mm Clay < 0.002 mm Textural class	 53.6 33.3 13.1 loamy sand
USDA Particle size distribution [%] Sand 0.050 – 2 mm Silt 0.002 – 0.050 mm Clay < 0.002 mm Textural class	 57.0 29.9 13.1 sandy loam
Organic C [%]	1.98
pH [H_2O] pH [$CaCl_2$]	7.9 7.4
Cation exchange capacity [$cmol^+ kg^{-1}$]	10.2
Max. water holding capacity [g /100 g dry weight]	27.0
Microbial biomass (start of study) [mg C/100 g dry soil]	27.8

B. STUDY DESIGN

1. Experimental conditions

For each test (photolysis and dark control), ten small steel dishes (88 mm x 44 mm x 10 mm) were filled with soil and arranged in a rectangular stainless steel bowl with a connected thermostat. Soil surfaces were then treated with the radio-labeled test item. The temperature of the dishes used for photolysis was adjusted to 22°C ± 1°C and controlled by an external tempering unit, while the dishes for the dark control were put into an incubator at 22°C ± 1°C. Subsequently, the bowl was closed airtight with a quartz glass covering. A continuous aeration of the whole incubation device was ensured with CO_2-depleted (0.5 M NaOH) and remoistened air applied via an air inlet and an air outlet. The exiting air was flushed through three gas washing flasks containing ethylene glycole, H_2SO_4 (0.5 M), and NaOH (0.5 M) to collect potentially evolving ^{14}C-volatiles.

The incubation bowl for photolysis was placed under a SUNTEST CPS plus (Atlas) equipped with a Xenon lamp emitting light with a sunlight similar spectrum at a light intensity of about 3 mW cm^{-2} (UVA range). This corresponds to a clear summer day in Southern Germany (about 48°N). Wavelengths < 290 nm were filtered off to simulate natural sunlight

To maintain the temperature especially on the quartz glass surface in order to avoid a rapid drying of the soil surface, the air space between lamp and quartz glass within the SUNTEST device was cooled by an external apparatus (Yeti, Seveso). This apparatus was connected to and controlled by the SUNTEST. To maintain the initial water content as constant as possible, dishes were weighed at each incubation day. The evaporated water was replaced.

The amount of test item to be applied on the soil surface was calculated based on a recommended field application rate of 250 g ha^{-1}. If a soil layer of 1 cm and bulk density of 1.5 kg L^{-1} is assumed, the application rate corresponds to about 1.67 mg test item per kg dry soil (and about 50 µg per dish).

2. Sampling

Sampling was performed after an incubation period of 0, 2, 5, 8, 12 and 15 days. At each sampling date, two dishes were taken from the photolysis test system and from the dark control (the day 0 samples were used for both experiments). At each sampling date, the respective volatile trapping solutions were removed.

3. Description of analytical procedures

Each soil sample was consecutively extracted three times with acetonitrile and three times with acetonitrile/water (1:1, v/v). After each extraction step, solid and extract were separated by centrifugation. The three corresponding acetonitrile and three acetonitrile/water extracts were combined, respectively, and analyzed for radioactivity.

After the last acetonitrile/water extraction, the soil residues were air-dried and homogenized by milling. Aliquots of each sample were combusted in a sample oxidizer. Trapped $^{14}CO_2$ was analyzed by LSC.

All extracts were analyzed by radio HPLC to determine the metabolite pattern. Prior to injection, the solvent of the extracts was completely evaporated by a rotary evaporator at 35°C. Then the residues were redissolved in a defined volume of the respective extraction solvent and subjected to HPLC analysis.

Non-extractable residues (NER) were found in amounts of 5-10% TAR at sampling days 8, 12 and 15 in the photolysis experiment as well as in the dark controls. The NER were further characterized by NaOH extraction. Samples were extracted three times with 0.5 M NaOH on a rotary shaker (200 rpm, extraction times: overnight, at least 7 h, overnight) and twice washed with water. Aliquots of the NaOH and the water extracts were analyzed by LSC. NaOH extracts and water extracts were pooled, representing together the fulvic and humic acid fraction. Since the amount of radioactivity did not exceed 5% TAR, no acidic precipitation of the humic acids from the pooled extracts was performed.

The remaining soil samples after NaOH and water extraction were air-dried at room temperature, aliquots were combusted, and the formed $^{14}CO_2$ was trapped and analyzed by LSC to determine the ^{14}C residues in the humin fraction.

4. Calculation of the degradation rate

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) as well as modeling endpoints. Kinetic analysis and calculation of $DegT_{50}$ and $DegT_{90}$ values was performed following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006)*]. The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting [*Schäfer et al. (2007)*]. The error tolerance and the number of iterations of the optimization tool were set to 0.00001 and 100, respectively.

For each data set, the kinetic models proposed by the FOCUS Kinetics guidance document were tested to identify the best-fit model. The recommended kinetic models, i.e. the single first order kinetics (SFO) and the Gustafson-Holden model (FOMC), are already implemented in KinGUI. The Goodness-of-fit was evaluated by visual assessment, χ^2 minimum error, and type-I-error rate (t-test) [for details see *Chapter 6.3 in FOCUS (2006)*].

Replicate measurements were considered for the parameter estimation. The initial concentration of the applied test item was set to the material balance recovered at day 0. The χ^2 value was calculated for the kinetic model as recommended by FOCUS, considering the average of replicate measurements. For the individual parameters, the t-test statistics were based on number of individual measurements.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Total recoveries of radioactivity extracted from soil are summarized in Table 7.1.1.3-2 and Table 7.1.1.3-3. The overall mean values for the material balance in the photolysis and in the dark control were in the range of 97.1-102.7% TAR.

Table 7.1.1.3-2: Recovery and distribution of radioactivity in soil LUFA 5M after treatment with ^{14}C-labeled pyraclostrobin and incubation under irradiated conditions [% TAR]

days after treatment		ACN	ACN/water	total extractable	non-extractable	volatiles*	material balance
0	I	98.6	0.9	99.5	0.0	n.a.	99.5
	II	99.5	1.0	100.5	0.0	n.a.	100.5
mean		99.0	0.9	100.0	0.0	n.a.	100.0
2	I	94.6	2.4	97.0	1.5	0.3	98.8
	II	93.6	2.9	96.4	1.6	0.3	98.3
mean		94.1	2.6	96.7	1.6	0.3	98.6
5	I	93.6	4.6	98.1	2.9	0.8	101.9
	II	90.0	4.6	94.6	2.8	0.8	98.2
mean		91.8	4.6	96.4	2.9	0.8	100.0
8	I	89.5	3.9	93.3	4.3	1.1	98.8
	II	89.5	4.2	93.7	4.7	1.1	99.6
mean		89.5	4.0	93.5	4.5	1.1	99.2
12	I	86.6	7.3	93.9	6.1	1.6	101.6
	II	87.5	7.1	94.5	5.6	1.6	101.8
mean		87.0	7.2	94.2	5.8	1.6	101.7
15	I	82.9	6.3	89.2	6.9	2.0	98.1
	II	82.8	6.9	89.7	7.7	2.0	99.4
mean		82.8	6.6	89.4	7.3	2.0	98.7

TAR = total applied radioactivity
ACN = acetonitrile
n.a. = not analyzed
* no other volatiles than CO_2 were found

Table 7.1.1.3-3: Recovery and distribution of radioactivity in soil LUFA 5M after treatment with ^{14}C-labeled pyraclostrobin and incubation under dark conditions [% TAR]

days after treatment		ACN	ACN/water	total extractable	non-extractable	volatiles*	material balance
0	I	98.6	0.9	99.5	0.0	n.a.	99.5
	II	99.5	1.0	100.5	0.0	n.a.	100.5
mean		99.0	0.9	100.0	0.0	n.a.	100.0
2	I	94.2	2.0	96.2	1.5	0.0	97.7
	II	93.2	1.8	95.0	1.4	0.0	96.5
mean		93.7	1.9	95.6	1.4	0.0	97.1
5	I	97.4	2.7	100.1	3.2	0.0	103.4
	II	95.5	2.8	98.4	3.6	0.0	102.0
mean		96.5	2.8	99.2	3.4	0.0	102.7
8	I	91.4	2.5	93.8	5.9	0.1	99.8
	II	91.9	2.5	94.4	5.2	0.1	99.7
mean		91.6	2.5	94.1	5.6	0.1	99.7
12	I	89.1	2.8	91.9	6.3	0.1	98.3
	II	88.8	3.0	91.7	7.3	0.1	99.1
mean		88.9	2.9	91.8	6.8	0.1	98.7
15	I	87.2	3.0	90.3	9.9	0.1	100.3
	II	88.4	3.2	91.6	9.6	0.1	101.4
mean		87.8	3.1	90.9	9.8	0.1	100.8

TAR = total applied radioactivity
ACN = acetonitrile
n.a. = not analyzed
* no other volatiles than CO_2 were found

B. EXTRACTABLE AND BOUND RESIDUES

The amount of extractable radioactive residues continuously decreased from 100.0% TAR at day 0 to 89.4 (photolysis) and 90.9% TAR (dark control) after 15 days of incubation. The majority of the extractable radioactive residues was always obtained with acetonitrile.

The amount of non-extractable residues increased from 0.0% TAR at day 0 to 7.3% TAR in the irradiated samples after 15 days, compared to 9.8% TAR in the control samples incubated in the dark.

C. VOLATILIZATION

Carbon dioxide was the only volatile degradation product detected during soil photolysis. It was found in amounts of maximum 2.0% TAR after 15 days of irradiation and only in trace amounts (0.1% TAR) after 15 days of dark incubation.

D. TRANSFORMATION OF PARENT COMPOUND

Aliquots of all sample extracts were analyzed by radio HPLC (see Table 7.1.1.3-4 and Table 7.1.1.3-5). After 15 days, the extracted amount of pyraclostrobin decreased to 82.1% TAR in samples of the photolysis experiment and to 76.7% TAR in the dark control samples (mean values of two replicates).

Several degradation products were detected in the extracts, but only the already known soil metabolite BF 500-6 appeared in amounts higher than 5% TAR. It was found up to 3.5% TAR under irradiated conditions and up to 7.9% TAR in the dark control samples. Metabolite BF 500-7 was identified in minor amounts reaching 1.3% TAR in irradiated soil and 3.0% TAR in the dark control soils. All other degradation products never exceeded 2.3% TAR (individual compound). The identity of the two known soil metabolites BF 500-6 and BF 500-7 was confirmed by the second radio HPLC method as well as by HPLC-MS/MS. Metabolite BF 500-3 was not detected in this study by any of the two described HPLC methods.

Table 7.1.1.3-4: Radio-HPLC analysis of soil extracts after treatment of soil LUFA 5M with ^{14}C-labeled pyraclostrobin and incubation under irradiated conditions (sum of ACN and ACN/water extracts), [% TAR]

days after treatment		total	BAS 500 F	BF 500-6 (500M01)	BF 500-7 (500M02)	sum others*
			t_R~69.3'	t_R~123.8'	t_R~131.5'	
0	I	99.5	99.5	n.d.	n.d.	n.d.
	II	100.5	100.5	n.d.	n.d.	n.d.
	mean	100.0	100.0	n.d.	n.d.	n.d.
2	I	97.0	97.0	n.d.	n.d.	n.d.
	II	96.4	96.4	n.d.	n.d.	n.d.
	mean	96.7	96.7	n.d.	n.d.	n.d.
5	I	98.1	95.8	1.6	n.d.	0.7
	II	94.6	93.6	1.0	n.d.	n.d.
	mean	96.4	94.7	1.3	n.d.	0.3
8	I	93.3	88.4	3.4	0.7	0.8
	II	93.7	87.5	2.4	0.7	3.1
	mean	93.5	88.0	2.9	0.7	2.0
12	I	93.9	86.7	2.3	0.9	4.1
	II	94.5	88.0	2.0	1.2	3.3
	mean	94.2	87.3	2.2	1.0	3.7
15	I	89.2	82.7	3.6	1.2	1.7
	II	89.7	81.5	3.5	1.5	3.2
	mean	89.4	82.1	3.5	1.3	2.5

ACN = acetonitrile
TAR = total applied radioactivity
n.d. = not detected
* sum of unknown peaks, each individual peak < 2.3% TAR

Table 7.1.1.3-5: Radio-HPLC analysis of soil extracts after treatment of soil LUFA 5M with ^{14}C-labeled pyraclostrobin and incubation under dark conditions (sum of ACN and ACN/water extracts) [% TAR]

days after treatment		total	BAS 500 F	BF 500-6 (500M01)	BF 500-7 (500M02)	sum others*
			t_R~69.3'	t_R~123.8'	t_R~131.5'	
0	I	99.5	99.5	n.d.	n.d.	n.d.
	II	100.5	100.5	n.d.	n.d.	n.d.
	mean	100.0	100.0	n.d.	n.d.	n.d.
2	I	96.2	96.2	n.d.	n.d.	n.d.
	II	95.0	95.0	n.d.	n.d.	n.d.
	mean	95.6	95.6	n.d.	n.d.	n.d.
5	I	100.1	96.3	2.5	1.4	n.d.
	II	98.4	94.9	2.5	1.0	n.d.
	mean	99.2	95.6	2.5	1.2	n.d.
8	I	93.8	93.8	n.d.	n.d.	n.d.
	II	94.4	89.5	3.7	0.9	0.3
	mean	94.1	91.7	1.8	0.4	0.1
12	I	91.9	81.2	7.3	1.8	1.6
	II	91.7	82.5	5.3	2.0	2.0
	mean	91.8	81.8	6.3	1.9	1.8
15	I	90.3	74.3	8.1	3.3	4.6
	II	91.6	79.1	7.7	2.8	2.0
	mean	90.9	76.7	7.9	3.0	3.3

ACN = acetonitrile
TAR = total applied radioactivity
n.d. = not detected
* sum of unknown peaks, each individual peak < 1.2% TAR

E. CHARACTERIZATION OF NON-EXTRACTABLE RESIDUES (NER)

Results of the non-extractable residues characterization performed by humic substance fractionation are given in Table 7.1.1.3-6.

The alkali-soluble radioactivity amounted to approximately 4% TAR at day 15 in both experiments (representing the sum of fulvic acids and humic acids). Since the alkali-soluble radioactivity did not exceed 5% TAR, no further separation into humic acids and fulvic acids was performed.

Table 7.1.1.3-6: Characterization of non-extractable residues in soil LUFA 5M after treatment with ^{14}C-pyraclostrobin under irradiated conditions [% TAR]

days after treatment	NER initial	NaOH extraction	Water extraction	Sum of NaOH and water extracts	Soil residues after extraction	Sum
irradiated						
8d II	4.71	2.42	0.16	2.58	1.85	4.43
12d I	6.05	3.45	0.17	3.63	2.09	5.72
15d II	7.70	3.84	0.23	4.07	2.71	6.78
dark control						
8d I	5.89	2.30	0.20	2.51	2.69	5.20
12d II	7.26	3.04	0.24	3.28	3.29	6.58
15d I	9.91	4.15	0.34	4.50	4.29	8.79

NER = non-extractable residues
TAR = total applied radioactivity

F. KINETIC MODELING RESULTS

The degradation of pyraclostrobin could be best described by the SFO kinetic fit. The $DegT_{50}$/$DegT_{90}$ values obtained are presented in Table 7.1.1.3-7. As a result, light did not have an effect on the degradation rate of pyraclostrobin in soil.

Table 7.1.1.3-7: Trigger and modeling endpoints for pyraclostrobin

Test system	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Best-fit model	χ^2 error
Photolysis	55.2	183.3	SFO	1.077
Dark control	41.5	137.8	SFO	1.893

III. CONCLUSION

Irradiation in the soil photolysis experiment with pyrazole-^{14}C-labeled pyraclostrobin did not show an influence on the degradation behavior and metabolite formation in soil. Under irradiated conditions, the formation of the known soil metabolites BF 500-6 and BF 500-7 was confirmed, and overall the pyraclostrobin degradation is similar as in the dark. A pyrazole-specific metabolite could not be detected.

Summary: Route of Degradation of pyraclostrobin in Soil

The overall understanding of soil degradation of pyraclostrobin did not change since the evaluation during the previous Annex I inclusion process (according to EU Directive 91/414/EEC).

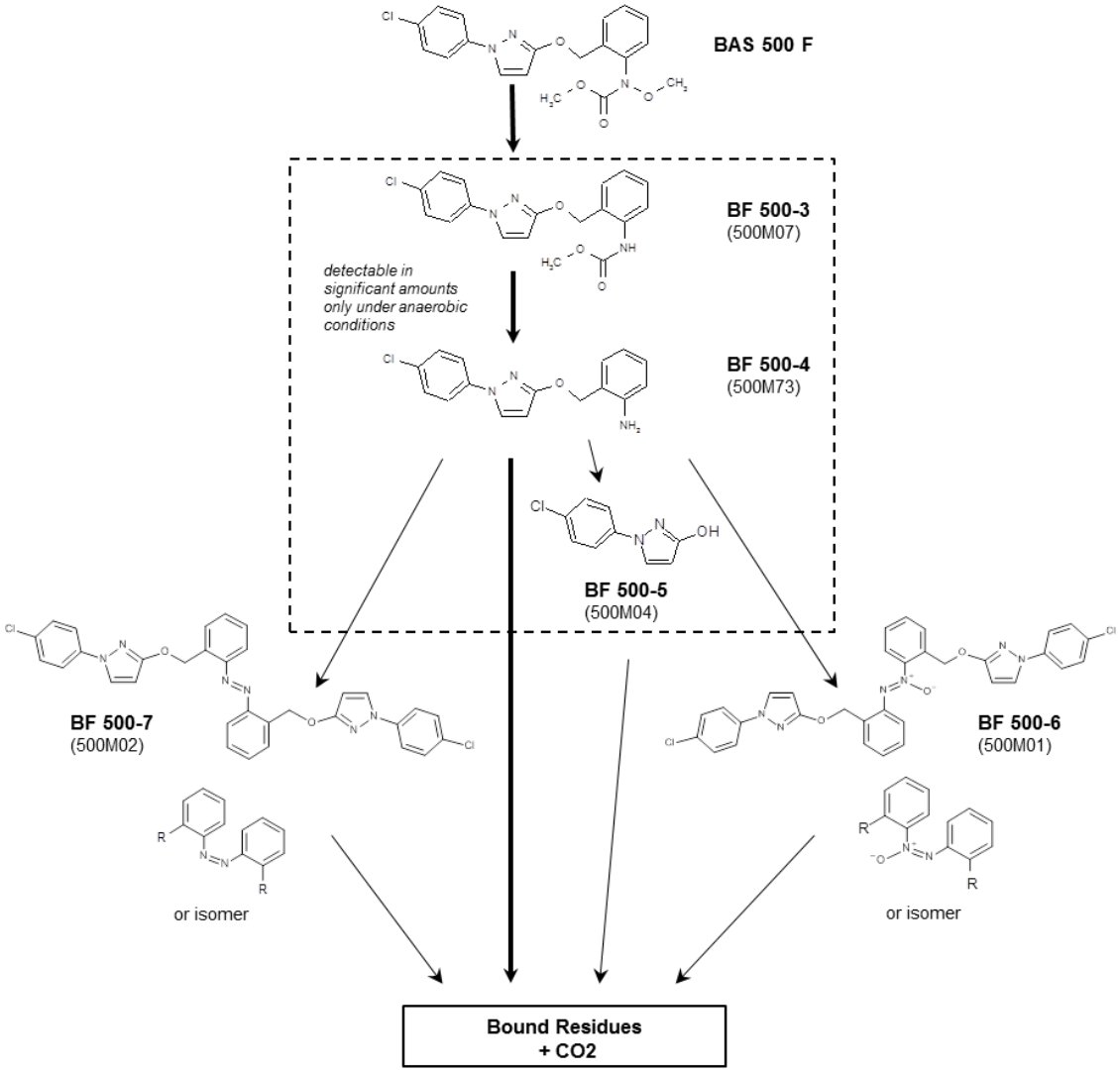
The behavior of pyraclostrobin in soil is characterized by a rather low mineralization rate and the formation of considerable amounts of bound residues.

The first degradation step is the demethoxylation of the carbamate group. The des-methoxy metabolite (BF 500-3) is transformed to the corresponding aniline (BF 500-4). In presence of oxygen, BF 500-4 reacts very fast either with another BF 500-4 molecule (forming metabolites BF 500-6 or BF 500-7) or with the organic matrix in soil leading to high amounts of bound residues. Under natural aerobic conditions, these reactions are very fast so that BF 500-3 or BF 500-4 can be detected, if at all, only in trace amounts. Under anaerobic conditions, the reactions are slowed down and BF 500-3 and BF 500-4 become detectable in soil extracts.

A further (minor) degradation reaction is the cleavage of the ether bond which results in the formation of metabolite BF 500-5. This metabolite further degrades to CO_2 or reacts with humic soil matrix. As with BF 500-3 and BF 500-4, these further degradation reactions are slowed down under anaerobic conditions.

A scheme on the proposed route of degradation is given in Figure 7.1.1.3-1.

Figure 7.1.1.3-1: Proposed route of degradation of pyraclostrobin in soil



CA 7.1.2 Rate of degradation in soil

CA 7.1.2.1 Laboratory studies

CA 7.1.2.1.1 Aerobic degradation of the active substance

In the new soil metabolism study performed with the pyrazole-labeled pyraclostrobin, besides information on the route of degradation also degradation rates were obtained. This study is described in detail in chapter M-CA 7.1.1.1 [*CA 7.1.1.1/1, BASF DocID 2013/1337273*].

Furthermore, a new degradation rate study was performed with pyraclostrobin in various soils, since the old degradation rate study did not provide a full material balance as required by the OECD 307 guideline.

Finally, pyraclostrobin degradation rates were re-calculated from the older, already peer-reviewed studies using their experimental data and analysing the kinetic parameters according to the current FOCUS guidance.

A summary table of all obtained laboratory soil degradation values for pyraclostrobin can be found at the end of this chapter (best-fit and normalized to 20°C, pF 2 and 10°C, pF 2).

Report:	CA 7.1.2.1.1/1 Kuhnke G.,Hassink J., 2014a Metabolism of (pyrazole-3-C14) BAS 500 F in soil under aerobic conditions 2013/1337273
Guidelines:	OECD 307, EPA 835.4100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This study is already described in detail in chapter M-CA 7.1.1.1 1 [*CA 7.1.1.1/1*]. Therefore, only the executive summary and the result of the kinetic evaluation is repeated here.

EXECUTIVE SUMMARY

The aerobic soil metabolism of pyrazole-3-^{14}C-labeled pyraclostrobin was investigated in LUFA 5M soil, a sandy loam (USDA). The nominal application rate was 0.67 mg kg^{-1} dry soil (corresponding to 250 g test item ha^{-1}). Soil aliquots of 100 g (dry weight basis) were weighed into test vessels and incubated at 20ºC and 53% maximum water holding capacity (pF 2.5) in the dark for 120 days. A closed incubation system with continuous aeration was used with an attached trapping system for the determination of volatile compounds.

Soil samples were taken at 0, 1, 3, 7, 10, 14, 31, 58, 91 and 120 days after treatment (DAT).

The soil samples were extracted three times with acetonitrile and three times with acetonitrile/water (1+1). The individual extracts were measured for radioactivity by LSC. The combined acetonitrile as well as the combined acetonitrile/water extracts were analyzed by HPLC. The remaining soil after extraction was combusted to determine the amount of non-extractable soil bound residues. The non-extractable residues were further characterized by NaOH and water extraction and subsequent fractionation into fulvic acids, humic acids, and humins. The fulvic acid fraction was furthermore partitioned with ethyl acetate. A full material balance was provided for each sampling interval.

The amount of extractable radioactivity in soil decreased from 99.8% TAR at time 0 to 39.0% TAR at 120 DAT. The amount of test item pyraclostrobin decreased throughout the incubation from 92.0% TAR at day 0 after treatment to 11.2% TAR at the end of the study. The two metabolites BF 500-6 (500M01) and BF 500-7 (500M02) including their isomers were identified by mass spectrometry and by comparison of retention times to reference items in two chromatographic systems. Metabolite BF 500-6 increased to about 16.2% TAR at 91 DAT and still accounted for 11.9% at the end of the study (mean of two replicates). Metabolite BF 500-7 was detected with max. 4.7% TAR at 31 DAT and decreased to 3.6% TAR (mean) at the end of the study. The corresponding isomers of BF 500-6 and BF 500-7 were found only in low amounts of maximum 1.1% TAR.

Non-extractable radioactive residues (NER) were formed in considerable amounts during incubation. They increased from 0.2% TAR on day 0 to a maximum of 52.7% TAR after 120 days. Characterization of the NER at 120 DAT revealed that about half of it was tightly bound to humins, while the rest was distributed in a ratio of about 1:1 between the humic and the fulvic acid fraction. The fulvic acid fraction was further characterized by partitioning with ethyl acetate. Amounts of 9.7% TAR of the fulvic acid fraction were soluble in ethyl acetate, whereas 3.0% TAR remained in the water phase. The ethyl acetate soluble fractions from samples of 120 DAT were investigated by HPLC. The main peak found by HPLC (accounting for 8.4% TAR) is metabolite BF 500-5 known to occur under alkaline conditions.

$^{14}CO_2$ was formed in low amounts up to 2.2% TAR. No other volatile compounds were detected in significant amounts. The material balance ranged from 93.8 to 110.3% TAR throughout the incubation period of 120 days, resulting in a mean value of 100.6% TAR.

Kinetic analysis and calculation of $DegT_{50}$ and $DegT_{90}$ values for pyraclostrobin and its metabolites BF 500-6 and BF 500-7 was performed following the recommendations of the FOCUS Kinetics workgroup. The analysis was done by non-linear regression methods using the software package KinGUI version 2.2012.320.1629.

$DegT_{50}$/$DegT_{90}$ values obtained are presented in Table 7.1.2.1.1-1 and Table 7.1.2.1.1-2.

Table 7.1.2.1.1-1: Trigger endpoints for pyraclostrobin, BF 500-6 and BF 500-7

Compound	Best-fit model	χ^2 error	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Pyraclostrobin	FOMC	2.20	22.3	115.2
BF 500-6	SFO	7.36	95.9	318.7
BF 500-7	SFO	10.55	81.5	270.7

Table 7.1.2.1.1-2: Modeling endpoints for pyraclostrobin, BF 500-6 and BF 500-7

Compound	Kinetic model	χ^2 error	$DegT_{50}$ [d]	k [d^{-1}]	Formation fraction [-]
Pyraclostrobin	SFO	5.05	26.2	0.0264	-
BF 500-6	SFO	8.42	68.0	0.0102	0.318
BF 500-7	SFO	10.60	59.0	0.0117	0.112

Report: CA 7.1.2.1.1/2
Staudenmaier H.,Kuhnke G., 2013a
Rate of degradation of ^{14}C-Pyraclostrobin (BAS 500 F) in aerobic soil
2011/1102370

Guidelines: OECD 307 (2002), EPA 835.4100, BBA IV 4-1, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995)

GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The objective of the study was to investigate the aerobic soil metabolism of pyraclostrobin in three soils originating from Germany.

The soils were treated with a nominal rate of 0.667 mg tolyl-^{14}C-labeled pyraclostrobin per kg of dry soil which corresponds to a field application rate of 250 g a.s. ha^{-1}. The incubation was carried out in the dark in the laboratory under aerobic conditions at a soil moisture of 40% of the maximum water holding capacity (MWHC) and at a temperature of 20°C. A closed incubation system with continuous aeration (moistened synthetic air) was used with an attached trapping system for the determination of volatile compounds. Samples were taken at 0, 3, 7, 14, 29/30, 59/62, 90 and 120 days after treatment (DAT).

The soil samples were extracted three times with acetonitrile and three times with acetonitrile/water (1:1). The individual extracts were analyzed by LSC. The combined acetonitrile as well as the combined acetonitrile/water extracts were analyzed by HPLC. The amount of non-extractable residues was determined by combustion of the remaining soil after extraction and subsequent LSC measurement.

The mass balance throughout the study ranged from 90.1 to 105.0% of the total applied radioactivity (TAR) for the three soils. The extractable radioactivity decreased from 95.5 - 98.9% TAR at day 0 to 39.0 - 50.9% after 120 days. The non-extractable radioactive residues (NER) increased from 0.6 - 1.0% TAR on day 0 to a maximum of 36.6 – 48.4% TAR after 120 days of incubation. Mineralization to $^{14}CO_2$ reached a total of 6.1 - 7.0% TAR. No other volatile compounds were detected.

The parent compound pyraclostrobin decreased continuously from 92.5 – 94.4% TAR at 0 DAT to 7.2 – 22.0% TAR after 120 days of incubation. Several metabolites were formed in moderate amounts: BF 500-6 (cis and trans isomers), BF 500-7 (cis and trans isomers), BF 500-3, 500M99, 500M97, 500M96 and 500M98. The most prominent metabolite, the trans isomer of BF 500-6, was formed in amounts up to 19.8% TAR. Only one other metabolite, the trans isomer of BF 500-7, reached amounts ≥ 5% TAR in one soil (max. 7.8% TAR).

The kinetic evaluation was re-analyzed in a separate report [*CA 7.1.2.1.1/2, BASF DocID 2014/1093424*], using a newer version of the kinetic software (KinGUI version 2 as compared to v1.1).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS code:	BAS 500 F
Common name:	Pyraclostrobin
Reg.No.:	304428
Chemical name (IUPAC):	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)-(N-methoxy) carbamate
Molecular weight:	387.8 g mol^{-1} (unlabeled)
Position of radiolabel:	Tolyl-ring-U-C^{14}
Specific radioactivity:	7.31 MBq mg^{-1}
Batch No.:	566-4040
Radiochemical purity:	98.7%

2. Soils

Three different soils were used for treatment and incubation. The soils were sieved through a 2 mm sieve before use, remoistened to approximately 8 – 12% soil moisture and stored at about 4°C in the dark. The soil characteristics are summarized in Table 7.1.2.1.1-3.

Table 7.1.2.1.1-3: Properties of soils used for investigation of pyraclostrobin degradation rates under aerobic conditions

Soil designation	Li 10 (10/1680/03)	LUFA 5M (10/1651/03)	Speyerer Wald 2 (10/1715/03)
Origin	Limburgerhof, RP, Germany	Mechtersheim, RP, Germany	Schifferstadt, RP, Germany
DIN Particle size distribution [%]			
Sand 0.063 – 2 mm	82.2	51.5	79.4
Silt 0.002 – 0.063 mm	13.5	36.5	16.5
Clay < 0.002 mm	4.3	12.0	4.1
Textural class	Silty sand	Loamy sand	Silty sand
USDA Particle size distribution [%]			
Sand 0.050 – 2 mm	84.1	56.8	82.6
Silt 0.002 – 0.050 mm	11.5	31.1	13.3
Clay < 0.002 mm	4.3	12.0	4.1
Textural class	Loamy sand	Sandy loam	Loamy sand
Total organic carbon [%]	0.97	1.19	0.83
pH (H_2O)	6.5	8.1	6.6
pH ($CaCl_2$)	5.5	7.1	5.5
Cation exchange capacity [$cmol^+ kg^{-1}$]	5.4	11.3	4.3
Maximum water holding capacity [g/100g dry soil]	25.9	26.7	22.9
Microbial biomass before incub. [mg C/100g dry soil]	22.7	35.3	16.4
Microbial biomass at 58/61 DAT [mg C/100g dry soil]	13.9	26.9	5.4
Microbial biomass at 121/124 DAT [mg C/100g dry soil]	11.3	23.4	3.5
Water retention characteristics (pF 2) [g soil moisture / g dry soil]	0.110 (11%)	0.202 (20.2%)	0.095 (9.5%)

DAT = days after treatment

B. STUDY DESIGN

1. Experimental conditions

The soils were adjusted to about 40% of the maximum water holding capacity and treated at a nominal concentration of 0.667 mg ^{14}C-pyraclostrobin per kg dry soil, which corresponds to a field application rate of 250 g a.s. ha^{-1} (assuming an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}).

For the soil treatment, 2.3 kg of each soil was treated with 1.075 mL of the treatment solution. The treated soils were homogenized and soil portions of 100 g dry soil equivalents were filled into suitable test vessels. The number of vessels was sufficient to allow duplicate sampling at each sampling time and to keep a limited number of reserve vessels.

The vessels were incubated in the dark for 120 days at a temperature of 20°C. Throughout the incubation period, the test vessels were continuously aerated with a slight stream of moistened synthetic air. The outgoing air was led through a series of gas-washing bottles containing trapping solutions for potential volatiles (ethylene glycol, 0.5M H_2SO_4, 0.5M NaOH). The water content of the soils was monitored throughout the incubation period by weighing the sampled vessels.

2. Sampling

Sampling times were 0, 3, 7, 14, 29 (Speyerer Wald 2 soil) / 30 (Li10 and LUFA 5M soil), 59 (Li10 and LUFA 5M soil) / 62 (Speyerer Wald 2 soil), 90 and 120 days after treatment (DAT).

At 0 DAT as well as at 59/62 DAT and 120 DAT two replicate samples were worked up. At the other sampling times only one replicate was worked up, the second one was stored in a freezer.

Suitable amounts of the trapping solutions from the gas washing bottles were subjected to LSC measurement. Measurements were performed in triplicate.

3. Description of the analytical procedures

The soil was extracted three times with 100 mL acetonitrile and three times with 100 mL acetonitrile/water (1:1, v:v) by shaking for about 30 minutes. After each extraction, the suspension was centrifuged and aliquots of each solution were radioassayed.

The three acetonitrile and the three acetonitrile/water extracts, respectively, were combined and the two combined extracts were concentrated to dryness, re-dissolved in the extraction solvent and analyzed by LSC. Aliquots of the combined extracts were analyzed by HPLC to obtain the metabolite pattern.

The extracted soil was dried in a drying oven at 60°C and homogenized. Aliquots were combusted in order to determine the amount of non-extractable radioactive residues (NER). The evolved $^{14}CO_2$ from each combusted aliquot was trapped in an Oxysolve C-400 scintillator and measured by LSC.

4. Calculation of the degradation rate

Kinetic analysis and calculations of best-fit DT_{50} and DT_{90} values was performed in the original study following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006)*], using KinGUI version 1.1. An updated kinetic evaluation using a newer version of the kinetic software (KinGUI version 2) was performed separately [*CA 7.1.2.1.1/2, BASF DocID 2014/1093424*].

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The detailed results on the extractability of radioactive residues in the soils are presented in Table 7.1.2.1.1-4 to Table 7.1.2.1.1-6. The material balance ranged from 90.1 to 105.0% TAR for all soils.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (ERR), non-extractable residues (NER), CO_2 and other volatiles for each soil is shown in Table 7.1.2.1.1-4 to Table 7.1.2.1.1-6. The amount of extractable radioactivity (ERR) decreased from 95.5 - 98.9% TAR (mean of two replicates) at 0 DAT to 39.0 - 50.9% TAR after 120 days of incubation.

The non-extractable radioactive residues increased during the study from 0.6 - 1.0% TAR at 0 DAT to a maximum of 36.6 - 48.4% TAR at the end of the study after 120 days of incubation.

Table 7.1.2.1.1-4: Distribution of radioactivity and mass balance in Li 10 soil after treatment with ^{14}C-pyraclostrobin [%TAR]

days after treatment	extractable residues			NER	Volatiles		Mass balance
	ACN	ACN + water	Total		NaOH ($^{14}CO_2$)	Other volatiles	
0 (rep 1)	94.8	1.7	96.5	1.0	n.d.	n.d.	97.5
0 (rep 2)	93.0	1.7	94.6	0.6	n.d.	n.d.	95.2
0 (mean)	93.9	1.7	95.5	0.8	n.d.	n.d.	96.4
3	86.4	2.7	89.1	7.3	0.2	0.0	96.5
7	76.9	2.7	79.6	14.7	0.7	0.0	95.0
14	66.7	3.2	69.9	21.8	1.1	0.0	92.8
30	55.3	3.7	59.0	31.8	2.5	0.0	93.2
59 (rep1)	45.5	3.6	49.1	38.6	4.4	0.0	92.1
59 (rep2)	45.5	3.6	49.1	38.7	4.4	0.0	92.2
59 (mean)	45.5	3.6	49.1	38.7	4.4	0.0	92.1
90	39.7	3.6	43.4	44.3	5.8	0.0	93.6
120 (rep1)	35.5	3.5	39.0	48.7	7.0	0.0	94.8
120 (rep2)	35.4	3.5	38.9	44.1	7.0	0.0	90.1
120 (mean)	35.5	3.5	39.0	46.4	7.0	0.0	92.4

TAR = total applied radioactivity
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determined

Table 7.1.2.1.1-5: Distribution of radioactivity and mass balance in LUFA 5M soil after treatment with ^{14}C-pyraclostrobin [%TAR]

days after treatment	extractable residues			NER	Volatiles		Mass balance
	ACN	ACN + water	Total		NaOH ($^{14}CO_2$)	Other volatiles	
0 (rep 1)	95.5	2.8	98.3	1.0	n.d.	n.d.	99.3
0 (rep 2)	96.7	2.9	99.6	1.0	n.d.	n.d.	100.6
0 (mean)	96.1	2.8	98.9	1.0	n.d.	n.d.	99.9
3	84.0	3.4	87.3	11.6	0.5	0.0	99.4
7	76.9	3.9	80.7	20.3	0.8	0.0	101.9
14	62.6	4.2	66.8	29.5	1.4	0.0	97.8
30	49.8	4.7	54.5	40.7	2.5	0.0.	97.7
59 (rep1)	40.7	4.9	45.6	47.4	4.0	0.0	97.0
59 (rep2)	43.9	4.9	48.9	47.6	4.0	0.0	100.5
59 (mean)	42.3	4.9	47.2	47.5	4.0	0.0	98.7
90	38.6	4.9	43.5	56.3	5.2	0.0	105.0
120 (rep1)	35.3	4.7	40.0	48.8	6.1	0.0	94.8
120 (rep2)	37.9	4.8	42.7	48.0	6.1	0.0	96.7
120 (mean)	36.6	4.7	41.3	48.4	6.1	0.0	95.8

TAR = total applied radioactivity
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determined

Table 7.1.2.1.1-6: Distribution of radioactivity and mass balance in Speyerer Wald 2 soil after treatment with ^{14}C-pyraclostrobin [%TAR]

days after treatment	extractable residues			NER	Volatiles		Mass balance
	ACN	ACN + water	Total		NaOH ($^{14}CO_2$)	Other volatiles	
0 (rep 1)	94.6	2.0	96.6	0.6	n.d.	n.d.	97.2
0 (rep 2)	94.9	2.0	96.8	0.6	n.d.	n.d.	97.4
0 (mean)	94.7	2.0	96.7	0.6	n.d.	n.d.	97.3
3	88.9	2.4	91.4	4.5	0.2	0.0	96.1
7	84.1	2.7	86.8	8.3	0.5	0.0	95.6
14	77.0	3.1	80.1	13.4	1.1	0.0	94.5
29	68.4	3.5	71.8	21.0	2.1	0.0	94.9
62 (rep1)	56.1	3.6	59.8	30.7	3.8	0.0	94.3
62 (rep2)	56.3	3.7	60.0	30.2	3.8	0.0	94.0
62 (mean)	56.2	3.7	59.9	30.5	3.8	0.0	94.2
90	50.9	3.6	54.6	33.2	5.0	0.0	92.7
120 (rep1)	47.1	3.6	50.7	33.3	6.1	0.0	90.1
120 (rep2)	47.6	3.6	51.2	39.9	6.1	0.0	97.2
120 (mean)	47.4	3.6	50.9	36.6	6.1	0.0	93.7

TAR = total applied radioactivity
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determined

C. VOLATILIZATION

Mineralization to CO_2 reached a total of 6.1 - 7.0% TAR after 120 days. No other volatile compounds were detected.

D. TRANSFORMATION OF PYRACLOSTROBIN

All combined soil extracts were analyzed by radio-HPLC. The results obtained with this system are summarized in Table 7.1.2.1.1-7 to Table 7.1.2.1.1-9.

Since the separation of the parent compound and all metabolites was not successful with one single HPLC system, all extracts were analyzed on two different systems: HPLC method 188-1 (Synergi Polar-RP) and HPLC method 188-2 (Synergi Hydro-RP) with a special long gradient. For a complete picture of the metabolism of the test item, the results of the two HPLC systems were combined: Amounts of pyraclostrobin and BF 500-3 were taken from the analyses with HPLC method 188.1 (Synergi Polar-RP) whereas the amounts of all other metabolites were taken from the analyses with HPLC method 188-2 (Synergi Hydro-RP).

The identity of the peaks of pyraclostrobin, BF 500-6, BF 500-7 and BF 500-3 was determined by co-chromatography with reference compounds and was confirmed by mass spectrometric analysis. The identity of further metabolites was determined by mass spectrometric analysis. The correct assignment of cis and trans metabolites BF 500-6 and BF 500-7 was confirmed by NMR spectroscopic analysis.

The parent compound pyraclostrobin decreased continuously from 92.5 - 94.4% TAR at 0 DAT to 7.2 - 22.0% TAR after 120 days of incubation.

Several metabolites were formed in moderate amounts: BF 500-6 (trans and cis isomers: max. 3.5 and 19.3% TAR, respectively), BF 500-7 (trans and cis isomers: 1.5 and 7.8% TAR, respectively) and BF 500-3 (max. 4.3% TAR). The metabolites 500M99, 500M97, 500M96 and 500M98 were found in amounts below 4% TAR.

Table 7.1.2.1.1-7: Summary results of radio-HPLC analysis of soil Li 10 after treatment of soils with ^{14}C-pyraclostrobin [%TAR]

days after treatment	total extract.	BF 500-3 t_R ~ 33.4	Pyraclostrobin 34.1	500M99 70.3	BF 500-6 cis 118.2	BF 500-6 trans 123.9	BF 500-7 trans 131.6	Others*
0 (rep 1)	96.5	2.6	93.7	n.d.	n.d.	n.d.	n.d.	0.1
0 (rep 2)	94.6	3.3	91.2	n.d.	n.d.	n.d.	n.d.	0.1
0 (mean)	95.5	2.9	92.5	n.d.	n.d.	n.d.	n.d.	0.1
3	89.1	4.3	83.6	n.d.	n.d.	1.4	n.d.	0.2
7	79.6	3.5	70.1	n.d.	n.d.	3.3	n.d.	0.1
14	69.9	3.3	56.3	1.3	0.8	5.9	0.9	0.2
30	59.0	3.4	39.5	2.2	1.3	8.6	0.7	1.6
59 (rep 1)	49.1	2.7	28.3	2.5	1.4	10.8	1.3	0.8
59 (rep 2)	49.1	3.3	28.3	2.2	1.1	11.2	1.4	2.1
59 (mean)	49.1	3.0	28.3	2.3	1.3	11.0	1.4	1.4
90	43.4	2.6	22.2	2.2	1.1	11.3	0.8	1.6
120 (rep 1)	39.0	2.6	17.4	1.9	2.0	12.1	1.1	0.9
120 (rep 2)	38.9	2.3	17.5	1.7	1.4	12.6	1.0	1.1
120 (mean)	39.0	2.4	17.4	1.8	1.7	12.3	1.0	1.0

TAR = total applied radioactivity
t_R = approx. retention time [min]
* sum of several peaks

Table 7.1.2.1.1-8: Summary results of radio-HPLC analysis of soil LUFA 5M after treatment of soils with ^{14}C-pyraclostrobin [%TAR]

days after treatment	total extract.	BF 500-3 t_R ~ 33.4	Pyraclo-strobin 34.1	500M99 70.3	BF 500-6 cis 118.2	BF 500-7 cis 123.2	BF 500-6 trans 123.9	500M97 130.1	BF 500-7 trans 131.6	Others*
0 (rep 1)	98.3	4.7	93.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.2
0 (rep 2)	99.6	3.5	95.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5
0 (mean)	98.9	4.1	94.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.3
3	87.3	3.2	76.0	n.d.	n.d.	n.d.	4.3	n.d.	1.8	0.2
7	80.7	2.9	61.6	n.d.	1.9	1.0	8.1	n.d.	2.5	0.3
14	66.8	2.7	40.5	n.d.	2.1	1.5	12.4	n.d.	4.8	0.5
30	54.5	2.1	24.0	n.d.	2.5	1.1	16.3	1.0	6.2	1.3
59 (rep 1)	45.6	1.0	12.1	0.3	3.1	0.8	17.8	0.7	7.8	1.1
59 (rep 2)	48.9	1.2	13.9	n.d.	3.2	0.9	19.0	1.1	7.7	1.6
59 (mean)	47.2	1.1	13.0	0.2	3.1	0.8	18.4	0.9	7.8	1.3
90	43.5	1.0	9.0	n.d.	3.5	1.2	19.8	n.d.	7.5	1.5
120 (rep 1)	40.0	1.1	6.7	n.d.	2.6	0.9	19.1	1.0	6.6	1.6
120 (rep 2)	42.7	1.0	7.7	n.d.	3.1	1.3	19.6	1.1	7.2	1.6
120 (mean)	41.3	1.0	7.2	n.d.	2.8	1.1	19.3	1.0	6.9	1.6

TAR = total applied radioactivity
t_R = retention time [min]
* sum of several peaks

Table 7.1.2.1.1-9: Summary results of radio-HPLC analysis of soil Speyerer Wald 2 after treatment with ^{14}C-pyraclostrobin [%TAR]

days after treatment	total extract.	BF 500-3 t_R ~ 33.4	Pyraclo-strobin 34.1	500M99 70.3	BF 500-6 cis 118.2	BF 500-6 trans 123.9	500M97 130.1	BF 500-7 trans 131.6	Others*
0 (rep 1)	96.6	3.4	93.0	n.d.	n.d.	n.d.	n.d.	n.d.	0.2
0 (rep 2)	96.8	3.8	92.9	n.d.	n.d.	n.d.	n.d.	n.d.	0.2
0 (mean)	96.7	3.6	92.9	n.d.	n.d.	n.d.	n.d.	n.d.	0.2
3	91.4	4.1	86.5	n.d.	n.d.	1.7	n.d.	n.d.	0.3
7	86.8	3.5	79.8	n.d.	n.d.	2.7	n.d.	n.d.	0.1
14	80.1	3.6	69.7	n.d.	n.d.	5.5	n.d.	n.d.	0.2
29	71.8	3.3	53.5	1.3	1.5	9.1	n.d.	1.6	0.4
62 (rep 1)	59.8	3.3	36.4	3.6	2.2	12.4	n.d.	n.d.	0.6
62 (rep 2)	60.0	3.2	35.6	3.8	1.8	12.4	n.d.	n.d.	0.5
62 (mean)	59.9	3.3	36.0	3.7	2.0	12.4	n.d.	n.d.	0.5
90	54.6	3.2	27.1	1.7	2.6	16.0	n.d.	1.6	1.9
120 (rep 1)	50.7	3.3	21.5	1.1	2.6	17.2	0.9	1.6	1.2
120 (rep2)	51.2	3.5	22.6	1.4	1.7	17.9	1.3	1.5	1.5
120 (mean)	50.9	3.4	22.0	1.3	2.1	17.5	1.1	1.5	1.4

TAR = total applied radioactivity
t_R = retention time [min]
* sum of several peaks

The kinetic evaluation was performed in a separate report [*CA 7.1.2.1.1/3, BASF DocID 2014/1093424*], using a newer version of the kinetic software (KinGUI version 2).

III. CONCLUSION

Pyraclostrobin was degraded rather fast in aerobic soil. The fraction of non-extractable residues increased reaching between 36.6 and 48.4% TAR at the end of the study after 120 days. $^{14}CO_2$ was formed in low amounts up to 7% TAR. No other volatile compounds were detected.

Several metabolites were formed in moderate amounts. The most prominent metabolite, the trans isomer of BF 500-6, was formed in amounts up to 19.3% TAR. Only one other metabolite, the trans isomer of BF 500-7, reached amounts ≥ 5% TAR in one soil.

Report: CA 7.1.2.1.1/3
Eickler B., 2014a
Kinetic evaluation of aerobic soil degradation of BAS 500 F - Pyraclostrobin: Determination of trigger and modeling endpoints according to Focus Degradation Kinetics
2014/1093424
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP: no

Note: This study, which was erroneously not listed in the application submitted for renewal of approval, is replacing BASF DocID 2010/1197290, which was listed in the application. The reason for submission is provided below.

Executive Summary

The degradation of the fungicide pyraclostrobin (BAS 500 F) in soil has been investigated in four aerobic laboratory soil degradation studies in nine experiments. The purpose of this evaluation was to analyze the degradation kinetics of pyraclostrobin and its metabolites BF 500-3, BF 500-6 and BF 500-7 observed in the studies according to current guidance of the FOCUS workgroup on degradation kinetics.

The appropriate kinetic models to derive trigger and modeling endpoints were identified considering the procedures and kinetic models proposed by the FOCUS kinetics guidance.

The best-fit model to derive trigger endpoints was selected based on a visual and statistical assessment. Modeling endpoints were normalized to reference conditions.

For pyraclostrobin, the kinetic evaluation showed that the SFO model is appropriate to derive trigger endpoints in two soils, while biphasic kinetic models (DFOP or FOMC) were selected as best-fit models in seven soils. The best-fit $DegT_{50}$ values were between 11.2 and 97.9 days, and $DegT_{90}$ values were between 87.7 and >1000 days. Modeling $DegT_{50}$ values range between 24.4 and 97.9 days, whereas normalized modeling endpoints (20°C, pF2) range between 15.6 and 95.4 days.

For metabolites BF 500-6 and BF 500-7 trigger endpoints as well as (normalized) modeling-$DegT_{50}$ values and corresponding formation fractions could be derived for each metabolite in at least two soils. For the transient metabolite BF 500-3, reliable trigger and modeling endpoints could not be derived from any of the soils.

I. MATERIAL AND METHODS

The degradation of pyraclostrobin and its soil metabolites BF 500-3, BF 500-6 and BF 500-7 in eight different soils in nine trials [*old EU Dossier, A II M 7.1.1.2.1/1, Ebert D. - BASF DocID 1998/11201; old EU dossier, A II M 7.1.1.2.1/2, Ebert D. - BASF DocID 1999/10090; old EU dossier, A II M 7.1.1.2.1/3, Ebert D. - BASF DocID 1999/1109; CA 7.1.2.1.1/1, Staudenmaier H., Kuhnke G. - BASF DocID 2011/1102370*] was analyzed taking into account the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*].

Kinetic modeling strategy

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) as well as modeling endpoints. The best-fit model was selected based on visual and statistical assessment and the corresponding $DegT_{50}$ and $DegT_{90}$ values are reported as trigger endpoints. Appropriate $DegT_{50}$ values for use in environmental fate models were derived depending on the kinetic model.

For the metabolites of pyraclostrobin, the procedure for deriving modeling and trigger endpoints for metabolites recommended by the FOCUS Kinetics workgroup [*FOCUS (2006)*] was followed. The metabolites were added to the appropriate kinetic model for the parent. In the evaluation of data from the studies [*Ebert D. - BASF DocID 1998/11201; Ebert D. - BASF DocID 1999/10090; Ebert D. - BASF DocID 1999/11091*], the degradation products BF 500-6 and BF 500-7 were considered. Simultaneous formation from the parent was assumed and considered as pathway in the kinetic evaluation. In the evaluation of data from the study [*Staudenmaier H., Kuhnke G. - BASF DocID 2011/1102370*], based on the known degradation pathway of pyraclostrobin, metabolite BF 500-3 was included as transient metabolite. Simultaneous formation of the degradation products BF 500-6 and BF 500-7 from the intermediate BF 500-3 was assumed and considered as pathway for the kinetic evaluation.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the best-fit model, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC) and the bi-exponential (DFOP) kinetics. The respective model descriptions and corresponding equations for calculating endpoints ($DegT_{50}$, $DegT_{90}$) are shown in the FOCUS Kinetics guidance [*FOCUS (2006), Box 5-1, Box 5.2 and Box 5-4*].

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling and software for kinetic evaluation

The initial value at day zero for the parent was set to material balance minus the measured amount of BF 500-3 at DAT 0, as this metabolite was a known impurity in the application solution. The metabolites BF 500-6 and BF 500-7 were considered in the evaluation and initial concentrations were set to zero. In *Staudenmaier H., Kuhnke G.* [*BASF DocID 2011/1102370*], the transient metabolite BF 500-3 was included in the evaluation. Measured values of BF 500-3 were used as initial concentrations. In case metabolite values were not reported and no LOQ or LOD were provided, metabolite values were conservatively set to 0.1% TAR.

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to $1x10^{-6}$ and 100, respectively. Note, that in this software package only SFO kinetic evaluation for the metabolites is considered for determining trigger and modeling endpoints.

Normalization to reference conditions

According to FOCUS *(2006)* the $DegT_{50}$ values obtained from laboratory studies should be normalized for modeling purposes to reference conditions at a temperature of 20°C and soil moisture at pF2 to account for the different soil temperature and moisture conditions during incubation. The laboratory trials were conducted at 20°C, therefore temperature correction was not necessary. The soil moisture was 40% MWHC, and normalization was conducted according to FOCUS *(2006)*. Reference moisture contents for the various soil types were derived from *FOCUS (2011)* [*Generic guidance for Tier 1 FOCUS ground water assessments, version 2.0. FOCUS groundwater scenarios working group*]. For *Staudenmaier H., Kuhnke G.* [*BASF DocID 2011/1102370*] reference water retention characteristics were provided in the study report and included in the normalization.

Experimental data

The kinetic evaluation was conducted for nine trials from four aerobic laboratory soil degradation studies [*Ebert D. - BASF DocID 1998/11201; Ebert D. - BASF DocID 1999/10090; Ebert D. - BASF DocID 1999/11091; Staudenmaier H., Kuhnke G. - BASF DocID 2011/1102370*]. The test substance was applied at a nominal rate of 0.33 and 0.667 mg kg^{-1} soil in all 3 studies of *Ebert D.* and in the study of *Staudenmaier H., Kuhnke G.*, respectively. The soil characteristics of these four studies are summarized in Table 7.1.2.1.1-10 to Table 7.1.2.1.1-13.

Table 7.1.2.1.1-10: Soil characteristics of study BASF DocID 1998/11201

Parameter	Bruch West
Soil type (USDA)	Sandy loam
pH ($CaCl_2$)	7.3
Organic carbon [%]	1.8
Particle size distribution [%]	
Clay (<2µm)	10
Silt (2 - 50 µm)	26
Sand (> 50 µm)	64
Max. water holding capacity [g/100g soil]	43.0

Table 7.1.2.1.1-11: Soil characteristics of study BASF DocID 1999/10090

Parameter	Bruch West
Soil type (USDA)	Sandy loam
pH ($CaCl_2$)	7.5
Organic carbon [%]	2.0
Particle size distribution [%]	
Clay (<2µm)	13
Silt (2 - 50 µm)	18
Coarse silt (> 50 µm)	69
Max. water holding capacity [g/100g soil]	44

Table 7.1.2.1.1-12: Soil characteristics of study BASF DocID 1999/11091

Parameter	US 771-15	Li 35 b	LUFA 2.2	Canadian
Soil type (USDA)	Loamy sand	Loamy sand	Loamy sand	Loam
pH ($CaCl_2$)	5.6	6.5	5.4	7.7
Organic carbon [%]	0.5	1.0	2.1	3
Particle size distribution [%]				
Clay (<2µm)	12	8	5	15
Silt (2 - 50 µm)	5	8	9	36
Coarse silt (> 50 µm)	83	84	86	49
Max. water holding capacity [g/100 g soil]	35	30	36	43

Table 7.1.2.1.1-13: Soil characteristics of study BASF DocID 2011/1102370

Parameter	Li 10	LUFA 5M	Speyerer Wald 2
Soil type (USDA)	Loamy sand	Sandy loam	Loamy sand
pH ($CaCl_2$)	5.5	7.1	5.5
Organic carbon [%]	0.97	1.19	0.83
Particle size distribution [%]			
Clay (<2µm)	4.3	12.0	4.1
Silt (2 - 50 µm)	11.5	31.1	13.3
Sand (50 µm – 2 mm)	84.1	56.8	82.6
Max. water holding capacity [g/100 g soil]	25.9	26.7	22.9
Water holding capacity at pF 2.0 [g/100 g soil]	11	20.2	9.5

An overview of the studies is given in Table 7.1.2.1.1-14.

Table 7.1.2.1.1-14: Overview on aerobic soil degradation studies with pyraclostrobin

Soil	Soil type	Incubation			Analyte	Sampling days [DAT]	Study (BASF DocID)
		Moisture	Temp. [°C]	Time [d]			
Bruch West	Sandy loam	40% MWHC	20	360	[tolyl-U-^{14}C]-pyraclostrobin	0, 1, 3, 7, 14, 33, 60, 87, 180, 270, 360	1998/11201
Bruch West	Sandy loam			360	[phenyl-U-^{14}C]-pyraclostrobin	0, 1, 3, 7, 14, 31, 59, 91, 180, 270, 360	1999/10090
US 771-15	Loamy sand			120	[tolyl-U-^{14}C]-pyraclostrobin	0, 3, 7, 14, 30, 60, 90, 120	1999/11091
Li 35 b	Loamy sand						
LUFA 2.2	Loamy sand						
Canadian soil	Loam						
Li 10	Loamy sand			120	[tolyl-U-^{14}C]-pyraclostrobin	0, 3, 7, 14, 29/30, 59/62, 90, 120	2011/1102370
LUFA 5M	Sandy loam						
Speyerer Wald 2	Loamy sand						

The measured data as well as resulting datasets submitted to kinetic analysis are given in Table 7.1.2.1.1-15 to Table 7.1.2.1.1-18.

Table 7.1.2.1.1-15: Experimental data from study BASF DocID 1998/11201 used for kinetic evaluation

DAT	Extractable radioactive residues of pyraclostrobin and its metabolites [% TAR]		
	Bruch West		
	Pyraclostrobin	BF 500-6 (sum of cis- and trans-isomer)	BF 500-7 (sum of cis- and trans-isomer)
0	96.2*	0.0**	0.0**
1	87.8	0.1$	0.1$
3	76.5	1.9	1.0
7	61.1	5.1	2.9
14	48.9	9.3	3.8
33	24.1	9.7	5.8
60	16.7	10.0	5.1
87	10.6	10.6	5.1
180	7.7	11.6	5.1
270	5.5	10.3	5.5
360	4.5	10.9	4.8

* Data at DAT0 set to material balance (102.6% TAR) minus amount of BF 500-3 (6.4% TAR) according to FOCUS (2006), because this metabolite was an impurity in the application solution

** Set to zero according to FOCUS (2006)

$ Set to 0.1 as the lowest value for reported decimal places, as no LOD or LOQ are provided

Table 7.1.2.1.1-16: Experimental data from study BASF DocID 1999/10090 used for kinetic evaluation

DAT	Extractable radioactive residues of pyraclostrobin and its metabolites [% TAR]		
	Bruch West		
	Pyraclostrobin	BF 500-6 (sum of cis- and trans-isomer)	BF 500-7 (sum of cis- and trans-isomer)
0	100.9*	0.0**	0.0**
1	91	1.9	0.6
3	80.7	3.1	0.1$
7	69.8	6.9	2.8
14	49.2	8.1	2.2
31	28.3	11.1	4.0
59	15.9	13.1	5.6
91	12.8	15.8	6.8
180	8.1	15.9	6.2
270	5.9	15.2	5.6
360	4.3	14.2	5.3

* Data at DAT0 set to material balance (102.8% TAR) minus amount of BF 500-3 (1.9% TAR) according to FOCUS (2006), because this metabolite was an impurity in the application solution

** Set to zero according to FOCUS (2006)

$ Set to 0.1 as the lowest value for reported decimal places, as no LOD or LOQ are provided

Table 7.1.2.1.1-17: Experimental data from study BASF DocID 1999/11091 used for kinetic evaluation

DAT	Extractable radioactive residues of pyraclostrobin and its metabolites [% TAR]		
	Pyraclostrobin	BF 500-6	BF 500-7
US 771-15 #			
0	101.0*	0.0**	0.0**
3	89.3	2.8	2.4
7	77.6	4.4	5.3
14	70.9	5.4	8.1
30	50.9	10.1	11.5
62	40.0	12.8	12.5
90	39.3	11.7	10.9
120	26.6	13.8	11.3
Li 35 b #			
0	100.8*	0.0**	0.0**
3	97.9	0.1$	0.1$
7	91.4	1.3	0.8
14	89.2	0.1$	0.1$
30	59.0	14.8	5.5
62	42.3	23.2	4.5
90	50.5	12.9	3.1
120	21.6	30.9	7.2
LUFA 2.2#			
0	101.5*	0.0**	0.0**
3	99.2	0.1$	0.1$
7	97.1	0.1$	0.1$
14	85.7	3.1	0.2
34	72.0	7.3	0.6
63	56.5	13.2	1.3
92	53.1	13.9	0.8
120	47.2	15.3	1.1
Canadian§			
0	99.1***	0.0**	0.0**
3	84.5	0.1$	0.1$
7	86.9	1.7	0.4
14	79.0	2.5	0.9
30	70.7	3.0	0.1$
60	61.5	4.2	0.5
90	48.3	13.3	1.4
120	38.3	16.7	4.0

* Data at DAT0 equal to material balance
** Set to zero according to FOCUS (2006)
*** Data at DAT0 set to material balance (99.8% TAR) minus amount of BF 500-3 (0.7% TAR), because this metabolite was an impurity in the application solution
# The values for BF 500-6 (sum of both isomers) include the values for the cis-isomer of BF 500-7 (maximum <1% TAR), which could not be separated from the trans-isomer of BF 500-6
§ BF 500-6 and BF 500-7 = sum of both isomers; separation of cis BF 500-7 and trans BF 500-6 could be achieved
$ Set to 0.1 as the lowest value for reported decimal places, as no LOD or LOQ are provided

Table 7.1.2.1.1-18: Experimental data from study BASF DocID 2011/1102370 used for kinetic evaluation

DAT	Extractable radioactive residues of pyraclostrobin and its metabolites [% TAR]			
	Pyraclostrobin[1]	BF 500-3[1]	BF 500-6[2,#]	BF 500-7[2,#]
Li 10				
0	(97.5) 94.9*	2.6**	0***	0***
0	(95.2) 91.9*	3.3**	0***	0***
3	83.6	4.3	1.4	NaN
7	70.1	3.5	3.3	0.1[$]
14	56.3	3.3	6.7	0.9
30	39.5	3.4	9.9	0.7
59	28.3	2.7	12.2	1.3
59	28.3	3.3	12.3	1.4
90	22.2	2.6	12.4	0.8
120	17.4	2.6	14.1	1.1
120	17.5	2.3	14.0	1.0
LUFA 5M				
0	(99.3) 94.6*	4.7**	0***	0***
0	(100.6) 97.1*	3.5**	0***	0***
3	76.0	3.2	4.3	1.8
7	61.6	2.9	10.0	3.5
14	40.5	2.7	14.5	6.3
30	24.0	2.1	18.8	7.3
59	12.1	1.0	20.9	8.6
59	13.9	1.2	22.2	8.6
90	9.0	1.0	23.3	8.7
120	6.7	1.1	21.7	7.5
120	7.7	1.0	22.7	8.5
Speyerer Wald 2				
0	(97.2) 93.8*	3.4**	0***	0***
0	(97.4) 93.6*	3.8**	0***	0***
3	86.5	4.1	1.7	NaN
7	79.8	3.5	2.7	NaN
14	69.7	3.6	5.5	0.1[$]
29	53.5	3.3	10.6	1.6
62	36.4	3.3	14.6	0.1[$]
62	35.6	3.2	14.2	0.1[$]
90	27.1	3.2	18.6	1.6
120	21.5	3.3	19.8	1.6
120	22.6	3.5	19.6	1.5

[1] Data from column Phenomenex Synergi Polar-RP

[2] Data from column Phenomenex Synergi Hydro-RP

* Data at DAT0 set to material balance (given in brackets) minus amount of BF 500-3, because this metabolite was an impurity in the application solution

** Measured values used as input, as BF 500-3 was an impurity in the application solution

*** Set to zero according to FOCUS (2006)

[#] BF 500-6 and BF 500-7 = sum of cis- and trans-isomer of each metabolite

[$] Set to 0.1 as the lowest value for reported decimal places, as no LOD or LOQ are provided

NaN default placeholder; metabolite not detected, therefore value omitted according to FOCUS (2006)

II. RESULTS AND DISCUSSION

Pyraclostrobin

The derived trigger endpoints (best-fit) for pyraclostrobin are summarized in Table 7.1.2.1.1-19. The kinetic evaluation showed that the SFO model is appropriate to derive trigger endpoints for additional work in two soils, while biphasic kinetic models (DFOP or FOMC) were selected as best-fit models in seven soils.

Table 7.1.2.1.1-19: Summary of endpoints for use as triggers for additional work of pyraclostrobin

BASF DocID - Soil	Soil type (USDA)	Best-fit kinetic model	χ^2 error	$DegT_{50}$ [d]	$DegT_{90}$ [d]
1998/11201 - Bruch West	Sandy loam	FOMC	3.7	12.4	117.0
1999/10090 - Bruch West	Sandy loam	DFOP	3.6	14.4	148.3
1999/11091 - US 711-15	Loamy sand	DFOP	4.8	34.7	338.0
1999/11091 - Li35b	Loamy sand	SFO	5.0	49.1	163.2
1999/11091 - LUFA 2.2	Loamy sand	FOMC	2.5	92.7	>1000*
1999/11091 - Canadian soil	Loam	SFO	4.8	97.9	325.2
2011/1102370 - Li10	Loamy sand	DFOP	1.3	21.2	204.0
2011/1102370 - LUFA 5M	Sandy loam	DFOP	3.4	11.2	87.7
2011/1102370 - Speyerer Wald 2	Loamy sand	FOMC	0.7	39.2	305.5

* Should be considered with care as value is far beyond the study duration

Modeling endpoints for pyraclostrobin were obtained using SFO and FOMC kinetics in six and three soils, respectively. The $DegT_{50}$ values suitable for modeling obtained under different incubation conditions were normalized to a reference moisture of pF2 and a temperature of 20°C. Parameters included in the normalization procedure are shown in Table 7.1.2.1.1-20 while derived modeling endpoints are summarized in Table 7.1.2.1.1-21.

Table 7.1.2.1.1-20: Factors for normalization to reference conditions (20°C, pF 2.0)

Analyte (BASF DocID)	Soil	T_{act} [°C]	T_{ref} [°C]	θ_{act} [g/ 100 g]	θ_{ref} [g/ 100 g]	f_{temp}	f_{moist}
[^{14}C]-pyraclostrobin (1999/11201)	Bruch West	20	20	17.2	19	1	0.93
[^{14}C]-pyraclostrobin (1999/10090)	Bruch West	20	20	17.6	19	1	0.95
[^{14}C]-pyraclostrobin (1999/11091)	US 771-15	20	20	14	14	1	1
	Li 35 b	20	20	12	14	1	0.9
	LUFA 2.2	20	20	14.4	14	1	1
	Canadian soil	20	20	17.2	25	1	0.77
[^{14}C]-pyraclostrobin (2011/1102370)	Li 10	20	20	10.4	11	1	0.96
	LUFA 5M	20	20	10.7	20.2	1	0.64
	Speyerer Wald 2	20	20	9.2	9.5	1	0.98

T_{act}	Actual temperature during incubation	[°C]
T_{ref}	Reference temperature (20°C)	[°C]
θ_{act}	Actual soil moisture	[g/100 g dry soil]
θ_{ref}	Reference soil moisture at field capacity (pF 2)	[g/100 g dry soil]
f_{temp}	Temperature correction factor	[-]
f_{moist}	Moisture correction factor	

Table 7.1.2.1.1-21: Summary of modeling endpoints of pyraclostrobin

BASF DocID - Soil	Soil type (USDA)	Kinetic model	χ^2 error	$DegT_{50}$ [d]	$DegT_{50norm}$ [d]**
1998/11201 - Bruch West	Sandy loam	FOMC	3.7	35.2*	32.7*
1999/10090 - Bruch West	Sandy loam	FOMC	4.0	34.8*	33.1*
1999/11091 - US 711-15	Loamy sand	SFO	11.0	55.6	55.6
1999/11091 - Li35b	Loamy sand	SFO	5.0	49.1	44.2
1999/11091 - LUFA 2.2	Loamy sand	SFO	5.0	95.4	95.4
1999/11091 - Canadian soil	Loam	SFO	4.8	97.9	75.4
2011/1102370 - Li10	Loamy sand	SFO	12.9	40.3	38.7
2011/1102370 - LUFA 5M	Sandy loam	FOMC	2.6	24.4*	15.6*
2011/1102370 - Speyerer Wald 2	Loamy sand	SFO	5.9	50.8	49.7

* Back-calculated from FOMC DT_{90}/3.32
** Normalized to reference conditions (20°C, pF2)

Metabolites of pyraclostrobin

The derived trigger endpoints for BF 500-6 and BF 500-7 are summarized in Table 7.1.2.1.1-22 and Table 7.1.2.1.1-23. The kinetic evaluation showed that the SFO model is appropriate to derive trigger endpoints (best-fit) for additional work in two soils, while for all other soils no reliable endpoints could be derived.

Table 7.1.2.1.1-22: Summary of endpoints for use as triggers for additional work of metabolite BF 500-6

BASF DocID - Soil	Soil type (USDA)	Best-fit kinetic model	χ^2 error	$DegT_{50}$ [d]	$DegT_{90}$ [d]
1998/11201 - Bruch West	Sandy loam	SFO	9.0	971.2*,**	>1000
1999/10090 - Bruch West	Sandy loam	No reliable endpoints derived**			
1999/11091 - US 711-15	Loamy sand	No reliable endpoints derived**			
1999/11091 - Li35b	Loamy sand	No reliable endpoints derived**			
1999/11091 - LUFA 2.2	Loamy sand	No reliable endpoints derived**			
1999/11091 - Canadian soil	Loam	No reliable endpoints derived**			
2011/1102370 - Li10	Loamy sand	No reliable endpoints derived**			
2011/1102370 - LUFA 5M	Sandy loam	SFO	2.7	>1000	>1000
2011/1102370 - Speyerer Wald 2	Loamy sand	No reliable endpoints derived**			

* Should be considered with care as value is far beyond the study duration
** No clear decline phase observed at the end of the study period

Table 7.1.2.1.1-23: Summary of endpoints for use as triggers for additional work of metabolite BF 500-7

BASF DocID - Soil	Soil type (USDA)	Best-fit kinetic model	χ^2 error	DT_{50} [d]	DT_{90} [d]
1998/11201 - Bruch West	Sandy loam	SFO	9.1	649.1*,**	>1000
1999/10090 - Bruch West	Sandy loam	No reliable endpoints derived**			
1999/11091 - US 711-15	Loamy sand	SFO	3.3	111.2	369.3
1999/11091 - Li35b	Loamy sand	No reliable endpoints derived**			
1999/11091 - LUFA 2.2	Loamy sand	No reliable endpoints derived#			
1999/11091 - Canadian soil	Loam	No reliable endpoints derived#			
2011/1102370 - Li10	Loamy sand	No reliable endpoints derived#			
2011/1102370 - LUFA 5M	Sandy loam	SFO	4.0	358.9*,**	>1000
2011/1102370 - Speyerer Wald 2	Loamy sand	No reliable endpoints derived#			

* Should be considered with care as value is far beyond the study duration
** No clear decline phase observed at the end of the study period
# Maximum occurrence < 5% TAR

For the transient metabolite BF 500-3, reliable trigger endpoints (SFO kinetics) could not be derived from any of the soils.

For the metabolites, the derived SFO-$DegT_{50}$ values are suitable as modeling endpoints after normalization to reference conditions. The same correction factors f_{temp} and f_{moist} as for the parent (see Table 7.1.2.1.1-20) were used for calculation of normalized $DegT_{50}$ values (Table 7.1.2.1.1-24 and Table 7.1.2.1.1-25).

Table 7.1.2.1.1-24: Summary of SFO endpoints for modeling of BF 500-6

BASF DocID / Soil	Soil type (USDA)	χ^2 error	$DegT_{50}$ [d]	$DegT_{50norm}$ [d]**	Formation fraction [-]
1998/11201 - Bruch West	Sandy loam	9.0	971.2*	903.2*	0.142
1999/10090 - Bruch West	Sandy loam	No reliable endpoints derived§			
1999/11091 - US 711-15	Loamy sand	No reliable endpoints derived§			
1999/11091 - Li35b	Loamy sand	No reliable DT_{50} derived§			0.371
1999/11091 - LUFA 2.2	Loamy sand	9.9	124.9	124.9	0.388
1999/11091 - Canadian soil	Loam	No reliable endpoints derived§			
2011/1102370 - Li10	Loamy sand	No reliable endpoints derived§			
2011/1102370 - LUFA 5M	Sandy loam	2.2	870.4*	557.9*	0.261
2011/1102370 - Speyerer Wald 2	Loamy sand	5.7	251.0	245.4	0.319

* Should be considered with care as value is far beyond the study duration
** Normalized to reference conditions (20°C, pF2)
§ No clear decline phase observed at the end of the study period / no degradation rate derived

Table 7.1.2.1.1-25: Summary of SFO endpoints for modeling of BF 500-7

BASF DocID / Soil	Soil type (USDA)	χ^2 error	$DegT_{50}$ [d]	$DegT_{50norm}$ [d]**	Formation fraction [-]
1998/11201 - Bruch West	Sandy loam	9.1	649.1*	603.7*	0.073
1999/10090 - Bruch West	Sandy loam	No reliable endpoints derived§			
1999/11091 - US 711-15	Loamy sand	No reliable endpoints derived§			
1999/11091 - Li35b	Loamy sand	No reliable endpoints derived§			
1999/11091 - LUFA 2.2	Loamy sand	No reliable endpoints derived#			
1999/11091 - Canadian soil	Loam	No reliable endpoints derived#			
2011/1102370 - Li10	Loamy sand	No reliable endpoints derived#			
2011/1102370 - LUFA 5M	Sandy loam	3.9	334.9*	214.7*	0.108
2011/1102370 - Speyerer Wald 2	Loamy sand	No reliable endpoints derived#			

* Should be considered with care as value is far beyond the study duration
** Normalized to reference conditions (20°C, pF2)
§ No clear decline phase observed at the end of the study period / no adequate fit achieved
# Maximum occurrence < 5% TAR

For the transient metabolite BF 500-3, no adequate fit could be derived using SFO kinetics and therefore no endpoints could be calculated.

III. CONCLUSION

Trigger and modeling endpoints were derived for pyraclostrobin in four laboratory degradation studies with nine trials. Trigger endpoints (best-fit) $DegT_{50}$ values were between 11.2 and 97.9 days, and $DegT_{90}$ values were between 87.7 and >1000 days. Modeling $DegT_{50}$ values range between 24.4 and 97.9 days, whereas normalized modeling endpoints (20°C, pF2) range between 15.6 and 95.4 days.

For metabolites BF 500-6 and BF 500-7 trigger endpoints as well as (normalized) modeling-$DegT_{50}$ values and corresponding formation fractions could be derived for each metabolite in at least two soils. For the transient metabolite BF 500-3, reliable trigger and modeling endpoints could not be derived from any of the soils.

Summary of degradation endpoints for pyraclostrobin in different soils under aerobic conditions

Table 7.1.2.1.1-26: Summary table on best-fit degradation endpoints of pyraclostrobin obtained in laboratory soil studies

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	Best-fit $DegT_{50}$ / $DegT_{90}$ [d]	Method of calculation
1998/11201 2014/1093424	Bruch West / Sandy loam (t)	7.3	1.8	20	40	12.4 / 117.0	FOMC
1999/10090 2014/1093424	Bruch West / Sandy loam (c)	7.5	2.0	20	40	14.4 / 148.3	DFOP
1999/10091 2014/1093424	US 771-15 / Loamy sand (t)	5.6	0.5	20	40	34.7 / 338.0	DFOP
	Li 35b / Loamy sand (t)	6.5	1.0	20	40	49.1 / 163.2	SFO
	LUFA 2.2 / Loamy sand (t)	5.4	2.1	20	40	92.7 / >1000	FOMC
	Canadian / Loam (t)	7.7	3.0	20	40	97.9 / 325.2	SFO
2011/1102370 2014/1093424	Li 10 / Loamy sand (t)	5.5	1.0	20	40	21.2 / 204.0	DFOP
	LUFA 5M / Sandy loam (t)	7.1	1.2	20	40	11.2 / 87.7	DFOP
	Speyerer Wald 2 / Loamy sand (t)	5.5	0.8	20	40	39.2 / 305.5	FOMC
2013/1337273	LUFA 5M / Sandy loam (p)	7.4	2.0	20	53	22.3 / 115.2	FOMC

(t), (c), (p) - tolyl, chlorophenyl, or pyrazole-labeled test item used
MWHC maximum water holding capacity

Table 7.1.2.1.1-27: Summary table on degradation endpoints for modeling of pyraclostrobin obtained in laboratory soil studies (20°C, pF2)

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	$DegT_{50}$ at study conditions [d]	Method of calculation	$DegT_{50}$ normalized to 20°C, pF2 [d]
1998/11201 2014/1093424	Bruch West / Sandy loam (t)	7.3	1.8	20	40	35.2[a]	FOMC	32.7
1999/10090 2014/1093424	Bruch West / Sandy loam (c)	7.5	2.0	20	40	34.8[a]	FOMC	33.1
1999/10091 2014/1093424	US 771-15 / Loamy sand (t)	5.6	0.5	20	40	55.6	SFO	55.6
	Li 35b / Loamy sand (t)	6.5	1.0	20	40	49.1	SFO	44.2
	LUFA 2.2 / Loamy sand (t)	5.4	2.1	20	40	95.4	SFO	95.4
	Canadian / Loam (t)	7.7	3.0	20	40	97.9	SFO	75.4
2011/1102370 2014/1093424	Li 10 / Loamy sand (t)	5.5	1.0	20	40	40.3	SFO	38.7
	LUFA 5M / Sandy loam (t)	7.1	1.2	20	40	24.4[a]	FOMC	15.6
	Speyerer Wald 2 / Loamy sand (t)	5.5	0.8	20	40	50.8	SFO	49.7
2013/1337273	LUFA 5M / Sandy loam (p)	7.4	2.0	20	53	26.2	SFO	22.8

(t), (c), (p) - tolyl, chlorophenyl, or pyrazole-labeled test item used
MWHC maximum water holding capacity
[a] calculated as $DegT_{50} = DegT_{90}/3.32$

Calculation of degradation rates at 10°C

$DegT_{50}$ values at a temperature of 10°C were calculated by multiplying the normalized $DegT_{50}$ values at 20°C and pF 2 with a default Q_{10} value of 2.58 [*EFSA (2007): "Opinion on a request from EFSA related to the default Q_{10} value used to describe the temperature effect on transformation rates of pesticides in soil. Scientific Opinion of the Panel on Plant Protection Products and their Residues (PPR-Panel). Question No EFSA-Q-2007-048. The EFSA Journal (2007) 622, 1-32*]. A summary of the calculated half-lives of pyraclostrobin at 10°C is given in the table below.

Table 7.1.2.1.1-28: Summary table on degradation endpoints for modeling of pyraclostrobin obtained in laboratory soil studies at 10°C and pF 2

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	DegT50 at 20°C, pF2 [d]	DegT50 at 10°C, pF2 [d]	Method of calculation
1998/11201 2014/1093424	Bruch West / Sandy loam (t)	7.3	1.8	20	40	32.7	84.5	FOMC
1999/10090 2014/1093424	Bruch West / Sandy loam (c)	7.5	2.0	20	40	33.1	85.3	FOMC
1999/10091 2014/1093424	US 771-15 / Loamy sand (t)	5.6	0.5	20	40	55.6	143.4	SFO
	Li 35b / Loamy sand (t)	6.5	1.0	20	40	44.2	114.0	SFO
	LUFA 2.2 / Loamy sand (t)	5.4	2.1	20	40	95.4	246.1	SFO
	Canadian / Loam (t)	7.7	3.0	20	40	75.4	194.5	SFO
2011/1102370 2014/1093424	Li 10 / Loamy sand (t)	5.5	1.0	20	40	38.7	100.0	SFO
	LUFA 5M / Sandy loam (t)	7.1	1.2	20	40	15.6	40.3	FOMC
	Speyerer Wald 2 / Loamy sand (t)	5.5	0.8	20	40	49.7	128.2	SFO
2013/1337273	LUFA 5M / Sandy loam (p)	7.4	2.0	20	53	22.8	58.8	SFO

(t), (c), (p) - tolyl, chlorophenyl, or pyrazole-labeled test item used
MWHC maximum water holding capacity

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

Degradation rates in three soils could not be reliably obtained for all metabolites from the parent studies. Therefore, separate degradation rate studies were performed using the respective metabolites as test items.

Summary tables on maximum occurrence and degradation rates of metabolites obtained in different parent and metabolite studies are provided at the end of this chapter.

Report: CA 7.1.2.1.2/1
Tornisielo A.,Sacchi R.R., 2011a
Rate of degradation of BF 500-6 on European soils under aerobic conditions
2011/1142307
Guidelines: OECD 307 (2002)
GLP: yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

The objective of the present study was to investigate the rate of degradation of BF 500-6 in three soils at a temperature of 20 ± 2°C.

The soils were treated with a nominal rate of 0.267 mg ^{14}C-labeled BF 500-6 per kg of dry soil which corresponds to a field application rate of 100 g a.s. ha^{-1}. The incubation was carried out in the dark in the laboratory under aerobic conditions at a soil moisture of 40% of the maximum water holding capacity. A closed incubation system with continuous aeration (moistened air) was used with an attached trapping system for the determination of volatile compounds. Samples were taken at 0, 3, 7, 14, 31, 60, 91, and 120 days after treatment (DAT).

The soil samples were extracted three times with acetonitrile and twice with acetonitrile/water (1:1) and the extracts were analyzed by LSC and HPLC. The amount of non-extractable residues was determined by combustion and subsequent LSC measurements.

The mass balance throughout the study ranged from 95.5 to 100.9% total applied radioactivity (TAR) for the three soils. The extractable radioactivity decreased from > 99.0% TAR at day 0 to 92.4 - 93.9% after 120 days. The majority of radioactivity in the extracts was always unchanged test item. At the end of incubation, BF 500-6 was detected in amounts of 86.6 - 90.6% TAR.

Seven minor unknown metabolites (UK) were detected showing maximum values of 1.6% (UK 2 and UK 6) and 2.3% (UK 5) TAR. Formation of CO_2 was observed in the three soils reaching 0.2 - 0.4% TAR after 120 days. No other volatile compounds were detected. Non-extractable residues were formed in amounts with a maximum of 4.1 to 4.9% TAR at the end of the study.

Degradation times of BF 500-6 were calculated with the computer program KinGUI 1.1 applying first order kinetics (SFO). DegT_{50} values were between 753 and 921 days.

I. MATERIAL AND METHODS

A. MATERIALS

BAS code:	BF 500-6
Synonym:	500M01
Reg.No.:	364380
Chemical name (IUPAC):	N,N'-bis-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl] oxymethyl} phenyl) diazene N-oxide
Molecular weight:	611.486 g mol^{-1} (unlabeled)
Position of radiolabel:	Tolyl-ring-U-C^{14}
Batch No.:	658-2004
Specific radioactivity:	8.42 MBq mg^{-1}
Radiochemical purity:	96.9% (according to certificate) 100.0% (determined by radio-HPLC prior to soil treatment in this study)

2. Soils

Three different soils were used for treatment and incubation. The soils were sieved through a 2 mm sieve before use. The soil characteristics are summarized in Table 7.1.2.1.2-1.

Table 7.1.2.1.2-1: Properties of soils used to investigate degradation rate of ^{14}C-BF 500-6

Soil designation	Li 10 (10/1680/03)	LUFA 5M (10/1651/03)	Speyerer Wald 2 (10/1715/03)
Origin	Limburgerhof, RP, Germany	Mechtersheim, RP, Germany	Schifferstadt, RP, Germany
DIN Particle size distribution [%]			
Sand 0.063 – 2 mm	82.2	51.5	79.4
Silt 0.002 – 0.063 mm	13.5	36.5	16.5
Clay < 0.002 mm	4.3	12.0	4.1
Textural class	Silty sand	Loamy sand	Silty sand
USDA Particle size distribution [%]			
Sand 0.050 – 2 mm	84.1	56.8	82.6
Silt 0.002 – 0.050 mm	11.5	31.1	13.3
Clay < 0.002 mm	4.3	12.0	4.1
Textural class	Loamy sand	Sandy loam	Loamy sand
Organic C [%]	0.97	1.19	0.83
pH (H_2O)	6.5	8.1	6.6
pH ($CaCl_2$)	5.5	7.1	5.5
Cation exchange capacity [$cmol^+ kg^{-1}$]	5.4	11.3	4.3
Maximum water holding capacity [g/100g dry soil]	25.9	26.7	22.9
Microbial biomass* [mg C/100g dry soil]	22.7	35.3	16.4
Microbial biomass at 60 DAT [mg C/100g dry soil]	27.0	41.0	20.0
Microbial biomass at 120 DAT [mg C/100g dry soil]	55.0	24.0	13.0

*data obtained before starting the study
DAT = days after treatment

The soils were transported from Germany to Brazil in a temperature range of 4 - 20°C and were stored in a refrigerator at 4 ± 2°C before use.

B. STUDY DESIGN

1. Experimental conditions

The soils were adjusted to about 40% MWHC and treated at a nominal concentration of 0.267 mg ^{14}C-BF 500-6 per kg dry soil, which corresponds to a field application rate of 100 g a.s. ha^{-1} (assuming an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}).

For the soil treatment, one kg of each soil was treated with 5 mL of the treatment solution. The treated soils were homogenized and soil portions of 50 g dry soil equivalents were filled into suitable test vessels. The number of vessels was sufficient to allow duplicate sampling at each sampling time and to keep a limited number of reserve vessels.

The vessels were incubated in the dark for 120 days at a temperature of 20 ± 2°C. Throughout the incubation period, the test vessels were continuously aerated with a slight stream of moistened and CO_2-free air. The outgoing air was led through a series of gas-washing bottles containing trapping solutions for potential volatiles (ethylene glycol, 0.5M H_2SO_4, 0.5M NaOH). The water content of the soils was monitored throughout the incubation period by weighing representative vessels at each sampling time.

For determination of the microbial biomass during (60 days) and at the end of incubation (120 days), additional soil samples without test item were incubated under the same conditions.

2. Sampling

Sampling times were 0 (no sampling of volatiles), 3, 7, 14, 31, 60, 91 and 120 days after treatment (DAT).

At 0 DAT as well as at 60 DAT and 120 DAT two replicate samples were worked up. At the other sampling times only one replicate was worked up, the second one was stored in a freezer.

3. Description of the analytical procedures

The soil was extracted consecutively three times with 100 mL acetonitrile and two times with 100 mL water/acetonitrile (1:1) by shaking for about 30 minutes. After each extraction, the suspension was centrifuged and two aliquots of the supernatant were measured by LSC.

After the last extraction, the soil was rinsed with 100 mL acetone. The acetone was also checked for radioactivity but no HPLC analysis was performed since the amounts of radioactive residues never exceeded 0.8% of the total applied radioactivity (TAR).

All the extracts were combined and concentrated to a small volume. Prior to HPLC analysis, the concentrated extracts were centrifuged. Then the supernatant was radio-assayed and analyzed by HPLC to obtain the metabolite pattern.

The extracted soil was dried at room temperature. Then, the soil was homogenized with a laboratory mill and at least aliquots combusted in order to determine the amount of non-extractable radioactive residues (NER, bound residues).

All liquid samples were measured for radioactivity by LSC. The radioactivity in the extracted and dried soil samples was determined by combustion of at least three aliquot parts. Combustion products were absorbed in the Oxysolve C-400 scintillation cocktail.

4. Kinetic modeling

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] in order to derive degradation kinetics and trigger endpoints. The analysis was conducted by non-linear regression methods using the software tool KinGUI 1.1.

For each data set, the kinetic models proposed by the FOCUS Kinetic guidance document were tested in order to identify the best-fit model. The recommended kinetic models, i.e. the single first order kinetics (SFO), the Gustafson-Holden model (FOMC) and the bi-exponential kinetic model (DFOP) are already implemented in KinGUI.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures recommended by the FOCUS guidance. The goodness-of-fit used for the identification of the best-fit kinetic model is the χ^2 minimum error level. The kinetic endpoints (DT_{50} and DT_{90} values) are reported for the kinetic models, which were selected based on statistical and visual assessment. The reliability of individual parameters was judged by means of a single-sided t-test. When available, replicate measurements were used for the parameter estimation.

In all soils, the measured value at DAT 0 was set to the material balance. At later sampling time points (3, 7, 14. 31, 60, 91 and 120 DAT), the measured values were set to the amount of extractable residues.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The detailed results on the extractability of radioactive residues in the soils are presented in Table 7.1.2.1.2-2 to Table 7.1.2.1.2-4. The material balance ranged from 95.5 to 100.9% TAR for all soils.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (ERR), non-extractable residues (NER), CO_2 and other volatiles for each soil is shown in Table 7.1.2.1.2-2 to Table 7.1.2.1.2-4. The amount of extractable radioactivity (ERR) moderately decreased from more than 99% TAR at 0 DAT to 92.4 - 93.9% TAR after 120 days of incubation.

Non-extractable radioactive residues (NER) reached maximum values of 4.1 to 4.9% TAR at the end of the study after 120 days of incubation.

Table 7.1.2.1.2-2: Distribution of radioactivity and mass balance in Li 10 soil after treatment with ^{14}C-BF 500-6 [%TAR]

days after treatment	extractable				NER	Volatiles				Mass balance
	ACN	ACN + water	Acetone	Total		NaOH ($^{14}CO_2$)	H_2SO_4	Ethylene glycol	Total	
0 (rep 1)	97.7	0.7	0.2	98.6	0.6	n.d.	n.d.	n.d.	n.d.	99.1
0 (rep 2)	99.1	0.9	0.2	100.1	0.7	n.d.	n.d.	n.d.	n.d.	100.9
0 (mean)	98.4	0.8	0.2	99.4	0.6	n.d.	n.d.	n.d.	n.d.	100.0
3	97.8	1.0	0.2	98.9	1.0	0.1	0.0	0.0	0.1	100.0
7	96.2	1.1	0.2	97.5	1.4	0.1	0.0	0.0	0.1	99.0
14	96.7	0.5	0.5	97.8	1.6	0.2	0.0	0.0	0.2	99.6
31	93.0	1.5	0.1	94.6	2.0	0.2	0.0	0.0	0.2	96.9
60 (rep 1)	93.0	1.5	0.2	94.7	3.1	0.2	0.0	0.0	0.2	98.0
60 (rep 2)	90.9	1.6	0.2	92.8	2.7	0.2	0.0	0.0	0.2	95.7
60 (mean)	92.0	1.5	0.2	93.7	2.9	0.2	0.0	0.0	0.2	96.9
91	90.8	1.9	0.2	93.0	2.8	0.3	0.0	0.0	0.3	96.1
120 (rep 1)	91.0	1.7	0.5	93.2	4.6	0.4	0.0	0.0	0.4	98.1
120 (rep 2)	91.7	1.7	0.5	93.9	4.1	0.4	0.0	0.0	0.4	98.4
120 (mean)	91.4	1.7	0.5	93.5	4.3	0.4	0.0	0.0	0.4	98.2
Arithmetic mean										**98.3**

TAR = total applied radioactivity
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determined

Table 7.1.2.1.2-3: Distribution of radioactivity and mass balance in LUFA 5M soil after treatment with ^{14}C-BF 500-6 [%TAR]

days after treatment	extractable				NER	Volatiles				Mass balance
	ACN	ACN + water	Acetone	Total		NaOH ($^{14}CO_2$)	H_2SO_4	Ethylene glycol	Total	
0 (rep 1)	98.7	1.0	0.1	99.8	0.5	n.d.	n.d.	n.d.	n.d.	100.3
0 (rep 2)	98.1	1.1	0.1	99.3	0.4	n.d.	n.d.	n.d.	n.d.	99.7
0 (mean)	98.4	1.0	0.1	99.6	0.4	n.d.	n.d.	n.d.	n.d.	100.0
3	97.6	1.5	0.1	99.3	0.9	0.0	0.0	0.0	0.0	100.2
7	96.1	1.8	0.2	98.1	1.1	0.1	0.0	0.0	0.1	99.3
14	96.8	1.1	0.8	98.7	1.5	0.1	0.0	0.0	0.1	100.3
31	93.3	2.3	0.2	95.8	2.1	0.2	0.0	0.0	0.2	98.1
60 (rep 1)	92.3	2.8	0.2	95.3	2.7	0.3	0.0	0.0	0.3	98.3
60 (rep 2)	92.6	2.9	0.2	95.7	3.1	0.3	0.0	0.0	0.3	99.1
60 (mean)	92.5	2.8	0.2	95.5	2.9	0.3	0.0	0.0	0.3	98.7
91	91.9	3.3	0.3	95.5	4.0	0.4	0.0	0.0	0.4	99.9
120 (rep 1)	89.9	3.5	0.5	93.9	4.9	0.4	0.0	0.0	0.4	99.2
120 (rep 2)	89.7	3.5	0.5	93.6	4.8	0.4	0.0	0.0	0.4	98.9
120 (mean)	89.8	3.5	0.5	93.7	4.9	0.4	0.0	0.0	0.4	99.1
Arithmetic mean										**99.4**

TAR = total applied radioactivity
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determined

Table 7.1.2.1.2-4: Distribution of radioactivity and mass balance in Speyerer Wald 2 soil after treatment with ^{14}C-BF 500-6 [%TAR]

days after treatment	extractable				NER	Volatiles				Mass balance
	ACN	ACN + water	Acetone	Total		NaOH ($^{14}CO_2$)	H_2SO_4	Ethylene glycol	Total	
0 (rep 1)	98.5	0.7	0.1	99.3	0.4	n.d.	n.d.	n.d.	n.d.	99.7
0 (rep 2)	98.9	0.7	0.1	99.8	0.5	n.d.	n.d.	n.d.	n.d.	100.3
0 (mean)	98.7	0.7	0.1	99.5	0.5	n.d.	n.d.	n.d.	n.d.	100.0
3	96.9	0.9	0.1	97.9	0.9	0.0	0.0	0.0	0.0	98.8
7	96.7	1.0	0.2	97.9	1.3	0.0	0.0	0.0	0.0	99.2
14	95.9	0.6	0.5	97.0	1.3	0.1	0.0	0.0	0.1	98.3
31	93.2	1.6	0.1	94.9	1.7	0.1	0.0	0.0	0.1	96.7
60 (rep 1)	90.7	1.7	0.2	92.6	2.7	0.2	0.0	0.0	0.2	95.5
60 (rep 2)	93.2	1.6	0.2	95.0	2.8	0.2	0.0	0.0	0.2	97.9
60 (mean)	91.9	1.7	0.2	93.8	2.7	0.2	0.0	0.0	0.2	96.7
91	90.3	1.7	0.2	92.2	3.5	0.2	0.0	0.0	0.2	95.9
120 (rep 1)	90.2	1.8	0.4	92.4	4.8	0.2	0.0	0.0	0.2	97.4
120 (rep 2)	90.4	1.8	0.5	92.7	4.9	0.2	0.0	0.0	0.2	97.7
120 (mean)	90.3	1.8	0.4	92.5	4.8	0.2	0.0	0.0	0.2	97.6
Arithmetic mean										**98.0**

TAR = total applied radioactivity
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determinated

C. VOLATILIZATION

Formation of CO_2 was negligible in all three soils reaching in total only 0.2 to 0.4% TAR after 120 days. No other volatile compounds were detected.

D. TRANSFORMATION OF BF 500-6

All combined soil extracts were analyzed by radio-HPLC. The results obtained with this system are summarized in Table 7.1.2.1.2-5.

The concentration of BF 500-6 decreased slowly in the three soils from 95.0% TAR at 0 DAT to approximately 86.6 - 90.6% TAR after 120 days of incubation. The identification of BF-500-6 was achieved by chromatographic comparison with the retention time of the ^{14}C-test item.

During the course of the study, seven minor unknown metabolites (UK) were visible in the chromatograms showing maximum values of 1.6% TAR (UK 2 and UK 6) and 2.3% TAR (UK 5).

Table 7.1.2.1.2-5: Summary results of radio-HPLC analysis of soil extracts (total of acetonitrile and acetonitrile/water extracts) after treatment of soils with ^{14}C-BF 500-6 [%TAR]

days after treatment	Total	BF 500-6 isomer t_R ~ 26.2	BF 500-6 29.4	BF 500-6 sum	UK 1 20.4	UK 2 21.0	UK 3 22.3	UK 4 22.7	UK 5 34.0	UK 6 35.3	UK 7 36.8
Li 10											
0 (rep 1)	98.6	8.3	86.0	94.2	0.7			1.1	2.0	0.5	
0 (rep 2)	100.1	8.6	87.1	95.7	0.7			0.8	2.3	0.6	
0 (mean)	99.4	8.4	86.6	95.0	0.7			1.0	2.1	0.5	
3	98.9	8.2	88.1	96.3	0.4			0.7	1.0	0.5	
7	97.5	9.0	86.8	95.8	0.2			0.1	0.3	1.1	
14	97.8	8.3	88.2	96.5	0.8					0.5	
31	94.6	7.8	85.0	92.8		0.5		0.7		0.6	
60 (rep 1)	94.7	7.5	85.8	93.3		0.6		0.4	0.2	0.3	
60 (rep 2)	92.8	7.5	83.6	91.1		0.6		0.2		0.9	
60(mean)	93.7	7.5	84.7	92.2		0.6		0.3	0.2	0.6	
91	93.0	6.7	83.9	90.6	0.1	0.7	0.4	0.6		0.3	0.3
120 (rep 1)	93.2	7.2	81.5	88.7	0.7	1.0	0.6	0.6	0.2	1.4	
120 (rep 2)	93.9	6.8	83.3	90.1	0.6	0.8	0.5	0.4		0.9	0.6
120 (mean)	93.5	7.0	82.4	89.4	0.7	0.9	0.5	0.5	0.2	1.1	0.6
LUFA 5M											
0 (rep 1)	99.8	9.4	87.3	96.7	0.9			0.4	1.6	0.2	
0 (rep 2)	99.3	9.1	86.1	95.2	0.9			0.8	1.7	0.7	
0 (mean)	99.6	9.3	86.7	95.9	0.9			0.6	1.7	0.5	
3	99.3	9.2	87.5	96.7	0.4			0.7	0.9	0.6	
7	98.1	10.1	86.7	96.8	0.3			0.7	0.2	0.1	
14	98.7	7.8	90.1	97.9				0.4	0.2	0.1	
31	95.8	11.3	82.6	93.9		0.4		0.5		1.0	
60 (rep 1)	95.3	6.9	85.9	92.8		0.4		0.2	0.0	0.8	1.0
60 (rep 2)	95.7	7.4	85.7	93.1		0.8		0.5		0.9	0.4
60(mean)	95.5	7.2	85.8	93.0		0.6		0.3	0.0	0.9	0.7
91	95.5	8.0	84.7	92.7	0.2	0.9	0.2			0.8	0.7
120 (rep 1)	93.9	8.9	81.7	90.6	0.2	1.5		0.4	0.3	0.2	0.8
120 (rep 2)	93.6	8.6	80.9	89.6	0.4	1.6	0.3	0.5		0.7	0.6
120 (mean)	93.7	8.7	81.3	90.1	0.3	1.5	0.3	0.5	0.3	0.4	0.7
Speyerer Wald 2											
0 (rep 1)	99.3	9.7	84.8	94.5	1.1			0.5	2.1	1.1	
0 (rep 2)	99.8	9.8	86.0	95.8	1.5			0.2	1.9	0.4	
0 (mean)	99.5	9.8	85.4	95.1	1.3			0.3	2.0	0.8	
3	97.9	8.1	87.0	95.2	0.7			0.6	0.7	0.8	
7	97.9	8.0	86.5	94.5		0.6		0.2	1.2	1.3	
14	97.0	6.8	88.0	94.8				1.1	0.4	0.7	
31	94.9	8.0	85.2	93.3		0.6		0.4		0.6	
60 (rep 1)	92.6	9.8	79.0	88.8	0.5	0.8	0.4	0.9		0.9	0.2
60 (rep 2)	95.0	9.4	81.5	90.9	0.7	0.7	0.3	0.9		1.0	0.6
60(mean)	93.8	9.6	80.3	89.9	0.6	0.7	0.3	0.9		1.0	0.4
91	92.2	6.5	83.1	89.6		1.0	0.7	0.4		0.4	0.1
120 (rep 1)	92.4	7.6	79.0	86.6	0.6	0.8	0.3	1.3		1.6	1.1
120 (rep 2)	92.7	8.5	80.7	89.1	0.6	0.6	0.6	0.8		0.2	0.8
120 (mean)	92.5	8.0	79.8	87.9	0.6	0.7	0.4	1.0		0.9	1.0

Total = acetonitrile and acetonitrile+water extracts
TAR = total applied radioactivity
UK = unknown compound
t_R = retention time [min]

Calculation of the degradation rates

All visual fits were good and the residuals were evenly distributed. The χ^2 errors were below 2 for all models. As no obvious biphasic degradation pattern was observed in any of the soils, SFO was selected as best fit model in all cases.

It was shown that the observed degradation of BF 500-6 in the different experiments could be well described by single first order (SFO) kinetics. In Table 7.1.2.1.2-6, the $DegT_{50}/DegT_{90}$ values of the selected kinetic models are presented.

Table 7.1.2.1.2-6: Best-fit kinetic $DegT_{50}$ and $DegT_{90}$ values of metabolite BF 500-6

Soil	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Kinetic model	χ^2 error
Li 10	855	>1000	SFO	1.18
LUFA 5M	921	>1000	SFO	1.04
Speyerer Wald 2	753	>1000	SFO	1.40

III. CONCLUSION

BF 500-6 degraded in three different soils with $DegT_{50}$ values between 753 and 921 days under aerobic conditions at 20 ± 2°C and a soil moisture of 40% of the maximum water holding capacity. Seven minor unknown metabolites appeared during the course of the study. However, none of them exceeded 2.3% of the total applied radioactivity (TAR). After 120 days of incubation, the mineralization rate reached 0.2 to 0.4% TAR and the non-extractable residues amounted to 4.1 - 4.9% TAR.

Normalization of degradation rates
Normalization to reference conditions (20°C, pF2) was performed as described for the parent (see paragraph after CA 7.1.1.1/1). A summary is provided below:

Table 7.1.2.1.2-7: Normalization of BF 500-6 $DegT_{50}$ values to reference conditions

Compound	Soil	pH ($CaCl_2$)	Kinetic Model	θ_{act}	θ_{ref}	f_{moist}	$DegT_{50,act}$	$DegT_{50,ref}$
BF 500-6	Li 10	5.5	SFO	10.4	11.0	0.96	855	822.1
	LUFA 5M	7.1	SFO	10.7	20.2	0.64	921	590.3
	Speyerer Wald 2	5.5	SFO	9.2	9.5	0.98	753	736.3

θ_{act}	actual soil moisture	[g / 100 g dry soil]
θ_{ref}	reference soil moisture at field capacity (pF 2) according to FOCUS (2012)	[g / 100 g dry soil]
f_{moist}	moisture correction factor	[-]
$DegT_{50,act}$	DT_{50} at study conditions	[d]
$DegT_{50,ref}$	DT_{50} at reference conditions	[d]

Report: CA 7.1.2.1.2/2
Tornisielo A.,Sacchi R.R., 2011b
Rate of degradation of BF 500-7 on European soils under aerobic conditions
2011/1142308
Guidelines: OECD 307 (2002)
GLP: yes
(certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

The objective of the present study was to investigate the rate of degradation of BF 500-7 in three soils at a temperature of 20 ± 2°C.

The soils were treated with a nominal rate of 0.267 mg ^{14}C-labeled BF 500-7 per kg of dry soil which corresponds to a field application rate of 100 g a.s. ha^{-1}. The incubation was carried out in the dark in the laboratory under aerobic conditions at a soil moisture of 40% of the maximum water holding capacity. A closed incubation system with continuous aeration (moistened air) was used with an attached trapping system for the determination of volatile compounds. Samples were taken at 0, 3, 7, 14, 29, 62, 87 and 121 days after treatment (DAT).

The soil samples were extracted three times with acetonitrile and twice with acetonitrile/water (1:1) and the extracts were analyzed by means of LSC and HPLC. The amount of non-extractable residues was determined by combustion and subsequent LSC measurements.

The mass balance throughout the study ranged from 94.3 to 101.5% of the total applied radioactivity (TAR) for the three soils. The extractable radioactivity decreased from > 97.0% TAR at day 0 to 89.4 – 94.1% after 121 days. The majority of radioactivity in the extracts was always unchanged test item. At the end of incubation, BF 500-7 was detected in amounts of 84.4 - 90.8% TAR.

Three metabolites were detected showing maximum values of about 2.6 - 3.2% TAR. Formation of CO_2 was observed in the three soils reaching 0.3 to 0.4% TAR after 121 days. No other volatile compounds were detected. Non-extractable residues were formed in amounts with a maximum of 6.3 to 7.4%TAR at the end of the study.

Degradation times of BF 500-7 were calculated with the computer program KinGUI 1.1 applying first order kinetics (SFO). DegT_{50} values were between 727 and >1000 days.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS code:	BF 500-7
Synonym:	500M02
Reg.No.:	369315
Chemical name (IUPAC):	N,N'-bis-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl} phenyl) diazene
Molecular weight:	595.4884 g mol^{-1} (unlabeled)
Position of radiolabel:	Tolyl-ring-U-C^{14}
Specific radioactivity:	8.90 MBq mg^{-1}
Batch No.:	933-2025
Radiochemical purity:	98.8% (according to certificate) 100.0% (determined by radio-HPLC prior to soil treatment in this study)

2. Soils

Three different soils were used for treatment and incubation. The soils were sieved through a 2 mm sieve before use. The soil characteristics are summarized in Table 7.1.2.1.2-8.

Table 7.1.2.1.2-8: Properties of soils used to investigate degradation rate of ^{14}C-BF 500-7

Soil designation	Li10 (10/1680/03)	LUFA 5M (10/1651/03)	Speyerer Wald 2 (10/1715/03)
Origin	Limburgerhof, RP, Germany	Mechtersheim, RP, Germany	Schifferstadt, RP, Germany
DIN Particle size distribution [%]			
Sand 0.063 – 2 mm	82.2	51.5	79.4
Silt 0.002 – 0.063 mm	13.5	36.5	16.5
Clay < 0.002 mm	4.3	12.0	4.1
Textural class	Silty sand	Loamy sand	Silty sand
USDA Particle size distribution [%]			
Sand 0.050 – 2 mm	84.1	56.8	82.6
Silt 0.002 – 0.050 mm	11.5	31.1	13.38
Clay < 0.002 mm	4.3	12.0	4.1
Textural class	Loamy sand	Sandy loam	Loamy sand
Organic C [%]	0.97	1.19	0.83
pH (H_2O)	6.5	8.1	6.6
pH ($CaCl_2$)	5.5	7.1	5.5
Cation exchange capacity [$cmol^+ kg^{-1}$]	5.4	11.3	4.3
Maximum water holding capacity [g/100g dry soil]*	25.9	26.7	22.9
Microbial biomass [mg C/100g dry soil]*	22.7	35.3	16.4
Microbial biomass at 62 DAT [mg C/100g dry soil]	19.0	25.0	23.0
Microbial biomass at 121 DAT [mg C/100g dry soil]	14.0	24.0	60.0

* data obtained before starting the study
DAT = days after treatment

The soils were transported from Germany to Brazil in a temperature range of 4 - 20°C and before use were stored in a refrigerator at 4 ± 2°C.

B. STUDY DESIGN

1. Experimental conditions

The soils were adjusted to about 40% max. water holding capacity and treated at a nominal concentration of 0.267 mg ^{14}C-BF 500-7 per kg dry soil, which corresponds to a field application rate of 100 g a.s. ha^{-1} (assuming an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}).

For the soil treatment, one kg of each soil was treated with 5 mL of the treatment solution. The treated soils were homogenized and soil portions of 50 g dry soil equivalents were filled into suitable test vessels. The number of vessels was sufficient to allow duplicate sampling at each sampling time and to keep a limited number of reserve vessels.

The vessels were incubated in the dark for 121 days at a temperature of 20°C ± 2°C. Throughout the incubation period, the test vessels were continuously aerated with a slight stream of moistened and CO_2-free air. The outgoing air was led through a series of gas-washing bottles containing trapping solutions for potential volatiles (ethylene glycol, 0.5M H_2SO_4, 0.5M NaOH). The water content of the soils was monitored throughout the incubation period by weighing representative vessels at each sampling time.

For determination of the microbial biomass during (62 days) and at the end of incubation (121 days), additional soil samples without test item were incubated under the same conditions.

2. Sampling

Sampling times were 0 (no sampling of volatiles), 3, 7, 14, 29, 62, 87 and 121 days after treatment (DAT).

At 0 DAT as well as at 62 DAT and 121 DAT two replicate samples were worked up. At the other sampling times only one replicate was worked up, the second one was stored in a freezer.

3. Description of the analytical procedures

The soil was extracted consecutively three times with 100 mL acetonitrile and two times with 100 mL acetonitrile/water (1:1) by shaking for about 30 minutes. After each extraction, the suspension was centrifuged and two aliquots of the supernatant were measured by LSC.

After the last extraction, the soil was rinsed with 100 mL acetone. The acetone was also checked for radioactivity but no HPLC analysis was performed since the amounts of radioactive residues never exceeded 1.1% of the total applied radioactivity (TAR).

All the extracts were combined and concentrated to a small volume. Prior to HPLC analysis, the concentrated extracts were centrifuged. After centrifugation, the supernatant was radio-assayed and analyzed by HPLC to obtain the metabolite pattern.

The extracted soil was dried at room temperature. Then, the soil was homogenized with a laboratory mill and aliquots combusted in order to determine the amount of non-extractable radioactive residues (NER, bound residues).

All liquid samples were measured for radioactivity by LSC. The radioactivity in the extracted and dried soil samples was determined by combustion of at least three aliquots. Combustion products were absorbed in the Oxysolve C-400 scintillation cocktail.

4. Calculation of the degradation rate of BF 500-7

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] in order to derive degradation kinetics and trigger endpoints. The analysis was conducted by non-linear regression methods using the software tool KinGUI 1.1.

For each data set, the kinetic models proposed by the FOCUS Kinetic guidance document were tested in order to identify the best-fit model. The recommended kinetic models, i.e. the single first order kinetics (SFO), the Gustafson-Holden model (FOMC) and the bi-exponential kinetic model (DFOP) are already implemented in KinGUI.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures recommended by the FOCUS guidance. The goodness-of-fit used for the identification of the best-fit kinetic model is the χ^2 minimum error level. The kinetic endpoints (DT_{50} and DT_{90} values) are reported for the kinetic models, which are selected based on statistical and visual assessment. The reliability of individual parameters was judged by means of a single-sided t-test. When available, replicate measurements were used for the parameter estimation.

In all soils, the measured value at DAT 0 was set to the material balance. At later sampling time points (3, 7, 14. 29, 62, 87 and 121 DAT), the measured values were set to the amount of extractable residues.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The detailed results on the extractability of radioactive residues in the soils are presented in Table 7.1.2.1.2-9 to Table 7.1.2.1.2-11. The material balance ranged from 94.3 to 101.5% TAR for all soils.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (ERR), non-extractable residues (NER), CO_2 and other volatiles for each soil is shown in Table 7.1.2.1.2-9 to Table 7.1.2.1.2-11. The amount of extractable radioactivity (ERR) decreased from more than 97% TAR at 0 DAT to 89.4 - 92.9% TAR after 121 days of incubation.

Non-extractable radioactive residues (NER) reached maximum values of 6.3 to 7.4%TAR at the end of the study after 121 days of incubation.

Table 7.1.2.1.2-9: Distribution of radioactivity and mass balance in Li 10 soil after treatment with ^{14}C-BF 500-7 [%TAR]

days after treatment	extractable				NER	Volatiles				Mass balance
	ACN	ACN + water	Acetone	Total		NaOH ($^{14}CO_2$)	H_2SO_4	Ethylene glycol	Total	
0 (rep 1)	95.8	0.9	0.4	97.2	1.6	n.d.	n.d.	n.d.	n.d.	98.8
0 (rep 2)	97.8	0.9	0.4	99.2	2.0	n.d.	n.d.	n.d.	n.d.	101.2
0 (mean)	96.8	0.9	0.4	98.2	1.8	n.d.	n.d.	n.d.	n.d.	100.0
3	97.2	0.5	0.4	98.1	2.0	0.0	0.0	0.0	0.0	100.1
7	94.7	0.6	0.9	96.2	2.1	0.1	0.0	0.0	0.1	98.4
14	94.2	1.5	0.4	96.0	3.6	0.1	0.0	0.0	0.1	99.7
29	92.6	1.9	0.5	95.0	4.0	0.2	0.0	0.0	0.2	99.2
62 (rep1)	87.2	1.5	0.6	89.2	6.3	0.3	0.0	0.0	0.3	95.9
62 (rep2)	88.4	1.4	0.5	90.3	5.7	0.2	0.0	0.0	0.2	96.2
62 (mean)	87.8	1.4	0.5	89.8	6.0	0.3	0.0	0.0	0.3	96.1
87	85.5	1.7	0.5	87.7	6.3	0.3	0.0	0.0	0.3	94.3
121 (rep1)	88.6	1.6	1.1	91.2	6.3	0.3	0.0	0.0	0.3	97.8
121 (rep2)	88.8	1.6	1.0	91.4	6.4	0.3	0.0	0.0	0.3	98.2
121 (mean)	88.7	1.6	1.1	91.3	6.3	0.3	0.0	0.0	0.3	98.0
Arithmetic mean										**98.1**

TAR = total applied radioactivity
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determined

Table 7.1.2.1.2-10: Distribution of radioactivity and mass balance in LUFA 5M soil after treatment with ^{14}C-BF 500-7 [%TAR]

days after treatment	extractable				NER	Volatiles				Mass balance
	ACN	ACN + water	Acetone	Total		NaOH ($^{14}CO_2$)	H_2SO_4	Ethylene glycol	Total	
0 (rep 1)	98.7	1.0	0.2	99.9	1.2	n.d.	n.d.	n.d.	n.d.	101.1
0 (rep 2)	96.7	1.0	0.2	97.9	1.0	n.d.	n.d.	n.d.	n.d.	98.9
0 (mean)	97.7	1.0	0.2	98.9	1.1	n.d.	n.d.	n.d.	n.d.	100.0
3	96.6	0.3	1.0	97.9	1.4	0.0	0.0	0.0	0.0	99.3
7	96.7	0.3	0.8	97.9	1.7	0.0	0.0	0.0	0.0	99.6
14	97.0	0.2	0.2	97.4	2.6	0.1	0.0	0.0	0.1	100.0
29	94.3	1.7	0.3	96.3	2.7	0.1	0.0	0.0	0.1	99.2
62 (rep1)	92.7	1.8	0.4	94.9	4.4	0.2	0.0	0.0	0.2	99.4
62 (rep2)	93.4	1.7	0.3	95.4	4.8	0.2	0.0	0.0	0.2	100.4
62 (mean)	93.0	1.7	0.4	95.1	4.6	0.2	0.0	0.0	0.2	99.9
87	89.3	2.1	0.3	91.7	5.7	0.2	0.0	0.0	0.2	97.6
121 (rep1)	90.0	2.1	0.8	92.9	7.4	0.3	0.0	0.0	0.3	100.6
121 (rep2)	89.8	2.1	0.9	92.7	6.7	0.3	0.0	0.0	0.3	99.8
121 (mean)	89.9	2.1	0.8	92.8	7.1	0.3	0.0	0.0	0.3	100.2
Arithmetic mean										**99.7**

TAR = total applied radioactivity
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determinated

Table 7.1.2.1.2-11: Distribution of radioactivity and mass balance in Speyerer Wald 2 soil after treatment with ^{14}C-BF 500-7 [%TAR]

days after treatment	extractable				NER	Volatiles				Mass balance
	ACN	ACN + water	Acetone	Total		NaOH ($^{14}CO_2$)	H_2SO_4	Ethylene glycol	Total	
0 (rep 1)	97.8	0.9	0.4	99.1	0.9	n.d.	n.d.	n.d.	n.d.	100.0
0 (rep 2)	97.4	0.8	0.4	98.6	1.4	n.d.	n.d.	n.d.	n.d.	100.0
0 (mean)	97.6	0.8	0.4	98.9	1.1	n.d.	n.d.	n.d.	n.d.	100.0
3	96.8	0.3	0.9	97.9	1.8	0.0	0.0	0.0	0.0	99.8
7	96.1	0.5	0.8	97.4	2.1	0.0	0.0	0.0	0.0	99.6
14	95.3	1.4	0.3	97.0	3.4	0.1	0.0	0.0	0.1	100.5
29	94.6	1.7	0.4	96.7	3.9	0.1	0.0	0.0	0.1	100.8
62 (rep1)	93.7	1.3	0.5	95.5	5.2	0.2	0.0	0.0	0.2	101.0
62 (rep2)	94.3	1.3	0.5	96.0	5.2	0.2	0.0	0.0	0.2	101.5
62 (mean)	94.0	1.3	0.5	95.8	5.2	0.2	0.0	0.0	0.2	101.2
87	90.5	1.6	0.5	92.6	6.8	0.3	0.0	0.0	0.3	99.7
121 (rep1)	86.9	1.4	1.0	89.4	7.0	0.3	0.0	0.0	0.3	96.7
121 (rep2)	91.6	1.4	1.0	94.1	7.0	0.3	0.0	0.0	0.3	101.4
121 (mean)	89.3	1.4	1.0	91.7	7.0	0.3	0.0	0.0	0.3	99.1
Arithmetic mean										**100.1**

TAR = total applied radioactivity
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determinated

C. VOLATILIZATION

Formation of CO_2 was negligible in all three soils reaching in total only 0.3% TAR after 121 days. No other volatile compounds were detected.

D. TRANSFORMATION OF BF 500-7

All combined soil extracts were analyzed by radio-HPLC. The results obtained with this system are summarized in Table 7.1.2.1.2-12.

The concentration of BF 500-7 decreased slowly in the three soils from more than 94.0% TAR at 0 DAT to approximately 84.4-90.8% TAR after 121 days of incubation. The identification of BF 500-7 was achieved by chromatographic comparison with the retention time of the ^{14}C-test item.

During the course of the study, three minor unknown metabolites (UK) were visible in the chromatograms showing maximum values of 1.4% TAR (UK 1 and UK 3) and 3.2% TAR (UK 2).

Table 7.1.2.1.2-12: Summary results of radio-HPLC analysis of soil extracts (total of acetonitrile and acetonitrile/water extracts) after treatment of soils with ^{14}C-BF 500-7 [%TAR]

days after treatment	Total	BF 500-7 isomer t_R ~ 28.7	BF 500-7 36.4	BF 500-7 sum	UK 1 23.1	UK 2 25.7	UK 3 27.5
Li 10							
0 (rep 1)	97.2	14.1	80.1	94.2	-	2.2	0.8
0 (rep 2)	99.2	13.4	83.4	96.8	-	1.5	0.9
0 (mean)	98.2	13.8	81.8	95.5	-	1.8	0.9
3	98.1	12.4	81.3	93.8	1.1	2.2	1.0
7	96.2	10.8	84.1	95.0	0.2	1.1	-
14	96.0	10.9	82.2	93.0	1.0	1.7	0.3
29	95.0	7.3	86.4	93.7	0.0	1.1	0.1
62 (rep 1)	89.2	12.6	74.6	87.2	0.7	1.4	-
62 (rep 2)	90.3	13.0	75.2	88.1	0.5	1.2	0.4
62 (mean)	89.8	12.8	74.9	87.7	0.6	1.3	0.4
87	87.7	9.1	76.9	86.0	0.3	0.9	0.5
121 (rep 1)	91.2	14.6	73.5	88.1	0.9	1.7	0.5
121 (rep 2)	91.4	14.9	74.0	88.8	0.6	1.3	0.7
121 (mean)	91.3	14.7	73.7	88.5	0.8	1.5	0.6
LUFA 5M							
0 (rep 1)	99.9	14.1	82.3	96.4	-	2.6	0.9
0 (rep 2)	97.9	14.5	80.7	95.2	-	2.0	0.7
0 (mean)	98.9	14.3	81.5	95.8		2.3	0.8
3	97.9	14.8	79.2	94.0	0.6	2.2	1.0
7	97.9	12.2	83.8	96.0	0.3	1.1	0.5
14	97.4	12.7	81.3	94.0	0.9	1.8	0.7
29	96.3	9.9	84.6	94.6	0.5	1.2	0.1
62 (rep 1)	94.9	14.5	79.0	93.5	0.2	0.9	0.2
62 (rep 2)	95.4	13.4	79.5	92.9	0.5	1.6	0.4
62 (mean)	95.1	13.9	79.2	93.2	0.4	1.3	0.3
87	91.7	7.1	83.1	90.2	0.4	0.9	0.2
121 (rep 1)	92.9	16.1	74.7	90.8	0.5	1.4	0.2
121 (rep 2)	92.7	15.3	74.7	90.0	0.6	1.7	0.5
121 (mean)	92.8	15.7	74.7	90.4	0.5	1.5	0.3
Speyerer Wald 2							
0 (rep 1)	99.1	14.7	81.4	96.1	0.6	1.6	0.8
0 (rep 2)	98.6	13.7	80.8	94.5	0.7	2.5	1.0
0 (mean)	98.9	14.2	81.1	95.3	0.6	2.0	0.9
3	97.9	14.2	79.8	94.0	1.3	2.0	0.6
7	97.4	10.1	84.8	94.9	0.6	1.9	-
14	97.0	13.0	82.3	95.3	0.2	1.2	0.4
29	96.7	8.3	86.7	95.0	0.7	0.9	0.1
62 (rep 1)	95.5	12.1	80.5	92.5	0.3	1.9	0.8
62 (rep 2)	96.0	12.1	81.2	93.3	1.0	1.6	0.1
62 (mean)	95.8	12.1	80.8	92.9	0.6	1.8	0.5
87	92.6	8.5	81.7	90.2	0.8	1.3	0.3
121 (rep 1)	89.4	13.2	71.2	84.4	0.3	3.2	1.4
121 (rep2)	94.1	13.4	75.7	89.1	1.4	2.8	0.7
121 (mean)	91.7	13.3	73.4	86.8	0.8	3.0	1.1

Total = acetonitrile and acetonitrile/water extracts
TAR = total applied radioactivity
UK = unknown compound
t_R = retention time [min]

Calculation of the degradation rates

All visual fits were good and the residuals were evenly distributed. The χ^2 errors were below 2 for all models. As no obvious biphasic degradation pattern was observed in any of the soils, SFO was selected as best fit model in all cases.

It was shown that the observed degradation of BF 500-7 in the different experiments could be well described by single first order (SFO) kinetics. In Table 7.1.2.1.2-13, the $DegT_{50}$/$DegT_{90}$ values of the selected kinetic models are presented.

Table 7.1.2.1.2-13: Best-fit kinetic $DegT_{50}$ and $DegT_{90}$ values of metabolite BF 500-7

Soil	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Kinetic model	χ^2 error
Li 10	727	>1000	SFO	2.08
LUFA 5M	>1000	>1000	SFO	1.49
Speyerer Wald 2	745	>1000	SFO	1.43

III. CONCLUSION

BF 500-7 degraded in three different soils with $DegT_{50}$ values between 727 and >1000 days under aerobic conditions at 20 ± 2°C and a soil moisture of 40% of the maximum water holding capacity. Three minor unknown metabolites appeared during the course of the study. However, none of them exceeded 3.2% of the total applied radioactivity (TAR). After 121 days of incubation, the mineralization rate had reached 0.3% TAR and the non-extractable residues amounted to 6.3 – 7.4% TAR.

Normalization of degradation rates

Normalization to reference conditions (20°C, pF2) was performed as described for the parent (see paragraph after *CA 7.1.1.1/1*). A summary is provided below:

Table 7.1.2.1.2-14: Normalization of BF 500-7 $DegT_{50}$ values to reference conditions

Compound	Soil	pH ($CaCl_2$)	Kinetic Model	θ_{act}	θ_{ref}	f_{moist}	$DegT_{50,act}$	$DegT_{50,ref}$
BF 500-7	Li 10	5.5	SFO	10.4	11	0.96	727	699.0
	LUFA 5M	7.1	SFO	10.7	20.2	0.64	1050 [a]	673.0
	Speyerer Wald 2	5.5	SFO	9.2	9.5	0.98	745	728.5

θ_{act} actual soil moisture [g / 100 g dry soil]
θ_{ref} reference soil moisture at field capacity (pF 2) according to FOCUS (2012) [g / 100 g dry soil]
f_{moist} moisture correction factor [-]
$DegT_{50,act}$ DT_{50} at study conditions [d]
$DegT_{50,ref}$ DT_{50} at reference conditions [d]
[a] Calculated from reported degradation rate as $DT_{50} = \ln 2 / k$

Report:	CA 7.1.2.1.2/3 Ebert D.,Dalkmann P., 2014a Rate of degradation of BF 500-4 (Reg. No. 358672) in soil under aerobic conditions 2013/1294779
Guidelines:	OECD 307, EPA 835.4100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The degradation rate of BF 500-4 (500M73, Reg. No. 358672), anaerobic soil metabolite of pyraclostrobin, was investigated under aerobic conditions in three German agricultural soils.

The soils were treated with radiolabelled test item BF 500-4 at a nominal rate of 0.27 mg per kg dry soil, which corresponds to a field application rate of 100 g a.s. ha^{-1} calculated on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}. This application rate is not related to any expected occurrence of this compound in soil, but was chosen for practical reasons to enable reliable quantification. Detectable concentrations of BF 500-4 in aerobic soil are generally not to be expected.

Soil aliquots of 100 g (dry weight basis) were weighed into test vessels and incubated in the dark under aerobic conditions with a soil moisture of 45% of the maximum water holding capacity and a temperature of 20°C ± 2°C.

Samples were taken after 0 and 2 hours and after 1, 3, 7, 14 and 28 days of incubation. All soil samples were worked up in duplicate. The soil samples were extracted three times with acetonitrile and once with acetonitrile/water (50/50, v/v). The individual extracts were analyzed by liquid scintillation counting (LSC). The extracts were then concentrated and analyzed by HPLC. The remaining soil after extraction was combusted to determine the amount of non-extractable soil bound residues. Additionally, the amount of formed volatiles was determined. A full material balance was provided for each sampling interval.

The amount of extractable radioactivity decreased continuously in the aerated soils from 89.4 to 93.1% TAR on hour 0 to 16.3 to 24.0% TAR after 28 days of incubation. The amount of non-extractable radioactive residues increased during the study from 2.1 to 3.3% TAR to 63.0 to 67.3% TAR after 28 days of incubation, representing the major route of degradation of BF 500-4. Mineralization to $^{14}CO_2$ reached between 5.1 and 6.3% TAR at the end of incubation. The material balance ranged from 85.9 to 98.1% TAR for all soils and sampling times.

The test item BF 500-4 degraded quickly already during the first day. From a level of 79.0 to 83.5% TAR at hour 0, the level dropped to 35.3 to 51.7% TAR after 1 day and decreased further to 1.2 to 3.0% TAR after 28 days.

The known pyraclostrobin metabolites BF 500-6 and BF 500-7 were detected in all three soils, reaching a maximum of 8.0% TAR (BF 500-6) and 2.2% TAR (BF 500-7) in soil LUFA 5M. Numerous unknown metabolites were formed over time, however, the majority of them in amounts < 2% TAR. Three unknown metabolites eluting at approximately 16, 33, and 40 min reached maximum amounts of 9.9, 8.6, and 5.8% TAR, respectively. Since BF 500-4 is itself a metabolite being detectable only under strict anaerobic soil conditions [old *EU Dossier, A II M 7.1.1.2.1/5, Kellner O. - BASF DocID 1999/10079*; old *EU Dossier, A II M 7.1.1.2.1/6, Kellner O. - BASF DocID 1999/11103*], these degradation products are considered to be not environmentally relevant. All other degradation products never exceeded 5% TAR.

Kinetic analysis and calculation of DT_{50} and DT_{90} values was performed following the recommendations of the FOCUS Kinetics workgroup. The analysis was conducted by non-linear regression methods employing the software tool KinGUII. Best-fit DT_{50} values ranged from 0.3 to 1.8 days (DT_{90}: 3.9 – 13.5 days) and modeling- DT_{50} values were between 2.2 and 6.1 days.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS code:	^{14}C-BF 500-4
Synonym:	500M73
Reg. No.:	358672
Chemical name (IUPAC):	2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenylamine
Molar mass:	299.76 g mol^{-1} (unlabeled)
Position of radiolabel:	Tolyl-U-C^{14}
Specific radioactivity of a.s.:	8.49 MBq mg^{-1}
Batch No.:	1072-1004
Radiochemical purity:	≥ 96.0%

2. Soils

Three agricultural soils were used for treatment and incubation. The soils were sieved through a 2 mm sieve before use. The soil characteristics are summarized in Table 7.1.2.1.2-15.

Table 7.1.2.1.2-15: Properties of soils used to investigate the degradation rate of ^{14}C-BF 500-4

Soil designation	LUFA 5M (14/1651/01)	Li10 (14/1680/01)	LUFA 2.2 (14/736/01)
Origin	Mechtersheim, RP, Germany	Limburgerhof, RP, Germany	Hanhofen, RP, Germany
DIN Particle size distribution [%]			
Sand 0.063 – 2 mm Silt 0.002 – 0.063 mm Clay < 0.002 mm	53.0 34.0 13.0	81.4 13.2 5.4	74.7 17.5 7.7
Textural class	Loamy sand Sl4	Loamy sand Sl2	Loamy sand Sl2
USDA Particle size distribution [%]			
Sand 0.050 – 2 mm Silt 0.002 – 0.050 mm Clay < 0.002 mm	58.9 28.2 13.0	82.6 12.0 5.4	76.6 15.7 7.7
Textural class	Sandy loam	Loamy sand	Sandy loam
Total organic carbon [%]	1.12	0.88	1.77
pH (H_2O) pH ($CaCl_2$)	7.9 7.2	6.8 6.1	5.9 5.3
Cation exchange capacity [$cmol^+ kg^{-1}$]	11.2	6.1	5.7
Max. water holding capacity [g/100g dry soil]	30.4	25.6	31.1
Microbial biomass before incub. [mg C/100g dry soil]	37.8	18.1	35.3
Microbial biomass after 32 / 33 days [mg C/100g dry soil]	30.4	24.7	27.4

B. STUDY DESIGN

1. Experimental conditions

Before treatment, soil moisture was adjusted to about 45% of the maximum water holding capacity. Each test vessel contained 100 g soil portions (dry weight equivalents) and was treated with ^{14}C-labeled BF 500-4 at a nominal concentration of 0.27 mg kg^{-1} dry soil, which corresponds to a field application rate of 100 g a.s. ha^{-1}. This application rate is not related to any expected occurrence of this compound in soil, but was chosen for practical reasons to enable reliable quantification. Detectable concentrations of BF 500-4 in aerobic soil are generally not to be expected.

The test vessels were incubated in the dark for up to 28 days at a temperature of 20 ± 2°C. Throughout the incubation period, the test vessels were continuously aerated with a slight stream of moistened synthetic air. The outgoing air was led through a series of gas-washing bottles containing trapping solutions for potential volatiles (0.5 M NaOH, ethylene glycol, 0.5 M H_2SO_4). H_2SO_4 traps were omitted after day three, since no radioactivity evolving from BF 500-4 was expected in this solution. The water content of the soils was monitored and re-adjusted if necessary throughout the incubation period by weighing the sampled vessels.

Since BF 500-4 showed instability in the soil extracts, treatment and incubation for the 1 d sampling had to be repeated for all soils. Also a 0 hour sampling (one replicate per soil) was repeated, because BF 500-4 amounted to < 80% TAR in some of the extracts. This treatment was done using non-treated spare vessels still kept in the incubator.

2. Sampling

Duplicate samples were taken at 0 and 2 hours and 1, 3, 7, 14 and 28 days after treatment (DAT). Except for 0 and 2 hours, the volatile trapping solutions were sampled and replaced by new flasks with fresh solutions.

3. Description of the analytical procedures

Several pre-tests were performed to optimize the extraction procedure. On the basis of the results in these pre-tests, an extraction procedure consisting of three steps of acetonitrile and one step of acetonitrile/water (50/50, v/v) was selected as extraction procedure for the main part of the study, since this procedure resulted in a high extraction yield of 92.9% TAR.

Therefore, the soil was consecutively extracted three times on a laboratory shaker for about 20 min, first with about 150 mL ACN, followed by twofold extraction with about 100 mL ACN and finally with about 100 mL of ACN/H_2O (50/50, v/v).

After each extraction step, the suspension was centrifuged, the supernatant was filtered and aliquots of each solution were radio-assayed. Since BF 500-4 showed some instability in soil extracts, the first ACN extract of the 0 – 24 h samples, which contained the majority of radioactivity, was immediately prepared for HPLC analysis. It was concentrated by rotary evaporation (temp. ≤ 40°C), redissolved in ACN and subjected to HPLC. The second and third ACN extract, which contained much less radioactivity, were combined, concentrated and analyzed by HPLC. For the 3 - 28 day samples, showing already a significantly reduced extractability, all three ACN extracts were combined, concentrated and analyzed by HPLC. Also the ACN/H_2O extracts were concentrated and subjected to HPLC analysis.

The procedural recoveries after concentration were checked by LSC. The recoveries were always ≥ 90% for the first ACN extract, and ≥ 79% for the pooled ACN extracts. The recovery for the ACN/H_2O extracts after concentration and redissolving was partially below 50%, however, this is considered negligible since the total amount of radioactivity never exceeded 3.4% in this fraction.

The extracted soil was dried at room temperature, homogenized in an analytical mill and aliquots were combusted to determine the amount of non-extractable radioactive residues (NER). Combustion products were trapped in an Oxysolve C-400 scintillator and measured by LSC.

The solutions from the volatile traps (0.5 M NaOH, ethylene glycol and 0.5 M H_2SO_4) were subjected to LSC measurement.

4. Calculation of the degradation rate of BF 500-4

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] in order to derive trigger and modeling endpoints. The software package KinGUII (version 2.2012.320.1629) was used for parameter fitting.

For each data set, the kinetic models proposed by the FOCUS Kinetic guidance document were tested in order to identify the best-fit model, i.e. single first order kinetics (SFO), the Gustafson-Holden model (FOMC) and bi-exponential kinetics (DFOP).

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures recommended by the FOCUS guidance. The goodness-of-fit used for the identification of the best-fit kinetic model is the χ^2 minimum error level. The reliability of individual parameters was judged by means of a single-sided t-test.

The initial concentration of the applied substance was set to the material balance recovered at day 0.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The detailed results on the extractability of radioactive residues in the soils are presented in Table 7.1.2.1.2-16 to Table 7.1.2.1.2-18. The material balance throughout the incubation period ranged from 85.9 to 95.2% TAR for soil LUFA 5M, from 88.3 to 94.3% TAR for soil Li10 and from 89.1 to 98.1% TAR for soil LUFA 2.2. The average material balance for all soil samples was 91.6% TAR for soil Li10, 91.2% TAR for soil LUFA 5M and 93.9% TAR for soil LUFA 2.2.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues, non-extractable residues (NER), CO_2 and other volatiles for each soil is shown in Table 7.1.2.1.2-16 to Table 7.1.2.1.2-18. Considering all three soils, the amount of extractable radioactivity decreased quickly already during the first day. From a level of 89.4 to 93.1% TAR at hour 0, it decreased to 58.7 to 63.6% TAR after 1 day and then decreased continuously further to 16.3 to 24.0% TAR after 28 days of incubation.

The amount of NER increased during the study from 2.1 - 3.3% TAR at hour 0 to 63.0 - 67.3% TAR after 28 days of incubation.

Table 7.1.2.1.2-16: Distribution of radioactivity and material balance after application of ^{14}C-BF 500-4 to soil LUFA 5M [%TAR]

time after treatment	extractable residues					NER	volatiles*			material balance
	ACN 1	ACN 2	ACN 3	ACN/ H_2O	total		NaOH	ethylene glycol	total	
0 h	77.3	10.6	2.7	1.3	91.9	2.1	–	–	–	94.0
0 h	77.4	10.9	2.8	1.2	92.3	2.1	–	–	–	94.4
0 h	80.3	10.8	2.7	1.3	95.1	2.2	–	–	–	97.2
0 h (mean)	**78.3**	**10.8**	**2.7**	**1.3**	**93.1**	**2.1**	**–**	**–**	**–**	**95.2**
2 h	67.9	9.7	2.5	2.2	82.3	8.0	–	–	–	90.3
2 h	69.5	10.5	2.6	2.1	84.7	8.2	–	–	–	92.9
2 h (mean)	**68.7**	**10.1**	**2.5**	**2.2**	**83.5**	**8.1**	**–**	**–**	**–**	**91.6**
24 h	45.8	8.9	2.5	2.9	60.1	26.9	0.1	0.0	0.1	87.1
24 h	45.3	7.2	2.1	2.7	57.3	27.2	0.1	0.0	0.1	84.7
24 h (mean)	**45.5**	**8.1**	**2.3**	**2.8**	**58.7**	**27.1**	**0.1**	**0.0**	**0.1**	**85.9**
3 d	36.4	7.2	2.2	3.3	49.1	43.6	0.1	0.0	0.1	92.8
3 d	35.8	7.4	2.0	3.2	48.3	41.3	1.9	0.0	1.9	91.6
3 d (mean)	**36.1**	**7.3**	**2.1**	**3.2**	**48.7**	**42.5**	**1.0**	**0.0**	**1.0**	**92.2**
7 d	22.0	3.8	1.3	3.4	30.4	58.4	2.3	0.0	2.3	91.1
7 d	22.1	3.7	1.3	3.4	30.6	57.4	3.8	0.0	3.8	91.7
7 d (mean)	**22.0**	**3.7**	**1.3**	**3.4**	**30.5**	**57.9**	**3.0**	**0.0**	**3.0**	**91.4**
14 d	16.5	3.0	0.9	3.2	23.5	64.3	4.1	0.0	4.1	91.9
14 d	16.6	3.2	0.9	3.2	24.0	65.4	5.7	0.0	5.7	95.1
14 d (mean)	**16.6**	**3.1**	**0.9**	**3.2**	**23.8**	**64.8**	**4.9**	**0.0**	**4.9**	**93.5**
28 d	11.2	1.9	0.5	2.3	15.8	66.8	4.5	0.0	4.5	87.2
28 d	11.8	2.0	0.6	2.4	16.8	67.7	8.0	0.0	8.0	92.5
28 d (mean)	**11.5**	**1.9**	**0.5**	**2.4**	**16.3**	**67.3**	**6.3**	**0.0**	**6.3**	**89.8**

ACN = acetonitrile
NER = non-extractable residues
NaOH = sodium hydroxide
* no radioactivity residues detected in H_2SO_4 trap
100% TAR = 0.290 mg kg^{-1}

Table 7.1.2.1.2-17: Distribution of radioactivity and material balance after application of ^{14}C-BF 500-4 to soil Li10 [%TAR]

time after treatment	extractable residues					NER	volatiles*			material balance
	ACN 1	ACN 2	ACN 3	ACN/ H_2O	total		NaOH	ethylene glycol	total	
0 h	78.0	10.9	2.2	1.0	92.0	2.0	–	–	–	94.0
0 h	77.5	10.9	2.3	1.1	91.8	2.7	–	–	–	94.5
0 h	78.6	10.8	2.2	1.1	92.7	1.9	–	–	–	94.5
0 h (mean)	**78.0**	**10.9**	**2.2**	**1.1**	**92.2**	**2.2**	**–**	**–**	**–**	**94.3**
2 h	68.7	11.0	2.4	2.3	84.4	8.4	–	–	–	92.8
2 h	69.6	10.9	2.5	2.3	85.3	8.9	–	–	–	94.1
2 h (mean)	**69.1**	**11.0**	**2.4**	**2.3**	**84.8**	**8.6**	**–**	**–**	**–**	**93.5**
24 h	49.5	9.1	2.3	3.3	64.1	26.8	0.0	–	0.0	90.9
24 h	48.1	9.4	2.3	3.3	63.1	28.7	0.0	–	0.0	91.8
24 h (mean)	**48.8**	**9.2**	**2.3**	**3.3**	**63.6**	**27.7**	**0.0**	**–**	**0.0**	**91.3**
3 d	32.8	6.7	1.9	3.2	44.6	47.1	0.8	0.0	0.8	92.6
3 d	31.7	6.9	1.9	3.2	43.7	46.6	0.0	0.0	0.0	90.4
3 d (mean)	**32.3**	**6.8**	**1.9**	**3.2**	**44.2**	**46.9**	**0.4**	**0.0**	**0.4**	**91.5**
7 d	25.4	4.5	1.3	3.3	34.5	53.5	1.7	0.0	1.7	89.7
7 d	24.0	4.3	1.3	3.2	32.9	54.5	1.8	0.0	1.8	89.2
7 d (mean)	**24.7**	**4.4**	**1.3**	**3.3**	**33.7**	**54.0**	**1.8**	**0.0**	**1.8**	**89.5**
14 d	18.4	3.8	1.0	2.9	26.1	57.8	3.1	0.0	3.1	87.0
14 d	18.6	3.7	1.0	3.0	26.3	60.0	3.2	0.0	3.2	89.5
14 d (mean)	**18.5**	**3.7**	**1.0**	**3.0**	**26.2**	**58.9**	**3.2**	**0.0**	**3.2**	**88.3**
28 d	13.5	2.7	0.6	2.5	19.3	71.0	5.1	0.0	5.1	95.4
28 d	12.2	2.3	0.6	2.1	17.2	59.1	5.1	0.0	5.1	81.4
28 d (mean)	**12.8**	**2.5**	**0.6**	**2.3**	**18.3**	**65.1**	**5.1**	**0.0**	**5.1**	**88.4**

ACN = acetonitrile
NER = non-extractable residues
NaOH = sodium hydroxide
* no radioactivity residues detected in H_2SO_4 trap
100% TAR = 0.290 mg kg^{-1}

Table 7.1.2.1.2-18: Distribution of radioactivity and material balance after application of ^{14}C-BF 500-4 to soil LUFA 2.2 [%TAR]

time after treatment	extractable residues					NER	volatiles*			material balance
	ACN 1	ACN 2	ACN 3	ACN/ H_2O	total		NaOH	ethylene glycol	total	
0 h	74.5	12.4	3.0	1.6	91.5	3.2	–	–	–	94.6
0 h	72.4	12.1	2.9	1.5	88.9	3.3	–	–	–	92.2
0 h	72.4	10.8	3.1	1.6	87.9	3.3	–	–	–	91.2
0 h (mean)	**73.1**	**11.8**	**3.0**	**1.6**	**89.4**	**3.3**	**–**	**–**	**–**	**92.7**
2 h	67.6	11.0	2.6	2.1	83.3	10.4	–	–	–	93.8
2 h	65.3	10.6	2.7	2.1	80.6	10.0	–	–	–	90.6
2 h (mean)	**66.4**	**10.8**	**2.6**	**2.1**	**82.0**	**10.2**	**–**	**–**	**–**	**92.2**
24 h	46.3	9.5	2.5	2.9	61.3	27.0	0.6	0.0	0.6	88.9
24 h	48.4	9.5	2.4	2.7	63.0	25.6	0.5	0.0	0.5	89.2
24 h (mean)	**47.4**	**9.5**	**2.4**	**2.8**	**62.1**	**26.3**	**0.6**	**0.0**	**0.6**	**89.1**
3 d	42.0	8.4	2.3	2.8	55.6	38.3	1.4	0.0	1.4	95.3
3 d	44.8	8.9	2.5	3.1	59.3	36.7	1.4	0.0	1.4	97.4
3 d (mean)	**43.4**	**8.7**	**2.4**	**3.0**	**57.4**	**37.5**	**1.4**	**0.0**	**1.4**	**96.4**
7 d	36.8	6.9	1.9	3.2	48.7	47.4	2.9	0.0	2.9	99.0
7 d	34.3	6.4	1.7	3.0	45.4	49.1	2.7	0.0	2.7	97.2
7 d (mean)	**35.5**	**6.6**	**1.8**	**3.1**	**47.1**	**48.2**	**2.8**	**0.0**	**2.8**	**98.1**
14 d	25.6	6.6	2.5	1.5	36.2	54.1	5.1	0.0	5.1	95.4
14 d	25.3	6.5	2.5	1.5	35.8	56.6	4.9	0.0	4.9	97.2
14 d (mean)	**25.4**	**6.5**	**2.5**	**1.5**	**36.0**	**55.3**	**5.0**	**0.0**	**5.0**	**96.3**
28 d	15.8	3.0	0.7	2.3	21.8	64.7	5.1	0.0	5.1	91.6
28 d	19.1	3.5	0.9	2.7	26.1	61.3	7.2	0.0	7.2	94.6
28 d (mean)	**17.5**	**3.2**	**0.8**	**2.5**	**24.0**	**63.0**	**6.1**	**0.0**	**6.1**	**93.1**

ACN = acetonitrile
NER = non-extractable residues
NaOH = sodium hydroxide
* no radioactivity residues detected in H_2SO_4 trap
100% TAR = 0.290 mg kg^{-1}

C. VOLATILIZATION

The formation of volatiles was exclusively related to the mineralization to $^{14}CO_2$, reaching a total of 5.1 to 6.3% TAR after 28 days of incubation. In ethylene glycol traps as well as in H_2SO_4 traps no radioactive residues were found.

D. TRANSFORMATION OF BF 500-4

All soil extracts were analyzed by radio-HPLC. The summarized results are presented in Table 7.1.2.1.2-19 to Table 7.1.2.1.2-21.

Considering all three soils, the concentration of the test item BF 500-4 decreased quickly already during the first day. From a level of 79.0 to 83.5% TAR at hour 0, it decreased to 35.3 to 51.7% TAR after 1 day and then decreased continuously further to 1.2 to 3.0% TAR after 28 days of incubation.

The known metabolites BF 500-6 as well as BF 500-7 were detected in all three soils, reaching maximum values of 8.0% TAR (BF 500-6) and 2.2% TAR (BF 500-7), respectively, in soil LUFA 5M.

Numerous unknown metabolites were formed over time, however, most of them in amounts < 2% TAR. Three unknown metabolites eluting at approximately 16, 33, and 40 min reached maximum amounts of 9.9, 8.6, and 5.8% TAR, respectively. The peaks with retention times 33 and 40 min were already present at low amounts in the application solution. None of the peaks were persistent and since all these compounds represent secondary metabolites, which can appear in the environment only if BF 500-4 is formed out of the active ingredient pyraclostrobin under anaerobic conditions, it is very unlikely that they will ever reach environmentally significant amounts.

Table 7.1.2.1.2-19: Summary results of radio-HPLC analysis of extracts of soil LUFA 5M after application of ^{14}C-BF 500-4 [%TAR]

time after treatment	replicate number	total extractable	unknown	unknown	unknown	BF 500-4	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	BF 500-6	BF 500-7	others*
		tRet ~ [min]	26.7 28.9	31.1 33.6	32.2 33.8	34.6 36.0	36.6 37.9	39.5 40.5	40.1 41.1	40.6 41.8	41.0 42.1	42.2 43.3	43.3 44.2	44.4 45.5	46.5 47.3	47.7 48.2	48.0 48.6	
0 h	1	91.9	0.1		0.6	82.2	1.9	0.2	3.5		0.6		0.8			0.1		1.9
	3**	95.1	0.5		2.2	84.9		2.0	2.2				1.1	0.4		0.3		1.6
	mean	**93.5**	**0.3**		**1.4**	**83.5**	**1.0**	**1.1**	**2.9**		**0.3**		**0.9**	**0.2**		**0.2**		**1.7**
2 h	1	82.3	2.0	0.4	3.1	62.9	2.8	2.2	1.5	0.3	1.0	0.7	1.7					3.7
	2	84.7	3.1		5.3	58.9	4.8	2.6			2.2	1.5	3.3	1.9				1.1
	mean	**83.5**	**2.5**	**0.2**	**4.2**	**60.9**	**3.8**	**2.4**	**0.8**	**0.1**	**1.6**	**1.1**	**2.5**	**0.9**				**2.4**
24 h	1	60.1	1.1	1.7	4.3	38.1	1.5	1.0			0.1	0.1	1.2	0.2		5.5		5.3
	2	57.3	0.8	2.7	4.5	32.5	0.3	3.6		0.2	1.4		1.6	0.1	0.1	3.9	0.1	5.7
	mean	**58.7**	**1.0**	**2.2**	**4.4**	**35.3**	**0.9**	**2.3**		**0.1**	**0.7**	**0.1**	**1.4**	**0.2**	**0.0**	**4.7**	**0.1**	**5.5**
3 d	1	49.1		2.5	3.9	15.1	1.6	5.0		1.2		2.0	2.7	2.2	0.3	6.3	4.2	2.0
	2	48.3		1.6	2.9	8.8	2.2	3.0		2.1		0.3	1.9	2.6	3.1	9.7	0.1	10.1
	mean	**48.7**		**2.1**	**3.4**	**11.9**	**1.9**	**4.0**		**1.6**		**1.2**	**2.3**	**2.4**	**1.7**	**8.0**	**2.2**	**6.1**
7 d	1	30.4		1.8	1.0	6.1	0.3	4.7		0.6	0.6	0.1	0.2		0.2	7.2	0.2	7.2
	2	30.6		1.8	0.8	6.3	0.6	5.7				0.2	0.9	0.1		5.5	2.7	5.9
	mean	**30.5**		**1.8**	**0.9**	**6.2**	**0.5**	**5.2**		**0.3**	**0.3**	**0.2**	**0.6**	**0.0**	**0.1**	**6.4**	**1.5**	**6.5**
14 d	1	23.5	0.2	1.1	0.5	4.3		6.2		0.6				0.1		6.6		3.9
	2	24.0		1.3	1.1	2.8		5.4		0.1			0.1	0.8	0.5	5.7	0.1	6.2
	mean	**23.8**	**0.1**	**1.2**	**0.8**	**3.5**		**5.8**		**0.4**			**0.0**	**0.5**	**0.2**	**6.1**	**0.0**	**5.0**
28 d	1	15.8		0.6	0.4	1.2		5.3		0.1				0.1	0.1	4.7	0.1	3.4
	2	16.8	0.1	0.6	0.4	1.3		5.7						0.1		4.3		4.2
	mean	**16.3**	**0.1**	**0.6**	**0.4**	**1.2**		**5.5**		**0.1**				**0.1**	**0.0**	**4.5**	**0.0**	**3.8**

* sum of several peaks, each single peak < 2% TAR
** HPLC result of second replicate considered as outlier; test item amounted to < 50% TAR
tRet = retention time (listed for both HPLC instruments used)
TAR = total applied radioactivity

Table 7.1.2.1.2-20: Summary results of radio-HPLC analysis of extracts of soil Li10 after application of ^{14}C-BF 500-4 [%TAR]

time after treatment	replicate number	total extractable	unknown	unknown	unknown	unknown	unknown	**BF 500-4**	unknown	unknown	unknown	unknown	**BF 500-6**	**BF 500-7**	others*
		tRet ~ [min]	16.1	19.1 21.0	26.7 28.9	31.3 32.7	32.1 33.6	34.6 36.0	36.6 37.9	39.5 40.5	40.6 41.8	43.3 44.2	47.8 48.6	48.3 49.4	
0 h	1	92.0		6.9	0.3	0.9	0.5	78.1	0.9	0.1	0.5	2.3			1.6
	2	91.8	2.3	0.5	1.4	0.6	2.3	79.2	1.2	0.2	0.7	1.1			2.3
	3	92.7	2.2		0.6		0.8	82.0	0.0	1.0	0.9	2.0			3.1
	mean	**92.2**	**1.5**	**2.5**	**0.8**	**0.5**	**1.2**	**79.7**	**0.7**	**0.4**	**0.7**	**1.8**			**2.3**
2 h	1	84.4	5.3	0.4	1.3	0.7	0.9	69.1	0.3	0.6	1.7	1.6			2.4
	2	85.3	14.4		2.3	4.4	3.1	45.2	5.2	1.4	2.5	1.2			5.5
	mean	**84.8**	**9.9**	**0.2**	**1.8**	**2.6**	**2.0**	**57.2**	**2.7**	**1.0**	**2.1**	**1.4**			**4.0**
24 h	1	64.1	2.9	0.0	2.0	1.8	2.6	48.7	0.3	0.4	0.2	0.1	2.6	1.0	1.3
	2	63.1	4.0		2.0	3.9	2.8	37.6	1.1	1.7	1.4		2.1	0.7	5.8
	mean	**63.6**	**3.5**	**0.0**	**2.0**	**2.9**	**2.7**	**43.2**	**0.7**	**1.1**	**0.8**	**0.1**	**2.4**	**0.8**	**3.6**
3 d	1	44.6		0.2		1.7	5.3	24.4	0.9	2.9	0.3		4.5		4.5
	2	43.7		0.2		1.9	6.5	26.1	0.5	3.1	0.4		3.9		1.2
	mean	**44.2**		**0.2**		**1.8**	**5.9**	**25.2**	**0.7**	**3.0**	**0.4**		**4.2**		**2.8**
7 d	1	34.5				1.2	2.4	20.6	0.3	2.7	0.3		3.5	0.1	3.6
	2	32.9				1.6	2.8	19.1		3.1			4.0	0.1	2.1
	mean	**33.7**				**1.4**	**2.6**	**19.8**	**0.2**	**2.9**	**0.1**		**3.7**	**0.1**	**2.8**
14 d	1	26.1				1.2	1.1	11.5	0.1	3.7	0.7		4.2		3.6
	2	26.3			0.1	1.3	1.4	11.4		3.4	0.3		3.4		5.0
	mean	**26.2**			**0.0**	**1.3**	**1.3**	**11.5**	**0.1**	**3.5**	**0.5**		**3.8**		**4.3**
28 d	1	19.3			0.4	2.0	2.1	3.3	0.2	5.4	0.3		2.6	0.1	2.9
	2	17.2			0.4	1.8	1.7	2.6	0.5	5.0	0.4	0.2	2.6	0.1	2.2
	mean	**18.3**			**0.4**	**1.9**	**1.9**	**2.9**	**0.4**	**5.2**	**0.4**	**0.1**	**2.6**	**0.1**	**2.5**

* sum of several peaks, each single peak < 2% TAR
tRet = retention time (listed for both HPLC instruments used)
TAR = total applied radioactivity

Table 7.1.2.1.2-21: Summary results of radio-HPLC analysis of extracts of soil LUFA 2.2 after application of ^{14}C-BF 500-4 [%TAR]

time after treatment	replicate number	total extractable	unknown	unknown	unknown	unknown	BF 500-4	unknown	unknown	unknown	unknown	BF 500-6	BF 500-7	others*
		tRet ~ [min]	20.6	28.8 30.5	31.0 32.7	32.2 33.9	34.6 36.0	36.6 37.9	39.5 40.5	40.6 41.8	43.3 44.2	48.0 48.6	49.5 49.7	
0 h	1**	91.5	2.1	0.2	1.6		79.0	0.8	0.6	2.0	2.8			2.5
	mean	**91.5**	**2.1**	**0.2**	**1.6**		**79.0**	**0.8**	**0.6**	**2.0**	**2.8**			**2.5**
2 h	1	83.3		1.3	1.2	0.2	74.9		1.4		0.6			3.8
	2	80.6		0.6	1.6		71.5	0.8	2.3		0.1			3.9
	mean	**82.0**		**0.9**	**1.4**	**0.1**	**73.2**	**0.4**	**1.8**		**0.4**			**3.9**
24 h	1	61.3		0.7	0.8		51.9	0.3	1.1	0.1		0.1		6.2
	2	63.0		0.4	1.8		51.6	0.9	1.3	0.1	0.2	0.1		6.6
	mean	**62.1**		**0.6**	**1.3**		**51.7**	**0.6**	**1.2**	**0.1**	**0.1**	**0.1**		**6.4**
3 d	1	55.6		1.6	0.4	8.0	40.4	0.4	1.7	1.2		0.1		1.7
	2	59.3	0.2	2.3	4.2	9.2	39.7	0.3	2.1	0.3				1.2
	mean	**57.4**	**0.1**	**1.9**	**2.3**	**8.6**	**40.0**	**0.3**	**1.9**	**0.7**		**0.1**		**1.5**
7 d	1	48.7		1.6	2.8	5.9	21.1	3.1	2.0	1.2		4.5		6.6
	2	45.4	1.5	1.9	2.1	5.1	16.6	2.1	2.8	3.1		3.2		7.1
	mean	**47.1**	**0.8**	**1.7**	**2.5**	**5.5**	**18.8**	**2.6**	**2.4**	**2.1**		**3.8**		**6.8**
14 d	1	36.2	0.1	1.3	3.4	3.5	3.3	1.9	3.1	8.3		1.2	0.6	9.4
	2	35.8		1.9	1.4	4.0	17.8	0.7	2.0	1.5	0.1	1.3		5.1
	mean	**36.0**	**0.1**	**1.6**	**2.4**	**3.7**	**10.6**	**1.3**	**2.5**	**4.9**	**0.1**	**1.3**	**0.3**	**7.2**
28 d	1	21.8			1.3	2.3	2.3	1.3	2.0	0.8		1.8	0.2	9.8
	2	26.1			1.3	3.0	3.7	1.0	2.1	1.3		1.9		12.0
	mean	**24.0**			**1.3**	**2.6**	**3.0**	**1.1**	**2.0**	**1.0**		**1.9**	**0.1**	**10.9**

* sum of several peaks, each single peak < 2% TAR
** HPLC result of other replicates considered as outlier; test item amounted to < 50% TAR
tRet = retention time (listed for both HPLC instruments used)
TAR = total applied radioactivity

Calculation of the degradation rates

The soil residues for LUFA 5M, Li10, and LUFA 2.2 could be best described by the DFOP kinetic fit approach. The calculated best-fit DT_{50}/DT_{90} values and the selected kinetic models are presented in Table 7.1.2.1.2-22, while modeling endpoints are summarized in Table 7.1.2.1.2-23.

Table 7.1.2.1.2-22: Best-fit kinetic DT_{50} and DT_{90} values of metabolite BF 500-4

Soil	DT_{50} [d]	DT_{90} [d]	Kinetic model	χ^2 error
LUFA 5M	0.3	3.9	DFOP	6.8
Li10	0.2	13.5	DFOP	7.6
LUFA 2.2	1.8	13.0	DFOP	3.9

Table 7.1.2.1.2-23: Modeling endpoints for BF 500-4

Soil	Kinetic model	χ^2 error	Modeling $DegT_{50}$ [d]
Soil Lufa 5M	FOMC	13.8	2.2*
Soil Li 10	DFOP	7.6	6.1**
Soil Lufa 2.2	SFO	14.7	2.8

* calculated as $DegT_{90}$ FOMC / 3.32 according to FOCUS (2006)
** calculated as $\ln(2)/k_2$ according to FOCUS (2006)

III. CONCLUSION

BF 500-4, metabolite of pyraclostrobin, degraded fast in aerobic soil. After 28 days of incubation, only 1.2 - 3% TAR remained as unchanged test item in soil. The degradation was dominated by the formation of non-extractable residues. $^{14}CO_2$ was formed in low amounts (5.1 - 6.3% TAR, tolyl-^{14}C-label). No other volatile residues were detected. BF 500-4 is not persistent in soil and degrades with half-lives of 0.2 - 1.8 days. The DT_{90} values were calculated with 3.0 - 13.5 days.

Numerous metabolites were formed, most of them in amounts <2% TAR. Three unknown metabolites reached maximum amounts of 9.9, 8.6, and 5.8% TAR, respectively. Since the test item BF 500-4 occurred only under strict anaerobic soil conditions in detectable amounts, it can be concluded that these metabolites will not reach significant amounts under realistic agricultural conditions.

Normalization of degradation rates

Normalization to reference conditions (20°C, pF2) was performed as described for the parent (see paragraph after CA 7.1.1.1/1). A summary is provided below:

Table 7.1.2.1.2-24: Normalization of BF 500-4 $DegT_{50}$ values to reference conditions

Compound	Soil	pH ($CaCl_2$)	Kinetic Model	θ_{act}	θ_{ref}	f_{moist}	$DegT_{50,act}$	$DegT_{50,ref}$
BF 500-4	LUFA 5M	7.2	FOMC [1]	13.7	21.1	0.74	2.2	1.6
	Li 10	6.1	DFOP [2]	11.5	10.1	1	6.1	6.1
	LUFA 2.2	5.3	SFO	14.0	18.5	0.82	2.8	2.3

θ_{act} actual soil moisture [g / 100 g dry soil]
θ_{ref} reference soil moisture at field capacity (pF 2) according to FOCUS (2012) [g / 100 g dry soil]
f_{moist} moisture correction factor [-]
$DegT_{50,act}$ DT_{50} at study conditions [d]
$DegT_{50,ref}$ DT_{50} at reference conditions [d]
[1] calculated as $DegT_{50} = DegT_{90} / 3.32$
[2] calculated as $DegT_{50} = \ln(2) / k_{slow}$

Report: CA 7.1.2.1.2/4
Schoof S.,Possienke M., 2013a
Rate of degradation of BF 500-5 (Reg.No. 298327) in soil under aerobic conditions
2013/1294780
Guidelines: OECD 307 (2002), EPA 835.4100
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The objective of the present study was to investigate the rate of degradation of BF 500-5, a metabolite of pyraclostrobin, in three soils under aerobic conditions.

The soils were treated with ^{14}C-labeled BF 500-5 at a nominal rate of 0.27 mg kg^{-1} dry soil which corresponds to a field application rate of 100 g a.s. ha^{-1} assuming equal distribution in the top 2.5 cm of soil layer and a soil bulk density of 1.5 g cm^{-3}. The incubation was carried out under aerobic conditions in the dark at 20 ± 2°C and a soil moisture of 40% of the maximum water holding capacity. A closed incubation system with continuous aeration was used with an attached trapping system for the determination of volatile compounds. Duplicate samples were taken at 0, 2 and 5 hours and after 1, 2, 4, 7, 14, 29 and 56 days after treatment (DAT).

The soil samples were extracted four times with acetonitrile/water (80:20, v/v) and the individual extracts were analyzed by Liquid Scintillation Counting (LSC). Combined extracts were concentrated and analyzed by HPLC. The amount of non-extractable residues was determined by combustion and subsequent LSC measurements.

The mass balance throughout the study ranged from 86.9 - 108.7% total applied radioactivity (TAR) for the three soils. The amount of extractable radioactivity (ERR) decreased quickly from 94.4 - 97.3% TAR on hour 0 to 25.7 - 32.6% TAR 1 DAT and decreased further to 8.4 - 11.8% TAR after 56 days of incubation.

One unknown metabolite was present in the soil extracts with up to 17.6% TAR. The maxima were reached after 5 hours and decreased thereafter. Since this compound can be regarded as secondary metabolite, which can appear in the environment only if BF 500-5 is formed out of pyraclostrobin under anaerobic conditions, it is very unlikely that it will ever reach environmentally significant amounts and it was not investigated further. All other peaks in the chromatograms never exceeded 1.3% TAR at any sampling time.

Formation of CO_2 was negligible in all three soils reaching in total only 2.0% TAR after 56 days. In ethylene glycol up to 0.2% TAR were detected, while no radioactive residues were found in the H_2SO_4 traps. The non-extractable residues increased during the study from 2.7 - 5.6% TAR at hour 0 to 83.1 - 88.2% TAR after 56 days of incubation.

The kinetic analysis and calculation of dissipation times of BF 500-5 were performed according to the FOCUS kinetic workgroup using the software tool KinGUI (version 2). The DT_{50} values ranged from 0.09 to 0.24 days.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS code:	BF 500-5
Synonym:	500M04
Reg.No.:	298327
Chemical name (IUPAC):	1-(4-chlorophenyl)-1H-pyrazol-3-ol
Molecular weight:	194.6 g mol^{-1} (unlabeled)
Position of radiolabel:	Phenyl-ring-U-C^{14}
Specific radioactivity of a.s.:	8.99 MBq mg^{-1}
Batch No.:	724-2102
Radiochemical purity:	99.8% (according to certificate) > 98.0% (determined in the application solution by radio-HPLC prior to soil treatment in this study)

2. Soils

Three different soils were used for treatment and incubation. The soils were sieved through a 2 mm sieve before use, remoistened to approximately 8 – 12% of the respective soil moisture and stored in the dark at about 4°C. The soil characteristics are summarized in Table 7.1.2.1.2-25.

Table 7.1.2.1.2-25: Properties of soils used to investigate degradation rate of ^{14}C-BF 500-5

Soil designation	Li 10 (13/1680/01)	LUFA 5M (13/1651/01)	LUFA 2.2 (13/736/01)
Origin	Limburgerhof, RP, Germany	Mechtersheim, RP, Germany	Hanhofen, RP, Germany
DIN Particle size distribution [%]			
Sand 0.063 – 2 mm	80.8	53.6	68.4
Silt 0.002 – 0.063 mm	13.7	33.3	20.7
Clay < 0.002 mm	5.5	13.1	10.9
Textural class	Loamy sand Sl2	Loamy sand Sl4	Loamy sand Sl3
USDA Particle size distribution [%]			
Sand 0.050 – 2 mm	82.2	57.0	69.5
Silt 0.002 – 0.050 mm	12.3	29.9	19.6
Clay < 0.002 mm	5.5	13.1	10.9
Textural class	Loamy sand	Sandy loam	Sandy loam
Total organic carbon [%]	0.96	1.98	1.87
pH (H_2O)	6.8	7.9	6.1
pH ($CaCl_2$)	6.3	7.4	5.5
Cation exchange capacity [$cmol^+ kg^{-1}$]	2.9	10.2	7.8
Maximum water holding capacity [g/100g dry soil]	24.7	27.0	36.4
Microbial biomass* [mg C/100g dry soil]	40.0	27.8	55.9
Microbial biomass at 55 DAT [mg C/100g dry soil]	19.9	16.3	36.2

* data obtained before starting the study
DAT = days after treatment

B. STUDY DESIGN

1. Experimental conditions

The soil moisture was adjusted to about 40% of the maximum water holding capacity (MWHC) and treated with ^{14}C-labeled BF 500-5 at a nominal concentration of 0.27 mg kg^{-1} dry soil, which corresponds to a field application rate of 100 g a.s. ha^{-1}.

For soil treatment, approximately 2.0 kg of each soil (dry weight) was treated with 2.0 mL of the treatment solution. The treated soils were homogenized and soil portions of 100 g dry soil equivalents were filled into 20 test vessels. The number of vessels was sufficient to allow duplicate sampling at each sampling time and to keep a limited number of reserve vessels.

During the soil incubation, a rapid degradation of BF 500-5 was observed. Therefore, single test vessels (6 vessels per soil type) were prepared and applied separately with test item for the three soil types in order to investigate very early time points. For these applications, aliquots of the soil (100 g dry weight) were spiked with 0.1 mL of the application solution and homogenized by shaking.

The test vessels were incubated in the dark for up to 56 days at a temperature of 20°C. Throughout the incubation period, the test vessels were continuously aerated with a slight stream of moistened synthetic air. The outgoing air was led through a series of gas-washing bottles containing trapping solutions for potential volatiles (0.5M NaOH, ethylene glycol, 0.5M H_2SO_4). The water content of the soils was monitored and re-adjusted if necessary throughout the incubation period by weighing the sampled vessels.

For the short incubation series, the test vessels were incubated in the dark at 20°C for up to 5 hours. No volatile traps were used for the short time incubation.

For determination of the microbial biomass at the end of the incubation period (55 days), additional soil samples were treated with the application solution and incubated under the same conditions. However, no trapping system for volatiles was used.

2. Sampling

Duplicate samples were taken at 0, 2 and 5 hours and 1, 2, 4, 7, 14, 29 and 56 days after treatment (DAT).

3. Description of the analytical procedures

The extractability of the test item from soil was investigated for one soil using different solvent systems: threefold extraction with acetonitrile, acetonitrile/water (80:20, v/v) or methanol, respectively. The best performing solvent mixture was acetonitrile/water (80:20, v/v) which resulted in a total extractable amount of 94.4% TAR. For the solvent mixture acetonitrile/water (80:20, v/v) it was additionally tested how many extraction steps were needed for an exhaustive extraction. Four extractions were shown to be sufficient for an exhaustive extraction.

The soil samples were extracted consecutively four times with about 100 mL acetonitrile/water (80:20, v/v) by shaking for about 20 minutes. Two replicates of soil LUFA 5M from the short time series were extracted five times with acetonitrile/water (80:20, v/v) shortly after the application (0 hours after treatment). An additional 0.5% TAR was extracted by the 5th step confirming that four extraction steps are sufficient to extract the majority of radioactivity from the soil.

After each extraction step, the suspension was centrifuged, the supernatant was filtered and aliquots of each solution were radio-assayed. The extracts were combined, concentrated to a small volume and analyzed by LSC. After an additional concentration step, aliquots of the combined extracts were analyzed by HPLC to obtain the metabolite pattern.

The extracted soil was dried at room temperature, homogenized in an analytical mill and aliquots were combusted to determine the amount of non-extractable radioactive residues (NER). Combustion products were trapped in an Oxysolve C-400 scintillator and measured by LSC.

4. Calculation of the degradation rate of BF 500-5

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] in order to derive trigger and modeling endpoints. The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting.

For each data set, the kinetic models proposed by the FOCUS Kinetic guidance document were tested in order to identify the best-fit model, i.e. single first order kinetics (SFO), the Gustafson-Holden model (FOMC) and bi-exponential kinetics (DFOP).

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures recommended by the FOCUS guidance. The goodness-of-fit used for the identification of the best-fit kinetic model is the χ^2 minimum error level. The kinetic endpoints (DT_{50} and DT_{90} values) are reported for the kinetic models, which are selected based on statistical and visual assessment. The reliability of individual parameters was judged by means of a single-sided t-test.

The initial concentration of the applied substance was set to the material balance recovered at day 0 minus the percentage of ‘Unknown 1’ as the latter was an impurity in the application solution.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The detailed results on the extractability of radioactive residues in the soils are presented in Table 7.1.2.1.2-26 to Table 7.1.2.1.2-28. The average material balance ranged from 86.9 to 108.7% TAR for all soils.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (ERR), non-extractable residues (NER), CO_2 and other volatiles for each soil is shown in Table 7.1.2.1.2-26 to Table 7.1.2.1.2-28. The amount of extractable radioactivity (ERR) decreased quickly from 94.4 - 97.3% TAR at hour 0 to 25.7 - 32.6% TAR after 1 DAT and decreased further to 8.4 to 11.8% TAR after 56 days of incubation.

The amount of NER increased during the study from 2.7 - 5.6% TAR at hour 0 to 83.1 - 88.2% TAR after 56 days of incubation.

Table 7.1.2.1.2-26: Distribution of radioactivity and material balance after application of ^{14}C-BF 500-5 to soil Li 10 [%TAR]

Time after treatment	ERR					NER	Volatiles				Material balance
	ACN/ H_2O 1	ACN/ H_2O 2	ACN/ H_2O 3	ACN/ H_2O 4	Total		NaOH ($^{14}CO_2$)	Ethylene glycol	H_2SO_4	Total	
0 h	74.0	20.1	4.6	1.2	99.9	2.9	n.d.	n.d.	n.d.	n.d.	102.8
0 h	70.7	18.6	4.3	1.0	94.7	2.5	n.d.	n.d.	n.d.	n.d.	97.2
0 h (mean)	72.4	19.3	4.5	1.1	97.3	2.7	n.d.	n.d.	n.d.	n.d.	100.0
2 h	52.1	15.8	4.5	1.1	73.5	17.6	n.d.	n.d.	n.d.	n.d.	91.1
2 h	46.9	14.5	4.0	1.1	66.5	16.3	n.d.	n.d.	n.d.	n.d.	82.8
2 h (mean)	49.5	15.1	4.2	1.1	70.0	17.0	n.d.	n.d.	n.d.	n.d.	86.9
5 h	39.1	11.4	2.6	1.0	54.1	37.4	n.d.	n.d.	n.d.	n.d.	91.5
5 h	42.2	12.2	2.3	1.1	57.9	41.1	n.d.	n.d.	n.d.	n.d.	99.0
5 h (mean)	40.6	11.8	2.5	1.0	56.0	39.3	n.d.	n.d.	n.d.	n.d.	95.3
1 d	17.7	4.5	1.8	1.6	25.6	65.9	0.0	0.0	0.0	0.0	91.5
1 d	17.9	5.8	1.8	1.5	27.1	69.8	0.1	0.0	0.0	0.1	97.0
1 d (mean)	17.8	5.2	1.8	1.5	26.3	67.9	0.0	0.0	0.0	0.1	94.2
2 d	11.1	3.9	1.6	0.9	17.5	82.1	0.1	0.0	0.0	0.1	99.8
2 d	11.0	4.7	1.7	0.9	18.3	99.2	0.1	0.0	0.0	0.1	117.6
2 d (mean)	11.1	4.3	1.6	0.9	17.9	90.7	0.1	0.0	0.0	0.1	108.7
4 d	8.8	3.7	1.4	1.2	15.1	79.6	0.1	0.0	0.0	0.2	94.8
4 d	9.0	3.4	1.3	1.2	15.0	91.0	0.2	0.0	0.0	0.2	106.2
4 d (mean)	8.9	3.6	1.4	1.2	15.0	85.3	0.2	0.0	0.0	0.2	100.5
7 d	7.4	3.3	1.2	0.6	12.6	87.1	0.3	0.0	0.0	0.3	100.0
7 d	7.3	3.2	1.2	0.7	12.3	79.6	0.4	0.0	0.0	0.4	92.3
7 d (mean)	7.3	3.3	1.2	0.6	12.4	83.4	0.3	0.0	0.0	0.3	96.1
14 d	6.9	2.4	1.2	0.6	11.1	84.1	0.5	0.0	0.0	0.5	95.7
14 d	6.8	2.5	1.2	0.6	11.0	85.3	0.7	0.0	0.0	0.7	97.0
14 d (mean)	6.8	2.4	1.2	0.6	11.0	84.7	0.6	0.0	0.0	0.6	96.4
29 d	5.5	2.3	1.3	0.6	9.7	81.2	0.6	0.0	0.0	0.6	91.5
29 d	5.2	2.3	1.3	0.6	9.4	79.4	1.2	0.0	0.0	1.2	90.0
29 d (mean)	5.3	2.3	1.3	0.6	9.6	80.3	0.9	0.0	0.0	0.9	90.8
56 d	4.5	2.3	0.8	0.7	8.4	84.3	1.2	0.0	0.0	1.2	93.9
56 d	4.5	2.4	0.8	0.8	8.5	91.3	1.4	0.0	0.0	1.4	101.1
56 d (mean)	4.5	2.3	0.8	0.8	8.4	87.8	1.3	0.0	0.0	1.3	97.5

TAR = total applied radioactivity
ERR = extractable radioactive residues
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determined

Table 7.1.2.1.2-27: Distribution of radioactivity and material balance after application of ^{14}C-BF 500-5 to soil LUFA 5M [%TAR]

Time after treatment	ERR						NER	Volatiles				Material balance
	ACN/ H_2O 1	ACN/ H_2O 2	ACN/ H_2O 3	ACN/ H_2O 4	ACN/ H_2O 5	Total		NaOH ($^{14}CO_2$)	Ethylene glycol	H_2SO_4	Total	
0 h	72.4	16.0	6.2	1.1	0.5	96.2	5.1	n.d.	n.d.	n.d.	n.d.	101.3
0 h	70.1	17.0	4.4	1.3	0.5	93.2	5.5	n.d.	n.d.	n.d.	n.d.	98.7
0 h (mean)	71.2	16.5	5.3	1.2	0.5	94.7	5.3	n.d.	n.d.	n.d.	n.d.	100.0
2 h	48.5	10.8	4.0	1.4	n.d.	64.8	36.8	n.d.	n.d.	n.d.	n.d.	101.6
2 h	50.6	10.7	3.8	1.4	n.d.	66.4	34.5	n.d.	n.d.	n.d.	n.d.	100.9
2 h (mean)	49.6	10.7	3.9	1.4	n.d.	65.6	35.7	n.d.	n.d.	n.d.	n.d.	101.2
5 h	30.3	8.6	3.1	1.8	n.d.	43.8	66.5	n.d.	n.d.	n.d.	n.d.	110.2
5 h	33.4	9.2	3.1	1.8	n.d.	47.5	58.0	n.d.	n.d.	n.d.	n.d.	105.5
5 h (mean)	31.9	8.9	3.1	1.8	n.d.	45.6	62.2	n.d.	n.d.	n.d.	n.d.	107.9
1 d	21.5	7.9	2.3	1.1	n.d.	32.8	68.5	0.1	0.0	0.0	0.1	101.4
1 d	21.0	8.0	2.2	1.1	n.d.	32.4	68.8	0.1	0.0	0.0	0.1	99.3
1 d (mean)	21.3	8.0	2.3	1.1	n.d.	32.6	67.7	0.1	0.0	0.0	0.1	100.3
2 d	18.0	4.7	2.3	1.0	n.d.	26.2	78.1	0.1	0.0	0.0	0.1	104.3
2 d	17.1	4.6	2.8	1.1	n.d.	25.6	76.8	0.2	0.0	0.0	0.2	102.6
2 d (mean)	17.6	4.7	2.6	1.0	n.d.	25.9	77.4	0.1	0.0	0.0	0.1	103.5
4 d	15.2	4.4	2.2	0.7	n.d.	22.6	77.2	0.1	0.0	0.0	0.1	99.9
4 d	14.8	4.0	2.2	0.8	n.d.	21.8	73.9	0.3	0.0	0.0	0.3	96.0
4 d (mean)	15.0	4.2	2.2	0.8	n.d.	22.2	75.6	0.2	0.0	0.0	0.2	97.9
7 d	12.9	4.1	2.0	0.9	n.d.	19.8	78.7	0.1	0.0	0.0	0.1	98.6
7 d	12.3	3.8	2.0	0.8	n.d.	18.9	78.5	0.4	0.0	0.0	0.4	97.8
7 d (mean)	12.6	3.9	2.0	0.8	n.d.	19.4	78.6	0.2	0.0	0.0	0.3	98.2
14 d	8.5	4.0	1.3	0.7	n.d.	14.5	85.4	0.1	0.0	0.0	0.1	100.0
14 d	9.3	3.6	1.1	0.7	n.d.	14.5	80.5	0.4	0.0	0.0	0.5	95.6
14 d (mean)	8.9	3.8	1.2	0.7	n.d.	14.5	83.0	0.3	0.0	0.0	0.3	97.8
29 d	11.0	3.3	1.6	0.8	n.d.	16.8	80.8	0.2	0.0	0.0	0.2	97.8
29 d	7.3	3.0	1.8	0.9	n.d.	13.0	83.1	0.7	0.1	0.0	0.8	96.9
29 d (mean)	9.2	3.1	1.7	0.9	n.d.	14.9	82.0	0.4	0.1	0.0	0.5	97.3
56 d	6.4	2.6	1.1	0.5	n.d.	10.7	81.2	0.5	0.1	0.0	0.6	92.5
56 d	6.0	2.6	1.1	0.5	n.d.	10.2	85.0	0.7	0.1	0.0	0.8	96.1
56 d (mean)	6.2	2.6	1.1	0.5	n.d.	10.4	83.1	0.6	0.1	0.0	0.7	94.3

TAR = total applied radioactivity
ERR = extractable radioactive residues
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determined

Table 7.1.2.1.2-28: Distribution of radioactivity and material balance after application of ^{14}C-BF 500-5 to soil LUFA 2.2 [%TAR]

Time after treatment	ERR					NER	Volatiles				Material balance
	ACN/ H_2O 1	ACN/ H_2O 2	ACN/ H_2O 3	ACN/ H_2O 4	Total		NaOH ($^{14}CO_2$)	Ethylene glycol	H_2SO_4	Total	
0 h	70.1	17.6	5.2	1.5	94.4	5.9	n.d.	n.d.	n.d.	n.d.	100.3
0 h	69.5	18.2	5.1	1.7	94.4	5.3	n.d.	n.d.	n.d.	n.d.	99.7
0 h (mean)	69.8	17.9	5.1	1.6	94.4	5.6	n.d.	n.d.	n.d.	n.d.	100.0
2 h	58.5	15.1	5.6	1.6	80.7	18.3	n.d.	n.d.	n.d.	n.d.	99.0
2 h	55.6	16.3	5.5	1.5	78.9	17.1	n.d.	n.d.	n.d.	n.d.	96.0
2 h (mean)	57.0	15.7	5.6	1.6	79.8	17.7	n.d.	n.d.	n.d.	n.d.	97.5
5 h	49.3	14.3	5.0	1.6	70.2	29.8	n.d.	n.d.	n.d.	n.d.	100.0
5 h	46.3	14.2	4.7	1.5	66.7	29.3	n.d.	n.d.	n.d.	n.d.	96.0
5 h (mean)	47.8	14.3	4.8	1.6	68.4	29.5	n.d.	n.d.	n.d.	n.d.	98.0
1 d	17.4	6.6	2.3	1.2	27.5	74.1	0.3	0.1	0.0	0.4	102.0
1 d	14.2	6.8	1.8	1.1	23.9	74.4	0.1	0.1	0.0	0.2	98.5
1 d (mean)	15.8	6.7	2.0	1.2	25.7	74.3	0.2	0.1	0.0	0.3	100.3
2 d	12.5	4.3	2.3	0.8	19.8	81.7	0.5	0.2	0.0	0.6	102.1
2 d	11.6	4.3	2.3	1.0	19.2	80.6	0.3	0.1	0.0	0.4	100.2
2 d (mean)	12.0	4.3	2.3	0.9	19.5	81.1	0.4	0.1	0.0	0.5	101.2
4 d	11.5	3.7	1.9	0.9	18.1	83.3	0.7	0.2	0.0	0.9	102.3
4 d	11.2	3.7	1.7	0.8	17.3	83.9	0.6	0.1	0.0	0.7	101.9
4 d (mean)	11.4	3.7	1.8	0.8	17.7	83.6	0.7	0.1	0.0	0.8	102.1
7 d	10.4	3.8	1.8	0.8	16.7	88.2	1.0	0.2	0.0	1.2	106.2
7 d	9.3	3.3	1.8	0.8	15.2	83.7	1.0	0.1	0.0	1.1	100.0
7 d (mean)	9.8	3.5	1.8	0.8	16.0	86.0	1.0	0.1	0.0	1.1	103.1
14 d	8.1	3.3	1.3	0.7	13.4	87.3	1.1	0.2	0.0	1.3	102.0
14 d	7.9	3.5	1.3	0.5	13.1	86.8	1.3	0.1	0.0	1.3	101.2
14 d (mean)	8.0	3.4	1.3	0.6	13.3	87.0	1.2	0.1	0.0	1.3	101.6
29 d	7.8	3.1	1.5	0.6	13.1	87.9	1.3	0.2	0.0	1.5	102.5
29 d	7.8	3.0	1.3	0.6	12.8	85.5	1.3	0.1	0.0	1.5	99.8
29 d (mean)	7.8	3.1	1.4	0.6	12.9	86.7	1.3	0.2	0.0	1.5	101.1
56 d	6.7	3.4	1.2	0.6	11.9	88.8	1.8	0.3	0.0	2.0	102.7
56 d	6.8	3.3	1.2	0.6	11.8	87.6	2.2	0.1	0.0	2.4	101.8
56 d (mean)	6.7	3.4	1.2	0.6	11.8	88.2	2.0	0.2	0.0	2.2	102.2

TAR = total applied radioactivity
ERR = extractable radioactive residues
NER = non-extractable radioactive residues
ACN = acetonitrile
n.d. = not determined

C. VOLATILIZATION

Formation of CO_2 was negligible in all three soils reaching in total only 2.0% TAR after 56 days. In ethylene glycol up to 0.2% TAR were detected, while no radioactive residues were found in the H_2SO_4 traps.

D. TRANSFORMATION OF BF 500-5

All combined soil extracts were analyzed by radio-HPLC. The results obtained with this system are summarized in Table 7.1.2.1.2-29 to Table 7.1.2.1.2-31.

The concentration of BF 500-5 decreased very quickly during the first day from 89.1 - 94.8% TAR at hour 0 to 15.0 - 19.7% TAR at 1 DAT, and then continuously to 0.6 - 1.4% TAR after 56 days of incubation.

One unknown metabolite was present in the soil extracts with up to 17.6% TAR. A peak with similar retention time was already present in the application solution. The maxima were reached after 5 hours and decreased thereafter. Since this compound can be regarded as secondary metabolite, which can appear in the environment only if BF 500-5 is formed out of pyraclostrobin under anaerobic conditions, it is very unlikely that it will ever reach environmentally significant amounts and it was not further investigated. All other peaks in the chromatograms never exceeded 1.3% TAR at any sampling time.

Table 7.1.2.1.2-29: Summary results of radio-HPLC analysis of extracts of soil Li 10 after application of ^{14}C-BF 500-5 [%TAR]

Time after treatment	Total	BF 500-5 t_R ~ 46.8	Unknown 1 t_R ~ 53.8	Unknown 2 t_R ~ 56.6	Others*
0 h	99.9	97.3	2.6	-	-
0 h	94.7	92.3	2.3	-	-
0 h (mean)	**97.3**	**94.8**	**2.5**	**-**	**-**
2 h	73.5	69.1	4.4	-	-
2 h	66.5	63.1	3.4	-	-
2 h (mean)	**70.0**	**66.1**	**3.9**	**-**	**-**
5 h	54.1	48.2	5.9	-	-
5 h	57.9	52.0	5.8	-	-
5 h (mean)	**56.0**	**50.1**	**5.8**	**-**	**-**
1 d	25.6	19.0	3.0	0.5	3.1
1 d	27.1	20.5	3.3	0.5	2.8
1 d (mean)	**26.3**	**19.7**	**3.2**	**0.5**	**2.9**
2 d	17.5	10.3	2.9	0.8	3.5
2 d	18.3	10.7	3.1	0.7	3.8
2 d (mean)	**17.9**	**10.5**	**3.0**	**0.7**	**3.7**
4 d	15.1	6.8	3.3	0.7	4.2
4 d	15.0	6.8	3.4	0.7	4.1
4 d (mean)	**15.0**	**6.8**	**3.3**	**0.7**	**4.2**
7 d	12.6	4.4	3.2	0.8	4.2
7 d	12.3	4.3	2.9	0.6	4.5
7 d (mean)	**12.4**	**4.4**	**3.0**	**0.7**	**4.3**
14 d	11.1	2.9	2.7	0.6	4.9
14 d	11.0	2.8	3.0	0.6	4.6
14 d (mean)	**11.0**	**2.8**	**2.8**	**0.6**	**4.7**
29 d	9.7	1.8	2.6	0.5	4.8
29 d	9.4	1.8	2.5	0.4	4.6
29 d (mean)	**9.6**	**1.8**	**2.6**	**0.5**	**4.7**
56 d	8.4	1.5	2.2	0.9	3.7
56 d	8.5	1.3	2.2	1.0	3.9
56 d (mean)	**8.4**	**1.4**	**2.2**	**1.0**	**3.8**

TAR = total applied radioactivity
t_R = retention time [min]
* each single peak in extract < 1.0% TAR
- means "not detected"

Table 7.1.2.1.2-30: Summary results of radio-HPLC analysis of extracts of soil LUFA 5M after application of ^{14}C-BF 500-5 [%TAR]

Time after treatment	Total	BF 500-5 t_R ~ 46.8	Unknown 1 t_R ~ 53.8	Unknown 2 t_R ~ 56.6	Others*
0 h	96.2	90.8	5.5	-	-
0 h	93.2	87.5	5.8	-	-
0 h (mean)	**94.7**	**89.1**	**5.6**	**-**	**-**
2 h	64.8	51.3	12.6	-	0.8
2 h	66.4	52.5	12.9	-	1.0
2 h (mean)	**65.6**	**51.9**	**12.8**	**-**	**0.9**
5 h	43.8	26.1	16.1	0.2	1.4
5 h	47.5	26.7	19.2	0.3	1.3
5 h (mean)	**45.6**	**26.4**	**17.6**	**0.2**	**1.3**
1 d	32.8	19.0	10.1	0.3	3.4
1 d	32.4	19.2	9.4	0.5	3.3
1 d (mean)	**32.6**	**19.1**	**9.7**	**0.4**	**3.3**
2 d	26.2	11.1	10.8	0.8	3.5
2 d	25.6	11.4	10.1	0.6	3.5
2 d (mean)	**25.9**	**11.2**	**10.4**	**0.7**	**3.5**
4 d	22.6	6.2	10.7	1.1	4.5
4 d	21.8	6.8	9.9	0.9	4.2
4 d (mean)	**22.2**	**6.5**	**10.3**	**1.0**	**4.4**
7 d	19.8	3.2	10.6	0.5	5.5
7 d	18.9	3.4	9.8	0.5	5.3
7 d (mean)	**19.4**	**3.3**	**10.2**	**0.5**	**5.4**
14 d	14.5	1.4	7.6	0.4	5.0
14 d	14.5	1.2	7.0	0.4	6.0
14 d (mean)	**14.5**	**1.3**	**7.3**	**0.4**	**5.5**
29 d	16.8	1.8	10.4	0.7	3.9
29 d	13.0	1.2	7.6	0.8	3.4
29 d (mean)	**14.9**	**1.5**	**9.0**	**0.8**	**3.7**
56 d	10.7	0.6	5.8	0.5	3.7
56 d	10.2	0.6	4.7	0.6	4.3
56 d (mean)	**10.4**	**0.6**	**5.3**	**0.6**	**4.0**

TAR = total applied radioactivity
t_R = retention time [min]
* each single peak in extract < 1.0% TAR
- means "not detected"

Table 7.1.2.1.2-31: Summary results of radio-HPLC analysis of extracts of soil LUFA 2.2 after application of ^{14}C-BF 500-5 [%TAR]

Time after treatment	Total	BF 500-5 t_R ~ 46.8	Unknown 1 t_R ~ 53.8	Unknown 2 t_R ~ 56.6	Others*
0 h	94.4	89.8	4.6	-	-
0 h	94.4	89.4	5.0	-	-
0 h (mean)	**94.4**	**89.6**	**4.8**	**-**	**-**
2 h	80.7	70.2	10.0	-	0.5
2 h	78.9	68.8	9.8	-	0.4
2 h (mean)	**79.8**	**69.5**	**9.9**	**-**	**0.5**
5 h	70.2	55.1	14.3	0.4	0.4
5 h	66.7	52.6	13.1	0.3	0.7
5 h (mean)	**68.4**	**53.9**	**13.7**	**0.3**	**0.5**
1 d	27.5	15.7	7.7	0.5	3.5
1 d	23.9	14.2	6.7	0.4	2.6
1 d (mean)	**25.7**	**15.0**	**7.2**	**0.5**	**3.0**
2 d	19.8	7.9	7.6	0.7	3.5
2 d	19.2	7.6	8.0	0.7	2.9
2 d (mean)	**19.5**	**7.7**	**7.8**	**0.7**	**3.2**
4 d	18.1	4.4	8.2	0.9	4.5
4 d	17.3	4.6	8.2	0.8	3.7
4 d (mean)	**17.7**	**4.5**	**8.2**	**0.9**	**4.1**
7 d	16.7	3.1	7.5	0.9	5.2
7 d	15.2	2.8	6.8	0.7	4.9
7 d (mean)	**16.0**	**3.0**	**7.1**	**0.8**	**5.0**
14 d	13.4	2.0	6.5	0.6	4.3
14 d	13.1	1.9	6.4	0.7	4.2
14 d (mean)	**13.3**	**1.9**	**6.4**	**0.7**	**4.3**
29 d	13.1	1.5	5.9	1.1	4.5
29 d	12.8	1.7	6.7	1.1	3.4
29 d (mean)	**12.9**	**1.6**	**6.3**	**1.1**	**3.9**
56 d	11.9	1.3	5.0	1.3	4.3
56 d	11.8	0.9	5.1	1.3	4.5
56 d (mean)	**11.8**	**1.1**	**5.0**	**1.3**	**4.4**

TAR = total applied radioactivity
t_R = retention time [min]
* each single peak in extract < 1.0% TAR
- means "not detected"

Calculation of the degradation rates

The residue data of soils Li 10 and LUFA 2.2 could be best described by the FOMC kinetic model, while the residue data of soil LUFA 5M was best described by the DFOP kinetic model which further improved the statistical and visual fit for the measured data. The calculated $DegT_{50}$/$DegT_{90}$ values and the selected kinetic models are presented in Table 7.1.2.1.2-32 (best-fit) and Table 7.1.2.1.2-33 (modeling).

Table 7.1.2.1.2-32: Best-fit kinetic DT_{50} and DT_{90} values of metabolite BF 500-5

Soil	DT_{50} [d]	DT_{90} [d]	Kinetic model	χ^2 error
Li 10	0.20	2.63	FOMC	3.74
LUFA 5M	0.09	2.95	DFOP	4.79
LUFA 2.2	0.24	1.77	FOMC	5.01

Table 7.1.2.1.2-33: Modeling endpoints ($DegT_{50}$) for metabolite BF 500-5

Soil	Kinetic model	χ^2 error	Modeling $DegT_{50}$ [d]*
Li 10	FOMC	3.74	0.79
LUFA 5M	FOMC	10.61	0.63
LUFA 2.2	FOMC	5.01	0.53

* calculated as $DegT_{90}$ FOMC / 3.32 according to FOCUS (2006)

III. CONCLUSION

BF 500-5, a metabolite of pyraclostrobin, degraded rather fast in three different soils with DT_{50} values of <1 day. After 56 days of incubation, the mineralization rate had reached 0.7 - 2.2% TAR and the non-extractable residues amounted to 83.1 – 88.2% TAR.

Normalization of degradation rates

Normalization to reference conditions (20°C, pF2) was performed as described for the parent (see paragraph after CA 7.1.1.1/1). A summary is provided below:

Table 7.1.2.1.2-34: Normalization of BF 500-5 $DegT_{50}$ values to reference conditions

Compound	Soil	pH ($CaCl_2$)	Kinetic Model	θ_{act}	θ_{ref}	f_{moist}	$DegT_{50,act}$	$DegT_{50,ref}$
BF 500-5	Li 10	6.3	FOMC[1]	9.9	11.0	0.93	0.8	0.7
	LUFA 5M	7.4	FOMC[1]	10.8	17.4	0.72	0.6	0.5
	LUFA 2.2	5.5	FOMC[1]	14.6	23.1	0.72	0.5	0.4

θ_{act} actual soil moisture [g / 100 g dry soil]
θ_{ref} reference soil moisture at field capacity (pF 2) according to FOCUS (2012) [g / 100 g dry soil]
f_{moist} moisture correction factor [-]
$DegT_{50,act}$ DT_{50} at study conditions [d]
$DegT_{50,ref}$ DT_{50} at reference conditions [d]
[1] calculated as $DegT_{50} = DegT_{90} / 3.32$

Summary on metabolite occurrence and degradation rates in aerobic soil

Table 7.1.2.1.2-35: Maximum occurrence of pyraclostrobin metabolites in laboratory aerobic soil studies (20°C, 40-50% MWHC)

Metabolite	BASF DocID	Study	Parent label	Soil	Maximum % AR
BF 500-3	1998/11201 [2]	aerobic soil	tolyl (~6%) [1]	Bruch West	6.4 (day 0)
	1999/10090 [2]	aerobic soil	chlorophenyl	Bruch West	1.9 (day 0)
	1999/11091 [2]	aerobic soil	tolyl	LUFA 2.2 Li35b US 771-15 Canadian	1.5 0.6 0.7 0.7
	2013/1348620	aerobic soil	pyrazole	LUFA 5M	0
	2011/1102370	aerobic soil	tolyl (3.7%) [1]	Li 10 LUFA 5M Speyerer Wald 2	4.3 4.1 4.1
BF 500-4	not found in aerobic soil				
BF 500-5	1999/10090 [2]	aerobic soil	chlorophenyl	Bruch West	2.8
BF 500-6	1998/11201 [2]	aerobic soil	tolyl	Bruch West	12
	1999/10090 [2]	aerobic soil	chlorophenyl	Bruch West	16
	1999/11091 [2]	aerobic soil	tolyl	LUFA 2.2 Li35b US 771-15 Canadian	15 31 14 17
	2013/1348620	aerobic soil	pyrazole	LUFA 5M	17.3
	2011/1102370	aerobic soil	tolyl	Li 10 LUFA 5M Speyerer Wald 2	14.1 23.3 19.8
BF 500-7	1998/11201 [2]	aerobic soil	tolyl	Bruch West	6
	1999/10090 [2]	aerobic soil	chlorophenyl	Bruch West	7
	1999/11091 [2]	aerobic soil	tolyl	LUFA 2.2 Li35b US 771-15 Canadian	1 7 13 4
	2013/1348620	aerobic soil	pyrazole	LUFA 5M	5.5
	2011/1102370	aerobic soil	tolyl	Li 10 LUFA 5M Speyerer Wald 2	2.5 8.7 3.0

MWHC maximum water holding capacity
[1] percent of BF 500-3 present in pyraclostrobin treatment solution (as impurity)
[2] already peer-reviewed during former Annex I listing

Table 7.1.2.1.2-36: Persistence endpoints of pyraclostrobin metabolites in aerobic soil studies (laboratory, 20°C, 40-50% MWHC)

Metabolite	Data source BASF DocID	Test item (label)	Soil	Persistence DegT50/DegT90 [d]	Method of calculation
BF 500-3	2011/1102370 2014/1093424	parent (t) (3.7%) [1]	Li 10 LUFA 5M Speyerer Wald 2	not calculated [3] not calculated [3] not calculated [3]	- - -
BF 500-4	2013/1294779	BF 500-4 (t)	LUFA 5M Li 10 LUFA 2.2	0.3 / 3.9 0.2 / 13.5 1.8 / 13.0	DFOP DFOP DFOP
BF 500-5	2013/1294780	BF 500-5 (c)	Li 10 LUFA 5M LUFA 2.2	0.20 / 2.63 0.09 / 2.95 0.24 / 1.77	FOMC DFOP FOMC
BF 500-6	1998/11201 [2] 2014/1093424	parent (t)	Bruch West	971.2 / >1000	SFO [4]
	1999/10090 [2] 2014/1093424	parent (c)	Bruch West	not calculated [3]	-
	1999/11091 [2] 2014/1093424	parent (t)	LUFA 2.2 Li 35b US 771-15 Canadian	not calculated [3] not calculated [3] not calculated [3] not calculated [3]	- - - -
	2013/1348620	parent (p)	LUFA 5M	95.9 / 318.7	SFO [4]
	2011/1102370 2014/1093424	parent (t)	Li 10 LUFA 5M Speyerer Wald 2	not calculated [3] >1000 / >1000 not calculated [3]	SFO [5]
	2011/1142307	BF 500-6 (t)	Li 10 LUFA 5M Speyerer Wald 2	855 / >1000 921 / >1000 753 / >1000	SFO SFO SFO
BF 500-7	1998/11201 [2] 2014/1093424	parent (t)	Bruch West	649.1 / >1000	SFO [4]
	1999/10090 [2] 2014/1093424	parent (c)	Bruch West	not calculated [3]	-
	1999/11091 [2] 2014/1093424	parent (t)	LUFA 2.2 Li 35b US 771-15 Canadian	not calculated [3] not calculated [3] 111.2 / 369.3 not calculated [3]	- - SFO [5] -
	2013/1348620	parent (p)	LUFA 5M	81.5 / 270.7	SFO [4]
	2011/1102370 2014/1093424	parent (t)	Li 10 LUFA 5M Speyerer Wald 2	not calculated [3] 358.9 / >1000 not calculated [3]	SFO [5]
	2011/1142308	BF 500-7 (t)	Li 10 LUFA 5M Speyerer Wald 2	727 / >1000 >1000 / >1000 745 / >1000	SFO SFO SFO

MWHC maximum water holding capacity
(t), (c), (p) - tolyl, chlorophenyl, or pyrazole-labeled test item used
[1] percent of BF 500-3 present in pyraclostrobin treatment solution (as impurity)
[2] already peer-reviewed during former Annex I listing
[3] no reliable endpoints derived in kinetic evaluation
[4] FOMC kinetics for parent
[5] DFOP kinetics for parent

Table 7.1.2.1.2-37: Modeling endpoints for pyraclostrobin metabolites in aerobic soil studies (laboratory, 20°C, 40-50% MWHC)

Metabolite	Data source BASF DocID	Test item (label)	Soil	DegT50 at study conditions [d]	Method of calculation	DegT50 SFO / 20°C / pF2 [d]	Formation fraction
BF 500-3	2011/1102370 2014/1093424	parent (t) (3.7%) [1]	Li 10 LUFA 5M Speyerer Wald 2	not calculated [3] not calculated [3] not calculated [3]	- - -	- - -	- - -
BF 500-4	2013/1294779	BF 500-4 (t)	LUFA 5M Li 10 LUFA 2.2	2.2 6.1 2.8	FOMC [4] DFOP [5] SFO	1.6 6.1 2.3	- - -
BF 500-5	2013/1294780	BF 500-5 (c)	Li 10 LUFA 5M LUFA 2.2	0.8 0.6 0.5	FOMC [4] FOMC [4] FOMC [4]	0.7 0.5 0.4	- - -
BF 500-6	1998/11201 [2] 2014/1093424	parent (t)	Bruch West	971.2	SFO [6]	903.2	0.142
	1999/10090 [2] 2014/1093424	parent (c)	Bruch West	not calculated [3]	-	-	-
	1999/11091 [2] 2014/1093424	parent (t)	LUFA 2.2 Li 35b US 771-15 Canadian	124.9 not calculated [3] not calculated [3] not calculated [3]	SFO [7] - - -	124.9 - - -	0.388 0.371 - -
	2013/1348620	parent (p)	LUFA 5M	68.0	SFO [7]	59.2	0.318
	2011/1102370 2014/1093424	parent (t)	Li 10 LUFA 5M Speyerer Wald 2	not calculated [3] 870.4 251.0	- SFO [6] SFO [7]	- 557.9 245.4	- 0.261 0.319
	2011/1142307	BF 500-6 (t)	Li 10 LUFA 5M Speyerer Wald 2	855.0 921.0 753.0	SFO SFO SFO	822.1 590.3 736.3	- - -
BF 500-7	1998/11201 [2] 2014/1093424	parent (t)	Bruch West	649.1	SFO [6]	603.7	0.073
	1999/10090 [2] 2014/1093424	parent (c)	Bruch West	not calculated [3]	-	-	-
	1999/11091 [2] 2014/1093424	parent (t)	LUFA 2.2 Li 35b US 771-15 Canadian	not calculated [3] not calculated [3] not calculated [3] not calculated [3]	- - - -	- - - -	- - - -
	2013/1348620	parent (p)	LUFA 5M	59.0	SFO [7]	51.3	0.112
	2011/1102370 2014/1093424	parent (t)	Li 10 LUFA 5M Speyerer Wald 2	not calculated [3] 334.9 not calculated [3]	- SFO [6] -	- 214.7 -	- 0.108 -
	2011/1142308	BF 500-7 (t)	Li 10 LUFA 5M Speyerer Wald 2	727 1050 [8] 745	SFO SFO SFO	699.0 673.0 728.5	- - -

MWHC maximum water holding capacity
(t), (c), (p) - tolyl, chlorophenyl, or pyrazole-labeled test item used
[1] percent of BF 500-3 present in pyraclostrobin treatment solution (as impurity)
[2] already peer-reviewed during former Annex I listing
[3] no reliable endpoints derived in kinetic evaluation
[4] calculated from FOMC kinetics as $DT_{50} = DT_{90}/3.32$
[5] calculated from DFOP kinetics as $DT_{50} = \ln(2)/k_{slow}$
[6] FOMC kinetics for parent
7 SFO kinetics for parent
[8] Calculated from reported degradation rate as $DT_{50} = \ln 2 / k$

CA 7.1.2.1.3 Anaerobic degradation of the active substance

No new experimental data were generated.

Although the available studies were done according to the previous guideline on anaerobic soil metabolism (without an aerobic pre-incubation phase), the results are still considered valid, since aerobic and anaerobic degradation follow the same metabolic pathway (see route of degradation, Figure 7.1.1.3-1).

Since the kinetic evaluations provided in the old reports are outdated, new kinetic evaluations for pyraclostrobin and metabolites were performed according to current FOCUS guidelines.

Report:	CA 7.1.2.1.3/1 Pape L., 2014a Kinetic evaluation of anaerobic soil degradation studies for BAS 500 F - Pyraclostrobin according to FOCUS 2014/1000701
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP:	no

Note: This study was not correctly listed in the application submitted for renewal of approval. Author and annex point has changed after submission of the application.

Executive Summary

The degradation of pyraclostrobin (BAS 500 F) in soil has been investigated in two anaerobic laboratory soil degradation studies. The purpose of this evaluation was to analyze the degradation kinetics of pyraclostrobin and its metabolites BF 500-3, BF 500-4, BF 500-5, 500M74 and 500M75 according to the current guidance of the FOCUS workgroup on degradation kinetics.

The appropriate kinetic models were selected based on visual and statistical assessment. The kinetic evaluation of the data showed that the DFOP model is the best-fit model for pyraclostrobin, while SFO can be used to derive modeling endpoints. The best-fit $DegT_{50}$ values were 1.5 and 2.3 days, and $DegT_{90}$ values were 6.5 and 8.4 days. The modeling $DegT_{50}$ values equal 2.0 and 2.4 days.

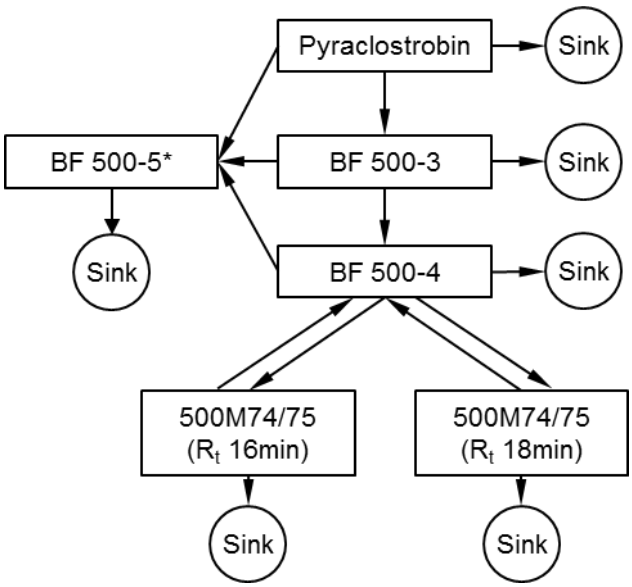
I. MATERIAL AND METHODS

The degradation of pyraclostrobin in soil under anaerobic laboratory conditions from two studies was analyzed taking into account the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*].

Kinetic modeling strategy

Kinetic evaluation was performed in order to derive i) degradation parameters as triggers for additional work (trigger endpoints) and ii) degradation parameters for environmental fate models (modeling endpoints). The best-fit model was selected based on visual and statistical assessment and the corresponding $DegT_{50}$ and $DegT_{90}$ values are reported as trigger endpoints. Appropriate $DegT_{50}$ values for use in environmental fate models were derived depending on the kinetic model.

For the metabolites of pyraclostrobin, the procedure for deriving modeling and trigger endpoints for metabolites recommended by the FOCUS Kinetics workgroup [*FOCUS (2006)*] was followed. The metabolites were added stepwise to the appropriate kinetic model for the parent. The conceptual degradation pathway considered is given in Figure 7.1.2.1.3-1. The metabolite BF 500-5 could be formed from different precursors (i.e. pyraclostrobin, BF 500-3 and BF 500-4). Therefore, all three formation routes were considered in the conceptual degradation pathway. The metabolites 500M74 and 500M75 are formed as isomers from BF 500-4 with possible reverse reactions. This was considered by including the reverse reaction routes in the conceptual degradation pathway. The pathway was stepwise adapted based on the results of the kinetic evaluation.



* BF 500-5 was only detected in 1999/11103

Figure 7.1.2.1.3-1: Setup of possible degradation pathways used for the kinetic evaluation

Kinetic models included in the evaluations

For the pyraclostrobin data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the best-fit model, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC) and the bi-exponential (DFOP) kinetics. For the assessment of the formation and degradation parameters of the metabolites, the SFO model was used.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling and software for kinetic evaluation

Degradation products which occurred at an amount of >5% TAR were included in the calculation. Degradation products which were detected at levels far below 5% TAR were not considered.

The initial concentrations of the metabolites BF 500-3 [old *EU Dossier, A II M 7.1.1.2.1/5, Kellner O. - BASF DocID 1999/10079*; old *EU Dossier, A II M 7.1.1.2.1/6, Kellner O. - BASF DocID 1999/11103*] and BF 500-4 [*Kellner O. - BASF DocID 1999/11103*] were found to be larger than zero. As both compounds occurred as impurities in the radiochemical purity analysis the initial concentration of the metabolites was set to the measured value. Accordingly, for the parent the initial concentration was set to difference between the material balance and the measured values of the metabolites. For all other metabolites the initial concentrations were set to zero.

According to *FOCUS (2006)* the first non-detect was set to ½ LOD and later sampling points were omitted. For the metabolites the last non-detect before the first reported value greater than zero was set to ½ LOD and earlier sampling points were omitted. In *Kellner O.* [*BASF DocID 1999/11103*] the LOD was not reported. Therefore, the lowest reported value for the HPLC analysis which was different from zero, i.e. 0.001 mg kg^{-1}, was assumed to represent the LOD. Hence, ½ LOD was assumed to be 0.0005 mg kg^{-1}, which corresponds to 0.15% TAR. In *Kellner O.* [*BASF DocID 1999/10079*] the reported LOD is 0.001 mg kg^{-1}. Hence, ½ LOD is 0.0005 mg kg^{-1}, which corresponds to 0.17% TAR.

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to $1x10^{-6}$ and 100, respectively.

Experimental data

The kinetic evaluation was based on the findings of two laboratory anaerobic soil degradation studies with German standard soils. The soil characteristics are summarized in Table 7.1.2.1.3-1.

Table 7.1.2.1.3-1: Soil characteristics

Study (BASF DocID)	**1999/11103**	**1999/10079**
Soil designation	**BASF 98/060/01**	**BASF 97/060/02**
Origin	Bruch West, Germany	Bruch West, Germany
Textural class (USDA)	Loamy sand	Loamy sand
Particle size distribution [%] sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm	 79 16 5	 73 17 10
Organic C [%]	1.4	1.5
Microbial biomass [mg C 100 g^{-1} dry soil]	40.3	38.3
CEC [meq 100 g^{-1}]	13	12.1
pH ($CaCl_2$) [-]	7.2	7.5
MWC [g H_2O 100 g^{-1} dry soil]	44	40
Soil moisture at field capacity (0.33 bar) [g H_2O 100 g^{-1} dry soil]	16.1	16.3

CEC = cation exchange capacity
MWC = maximum water holding capacity

The test soils were treated with chlorophenyl-^{14}C-pyraclostrobin [*Kellner O. - BASF DocID 1999/11103*] or tolyl-^{14}C-pyraclostrobin [*Kellner O. - BASF DocID 1999/10079*] at a nominal application rate of 0.33 mg kg^{-1} dry soil (corresponding to 250 g a.s. ha^{-1}) and incubated anaerobically at 20°C in the dark for 120 days. Soil samples were taken at 0, 3, 7, 14, 28, 63, 88 and 120 days after treatment (DAT) [*BASF DocID 1999/11103*] or 0, 7, 14, 28, 62, 90 and 120 DAT [*BASF DocID 1999/10079*].

The measured data as well as resulting datasets submitted to kinetic analysis are given in Table 7.1.2.1.3-2 to Table 7.1.2.1.3-5.

Table 7.1.2.1.3-2: Experimental data [% TAR] of study BASF DocID 1999/11103

DAT	Pyraclostrobin	BF 500-3	BF 500-4	500M74/75 (R_t 16min) [a]	500M74/75 (R_t 18min) [a]	BF 500-5	Material balance
0	95.2	2.5	1.1	0	0	0	100.0
3	39.4	52.6	3	0	0	0.9	100
7	13.1	68.7	4.9	0	0.6	1.3	95
14	3.6	79.9	7	0.9	0.7	0.9	102.7
28	0.2	63	6.9	2.6	2	4.4	100
63	0	45.2	5.7	5.5	3.9	7.5	99.1
88	0	39.2	4.4	7.3	3.6	7.1	100.9
120	0	36.4	3.4	6.9	3.1	7.7	94.9

DAT Days after treatment
TAR Total applied radioactivity
[a] Metabolites could not be unambiguously assigned to HPLC peaks with retention times of 16 and 18 min.

Table 7.1.2.1.3-3: Input data used for kinetic analysis [% TAR] of study BASF DocID 1999/11103

DAT	Pyraclostrobin	BF 500-3	BF 500-4	500M74/75 (R_t 16min) [a]	500M74/75 (R_t 18min) [a]	BF 500-5
0	96.4 [b]	2.5	1.1	0	0	0
3	39.4	52.6	3	NA [d]	0.15 [c]	0.9
7	13.1	68.7	4.9	0.15 [c]	0.6	1.3
14	3.6	79.9	7	0.9	0.7	0.9
28	0.2	63	6.9	2.6	2	4.4
63	0.15 [c]	45.2	5.7	5.5	3.9	7.5
88	NA [d]	39.2	4.4	7.3	3.6	7.1
120	NA [d]	36.4	3.4	6.9	3.1	7.7

DAT Days after treatment
TAR Total applied radioactivity
[a] Metabolites could not be unambiguously assigned to HPLC peaks with retention times of 16 and 18 min.
[b] Difference between material balance and the measured values for BF 500-3 and BF 500-4
[c] Set to 0.5 LOD
[d] Omitted

Table 7.1.2.1.3-4: Experimental data [% TAR] of study BASF DocID 1999/10079

DAT	Pyraclostrobin	BF 500-3	BF 500-4	500M74/75 (R_t 16min) [a]	500M74/75 (R_t 18min) [a]	Material balance
0	95.4	2.9	0	0	0	100
7	8.7	95.8	0	0	0	111.5
14	3.8	84.8	5.6	0	1.1	106.6
28	1.2	73.5	5.4	2.9	2.4	106.6
62	0	40.4	11.1	11.4	5.4	110.5
90	0	37.1	7.6	10.5	4.4	108
120	0	31.7	1.8	9	2.8	108.4

DAT Days after treatment
TAR Total applied radioactivity
[a] Metabolites could not be unambiguously assigned to HPLC peaks with retention times of 16 and 18 min.

Table 7.1.2.1.3-5: Input data used for kinetic analysis [% TAR] of study BASF DocID 1999/10079

DAT	Pyraclostrobin	BF 500-3	BF 500-4	500M74/75 (R_t 16min) [a]	500M74/75 (R_t 18min) [a]
0	97.1 [b]	2.9	0	0	0
7	8.7	95.8	0.17 [c]	NA [d]	0.17 [c]
14	3.8	84.8	5.6	0.17 [c]	1.1
28	1.2	73.5	5.4	2.9	2.4
62	0.17 [c]	40.4	11.1	11.4	5.4
90	NA [d]	37.1	7.6	10.5	4.4
120	NA [d]	31.7	1.8	9	2.8

DAT Days after treatment
TAR Total applied radioactivity
[a] Metabolites could not be unambiguously assigned to HPLC peaks with retention times of 16 and 18 min.
[b] Difference between material balance and the measured value for BF 500-3
[c] Set to 0.5 LOD
[d] Omitted

II. RESULTS AND DISCUSSION

Kinetic evaluation of BASF DocID 1999/11103 - derivation of trigger endpoints

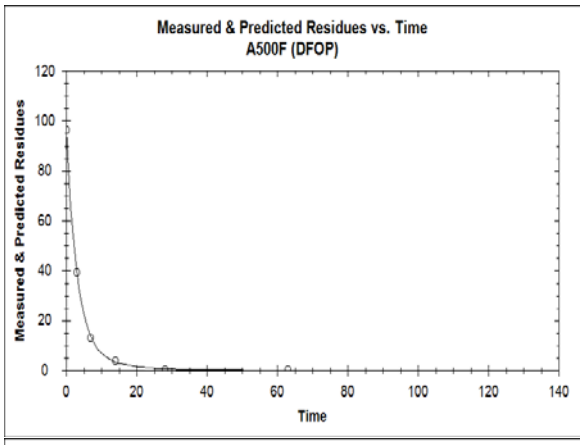
Metabolites were included stepwise into the best-fit model for the parent.

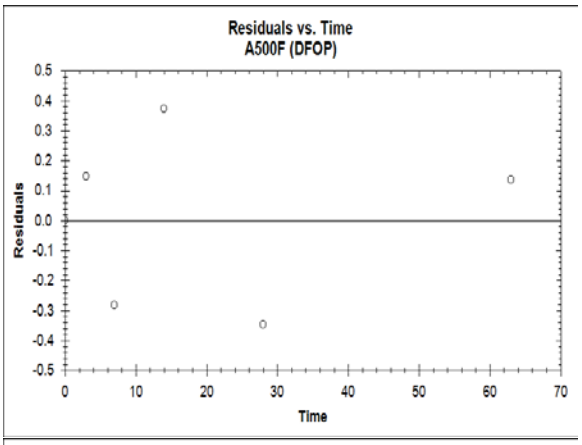
Table 7.1.2.1.3-6: Statistical and visual assessment of different kinetic models for pyraclostrobin and its metabolites (BASF DocID 1999/11103)

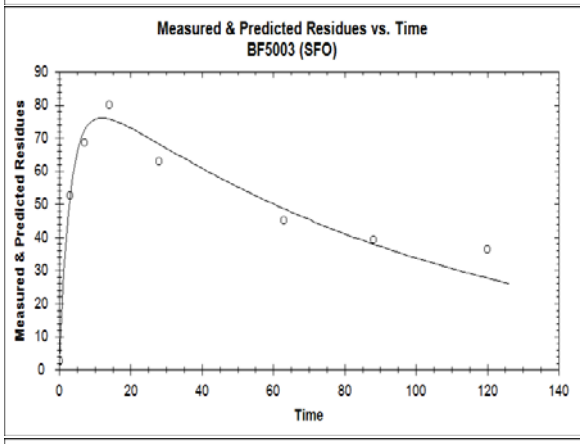
Step in FOCUS flowchart	Kinetic model	χ^2 error	p (t-test)	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
(1) & (2) Run parent only SFO & FOMC	SFO	2.8	k: <0.01	good	2.4	7.9
	FOMC	1.3	β: <0.05	excellent	2.3	8.5
(3) Run DFOP (parent only)	DFOP	1.0	k1: <0.01 k2: not sig. g: <0.01	excellent	2.3	8.4
⇨ Overall, the DFOP model results in the best fit. ⇨ **Conclusion**: Use DFOP for derivation of trigger endpoints; use SFO for derivation of modeling endpoints.						
(4) Run parent DFOP and BF 500-3 SFO	Parent DFOP	1.0	k1: <0.01 k2: <0.01 g: <0.01	excellent	2.3	8.4
	BF 500-3 SFO	6.5	k: <0.01	good	78.5	260.8
⇨ DFOP fit for parent is visually acceptable, χ^2 error is low, k1, k2 are significantly different from zero. SFO fit for BF 500-3 is visually acceptable, χ^2 error is low, k is significantly different from zero. ⇨ Include BF 500-4.						
(5) Run parent DFOP and BF 500-3 and BF 500-4 SFO	Parent DFOP	2.5	k1: not sig. k2: <0.01 g: <0.05	good	2.2	8.4
	BF 500-3 SFO	7.1	k: <0.01	good	68.4	227.3
	BF 500-4 SFO	14.5	k: <0.01	moderate	7.6	25.2
⇨ DFOP fit for parent is visually acceptable, χ^2 error is low, k1 is not significantly different from zero and changed from 0.3348 in run (4) to 8.5551. The resulting $DegT_{50}$ and $DegT_{90}$ values are similar to run (4). ⇨ Repeat run with fixed parameters from run (4) for the parent.						
(6) Run parent DFOP (fixed M0, k1, k2, g) and BF 500-3 and BF 500-4 SFO	Parent DFOP	0.7	k1: fixed k2: fixed g: fixed	excellent	2.3	8.4
	BF 500-3 SFO	7.4	k: <0.01	good	67.8	225.1
	BF 500-4 SFO	14.7	k: <0.01	moderate	7.5	25.0
⇨ SFO fit for BF 500-3 is visually acceptable, χ^2 error is low, k is significantly different from zero. SFO fit for BF 500-4 is visually acceptable, χ^2 error is acceptable, k is significantly different from zero. ⇨ Include BF 500-5 as degradate from parent, BF 500-3 and BF 500-4.						
(7) Run parent DFOP (fixed M0, k1, k2, g) and BF 500-3, BF 500-4 and BF 500-5 SFO	Parent DFOP	0.7	k1: fixed k2: fixed g: fixed	excellent	2.3	8.4
	BF 500-3 SFO	7.4	k: <0.01	good	67.6	224.5
	BF 500-4 SFO	14.6	k: <0.01	moderate	7.5	24.8
	BF 500-5 SFO	12.0	k: <0.05	good	42.6	141.5
⇨ SFO fit for BF 500-3, BF 500-4 and BF 500-5 are visually acceptable, χ^2 errors are below 15%, k values are significantly different from zero. The formation fractions of BF 500-5 from parent and BF 500-3 are below 0.01 and not significantly different from zero. ⇨ Repeat run with BF 500-5 only formed from BF 500-4						

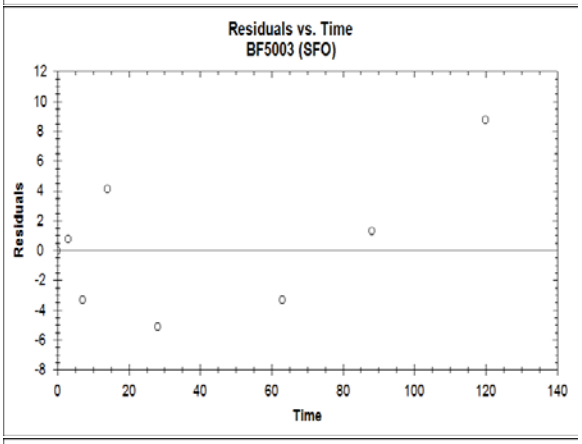
Step in FOCUS flowchart	Kinetic model	χ^2 error	p (t-test)	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
(8) Run parent DFOP (fixed M0, k1, k2, g) and BF 500-3, BF 500-4 and BF 500-5 SFO	Parent DFOP	0.7	k1: fixed k2: fixed g: fixed	excellent	2.3	8.4
	BF 500-3 SFO	7.4	k: <0.01	good	67.7	225.0
	BF 500-4 SFO	14.7	k: <0.01	moderate	7.4	24.7
	BF 500-5 SFO	11.9	k: <0.01	good	34.0	113.0
⇨ SFO fit for BF 500-3, BF 500-4 and BF 500-5 are visually acceptable, χ^2 errors are below 15%, k values are significantly different from zero. The formation fraction of BF 500-5 from BF 500-4 is significantly different from zero. ⇨ Include 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) as degradates of BF 500-4 with possible reverse reaction.						
(9) Run parent DFOP (fixed M0, k1, k2, g) and BF 500-3, BF 500-4, BF 500-5, 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) SFO	Parent DFOP	0.7	k1: fixed k2: fixed g: fixed	excellent	2.3	8.4
	BF 500-3 SFO	7.0	k: <0.01	good	69.9	232.1
	BF 500-4 SFO	17.9	k: not sig.	moderate	7.7	25.7
	BF 500-5 SFO	11.9	k: <0.01	good	32.1	106.5
	500M74/75 (R_t 16min)	5.0	k: <0.05	good	64.3	213.7
	500M74/75 (R_t 18min)	6.4	k: <0.01	good	23.4	77.7
⇨ All fits are visually acceptable, for BF 500-3, BF 500-5, 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) χ2 errors are below 15% and k values are significantly different from zero. For BF 500-4, χ2 error is above 15% and k is not significantly different from zero. The formation fractions for the reverse reactions from 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) to BF 500-4 are very low and not significantly different from zero. ⇨ Repeat run without reverse reaction of 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) to BF 500-4.						
(10) Run parent DFOP (fixed M0, k1, k2, g) and BF 500-3, BF 500-4, BF 500-5, 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) SFO	Parent DFOP	0.7	k1: fixed k2: fixed g: fixed	excellent	2.3	8.4
	BF 500-3 SFO	7.0	k: <0.01	good	70.1	232.9
	BF 500-4 SFO	15.5	k: <0.01	moderate	7.8	25.9
	BF 500-5 SFO	11.9	k: <0.01	good	32.2	107.1
	500M74/75 (R_t 16min)	5.0	k: <0.01	good	64.6	214.7
	500M74/75 (R_t 18min)	6.4	k: <0.01	good	23.5	78.0
⇨ SFO fits for BF 500-3, BF 500-5, 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) are visually acceptable, χ2 errors are below 15%, k values are significantly different from zero. SFO fit for BF 500-4, is visually acceptable, χ2 error is slightly above 15%, k is significantly different from zero. ⇨ **Conclusion**: The pathway is reliable. The results are adequate to be used as trigger endpoints.						

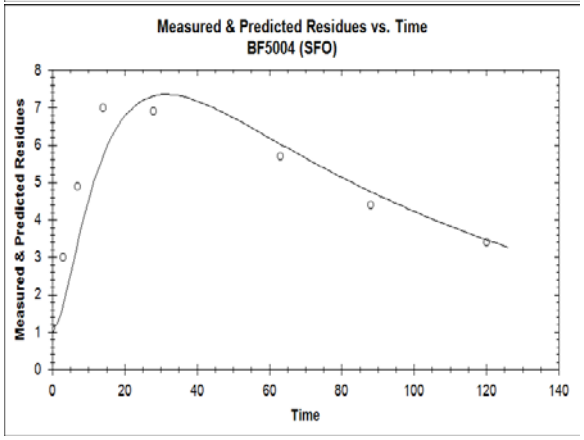
The fits of the final run are presented in Figure 7.1.2.1.3-2: .

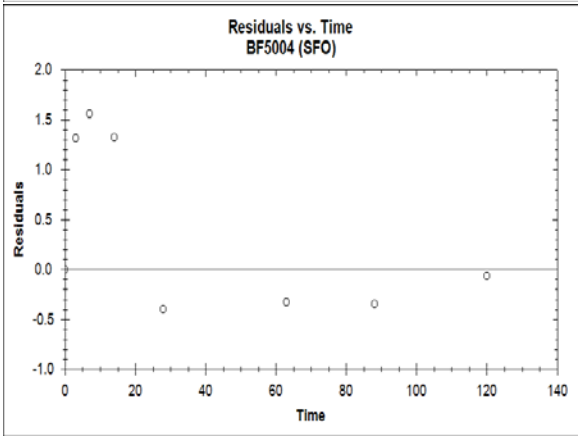












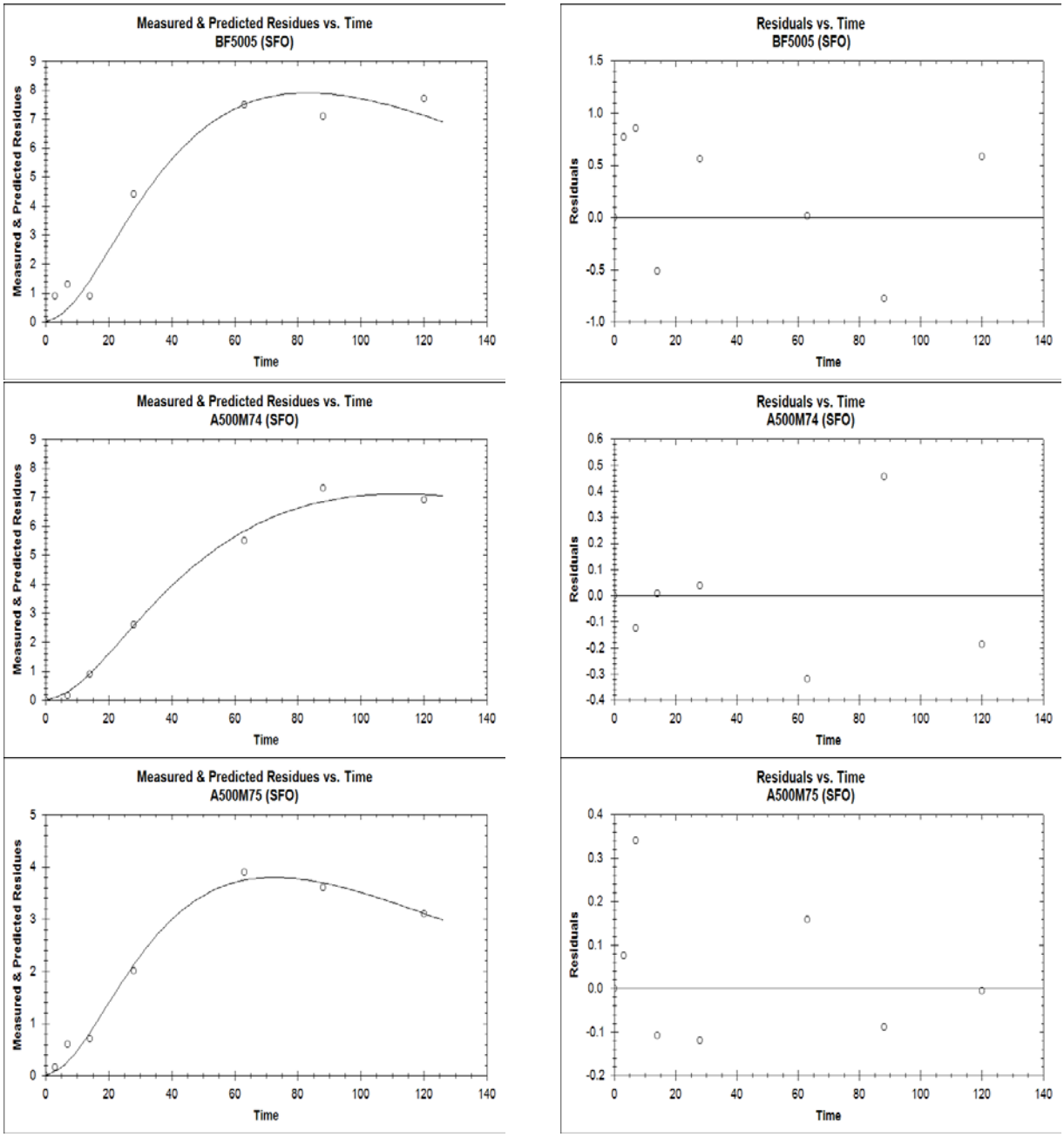


Figure 7.1.2.1.3-2: Final Kingui fit for pyraclostrobin, BF 500-3, BF 500-4, BF 500-5, 500M74 and 500M75, DFOP kinetics for the parent, fixed parameters for parent, adapted pathway (BASF DocID 1999/11103)

The adapted degradation pathway is given in Figure 7.1.2.1.3-3 and the complete parameter estimation for derivation of trigger endpoints of pyraclostrobin and its metabolites BF 500-3, BF 500-4, BF 500-5, 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) are presented in Table 7.1.2.1.3-7.

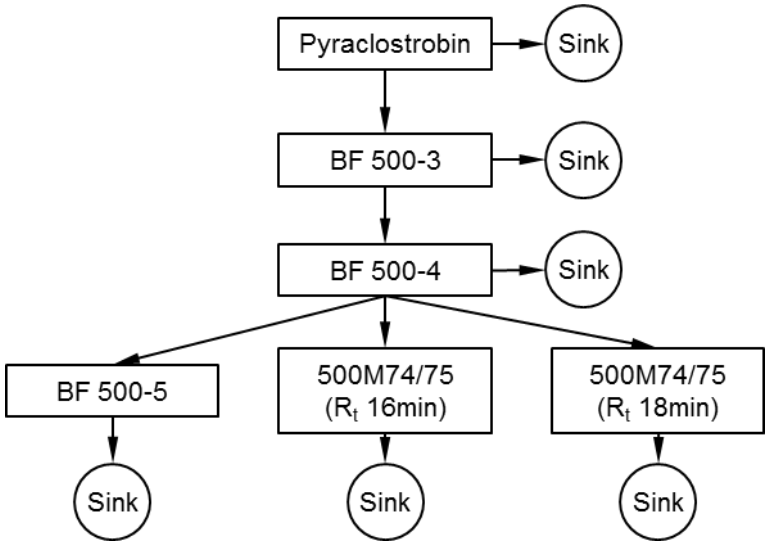


Figure 7.1.2.1.3-3: Degradation pathway of pyraclostrobin derived from the kinetic evaluation of BASF DocID 1999/11103

Table 7.1.2.1.3-7: Parameters for pyraclostrobin and its metabolites for derivation of trigger endpoints (BASF DocID 1999/11103)

Parameter	Pyraclostrobin [a)]	BF 500-3	BF 500-4	BF 500-5	500M74/75 (R_t 16min) [b)]	500M74/75 (R_t 18min) [b)]
Kinetic model	DFOP	SFO	SFO	SFO	SFO	SFO
χ^2 [%]	0.7	7.0	15.5	11.9	5.0	6.4
k-rate [d^{-1}]	k1: 0.3348 k2: 0.1077 g: 0.8856	0.0099	0.0888	0.0215	0.0107	0.0295
$DegT_{50}$ [d]	2.3	70.1	7.8	32.2	64.6	23.5
$DegT_{90}$ [d]	8.4	232.9	25.9	107.1	214.7	78.0

a) The values from Step (4) are reported (see Table 7.1.2.1.3-6) as degradation parameters were fixed during the assessment of the pathway fit.

b) Metabolites could not be unambiguously assigned to HPLC peaks with retention times of 16 and 18 min.

Kinetic evaluation of BASF DocID 1999/11103 - derivation of modeling endpoints

The same degradation pathway derived from the derivation of trigger endpoints was considered. All metabolites were included simultaneously into the SFO model for the parent.

The complete parameter estimation for derivation of modeling endpoints of pyraclostrobin and its metabolites BF 500-3, BF 500-4, BF 500-5, 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) is given in Table 7.1.2.1.3-8.

Table 7.1.2.1.3-8: Parameters for pyraclostrobin and its metabolites for derivation of modeling endpoints (BASF DocID 1999/11103)

Parameter	Pyraclostrobin	BF 500-3	BF 500-4	BF 500-5	500M74/75 (R_t 16min) [a]	500M74/75 (R_t 18min) [a]
Kinetic model	SFO	SFO	SFO	SFO	SFO	SFO
χ^2 [%]	2.8	6.7	16.5	11.9	5.0	6.4
k-rate [d^{-1}]	0.2918	0.0096	0.0857	0.0224	0.0112	0.0305
$DegT_{50}$ [d]	2.4	72.6	8.1	31.0	62.1	22.7
ff [-]	-	0.867	1 [b]	0.409 [c]	- [d]	- [d]
ff sig.	-	<0.01	<0.01	<0.05	not sig.	not sig.

[a] Metabolites could not be unambiguously assigned to HPLC peaks with retention times of 16 and 18 min.

[b] from BF 500-3

[c] from BF 500-4

[d] No reliable formation fraction could be determined.

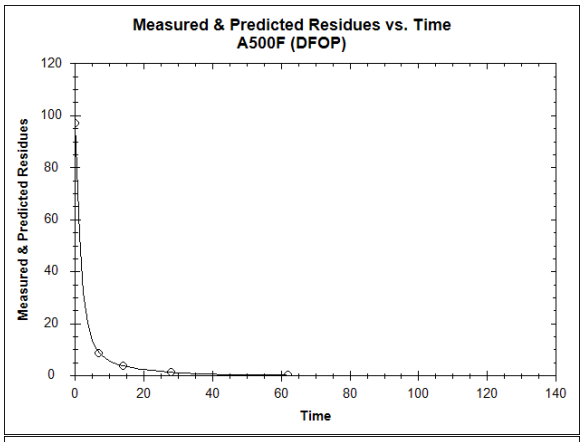
Kinetic evaluation of BASF DocID 1999/10079 - derivation of trigger endpoints

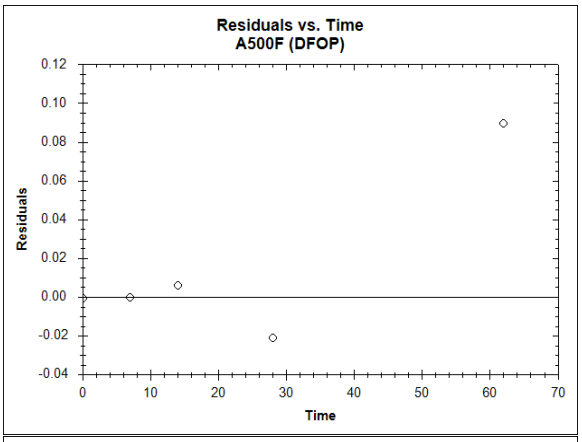
Metabolites were included stepwise into the best-fit model for the parent. The conceptual degradation pathway was adapted according to the results of the kinetic evaluation of BASF DocID 1999/11103, i.e. the possible reverse reaction of the metabolites 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) to BF 500-4 was not considered.

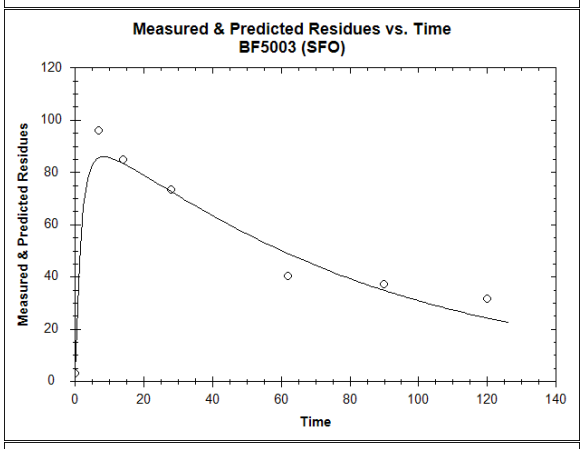
Table 7.1.2.1.3-9: Statistical and visual assessment of different kinetic models for pyraclostrobin and its metabolites (BASF DocID 1999/10079)

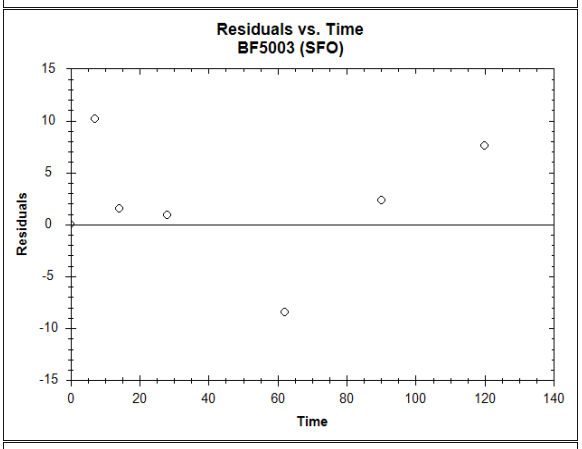
Step in FOCUS flowchart	Kinetic model	χ^2 error	p (t-test)	Visual assessment	$DegT_{50}$ [d]	$DegT_{90}$ [d]
(1) & (2) Run parent only SFO & FOMC	SFO	5.2	k: <0.01	good	2.1	6.9
	FOMC	0.7	β: <0.05	excellent	1.1	6.4
(3) Run DFOP (parent only)	DFOP	0.2	k1: <0.05 k2: <0.05 g: <0.01	excellent	1.5	6.5
⇨ Overall, the DFOP model results in the best fit ⇨ **Conclusion**: Use DFOP for derivation of trigger endpoints; use SFO for derivation of modeling endpoints.						
(4) Run parent DFOP and BF 500-3 SFO	Parent DFOP	0.2	k1: <0.01 k2: <0.01 g: <0.01	excellent	1.5	6.5
	BF 500-3 SFO	8.8	k: <0.01	good	58.9	195.6
⇨ DFOP fit for parent is visually acceptable, χ^2 error is low, k1, k2 are significantly different from zero. SFO fit for BF 500-3 is visually acceptable, χ^2 error is low, k is significantly different from zero. ⇨ Include BF 500-4.						
(5) Run parent DFOP and BF 500-3 and BF 500-4 SFO	Parent DFOP	0.2	k1: <0.01 k2: <0.01 g: <0.01	excellent	1.5	6.5
	BF 500-3 SFO	8.8	k: <0.01	good	58.4	193.9
	BF 500-4 SFO	33.1	k: <0.05	poor	19.2	63.7
⇨ The fits for the parent and BF 500-3 are visually acceptable, χ^2 errors are low, k values are significantly different from zero. SFO fit for BF 500-4 is poor, χ^2 error is above 15% but k is significantly different from zero. ⇨ Include 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min).						
(6) Run parent DFOP and BF 500-3, BF 500-4, 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) SFO	Parent DFOP	0.2	k1: <0.01 k2: <0.01 g: <0.01	excellent	1.5	6.5
	BF 500-3 SFO	8.9	k: <0.01	good	56.9	189.0
	BF 500-4 SFO	39.4	k: <0.01	poor	11.3	37.7
	500M74/75 (R_t 16min)	14.3	k: <0.01	moderate	36.6	121.5
	500M74/75 (R_t 18min)	11.1	k: <0.01	good	15.4	51.0
⇨ The fits for the parent, BF 500-3, 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) are visually acceptable, χ^2 errors are below 15%, k values are significantly different from zero. The fit for BF 500-4 is visually poor, χ^2 error is above 15% but k is significantly different from zero. As the fit provides a conservative estimate for k it is considered to be acceptable for derivation of trigger endpoints. ⇨ **Conclusion**: The pathway is reliable. The results are adequate to be used as trigger endpoints.						

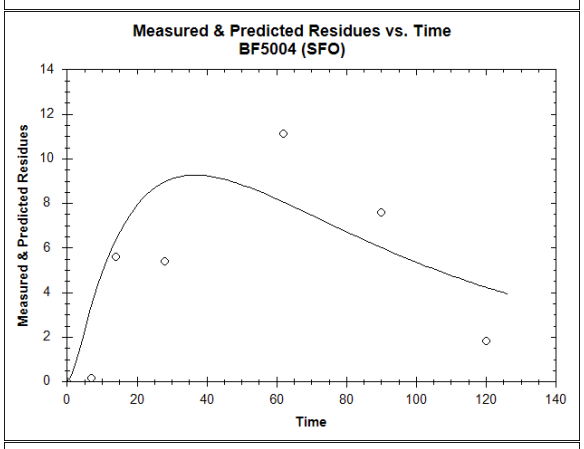
The fits of the final run are presented in Figure 7.1.2.1.3-4.

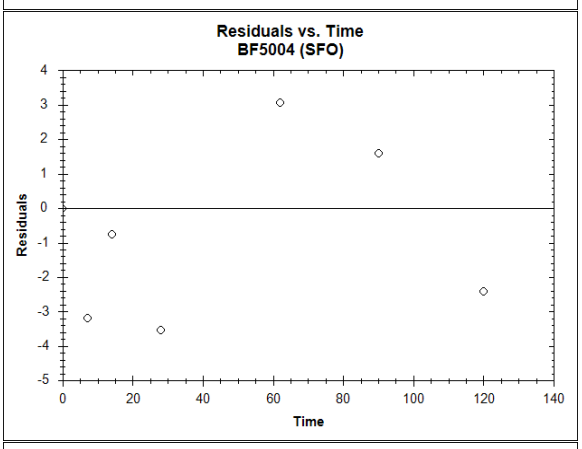

Measured & Predicted Residues vs. Time
A500F (DFOP)
Measured & Predicted Residues
Time

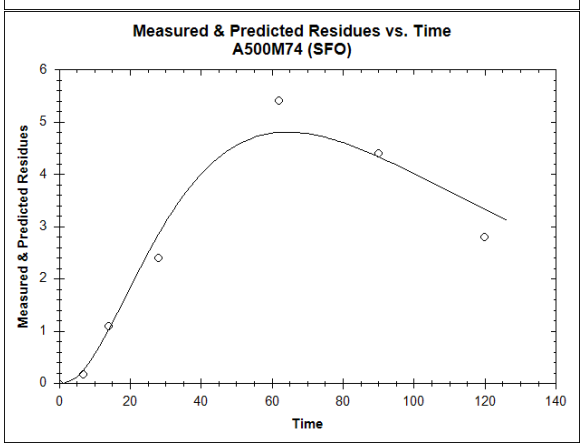

Residuals vs. Time
A500F (DFOP)
Residuals
Time

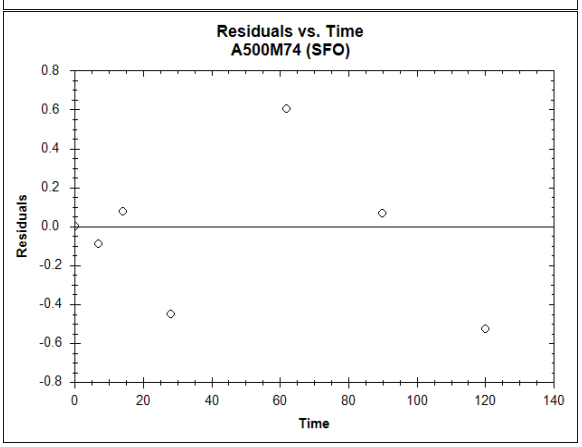

Measured & Predicted Residues vs. Time
BF5003 (SFO)
Measured & Predicted Residues
Time


Residuals vs. Time
BF5003 (SFO)
Residuals
Time


Measured & Predicted Residues vs. Time
BF5004 (SFO)
Measured & Predicted Residues
Time


Residuals vs. Time
BF5004 (SFO)
Residuals
Time


Measured & Predicted Residues vs. Time
A500M74 (SFO)
Measured & Predicted Residues
Time


Residuals vs. Time
A500M74 (SFO)
Residuals
Time

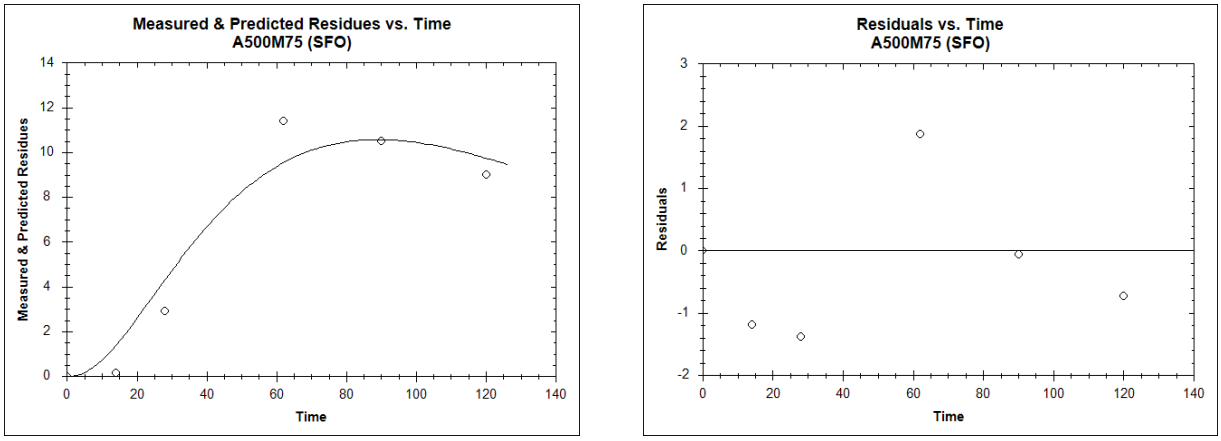


Figure 7.1.2.1.3-4: Final Kingui fit for pyraclostrobin, BF 500-3, BF 500-4, 500M74 and 500M75, DFOP kinetics for the parent (BASF DocID 1999/10079)

The adapted degradation pathway is given in Figure 7.1.2.1.3-5 and the complete parameter estimation for derivation of trigger endpoints of pyraclostrobin and its metabolites BF 500-3, BF 500-4, 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) are presented in Table 7.1.2.1.3-10.

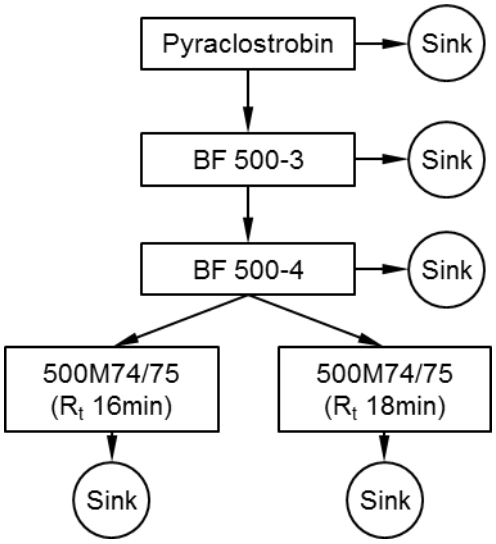


Figure 7.1.2.1.3-5: Degradation pathway of pyraclostrobin derived from the kinetic evaluation of BASF DocID 1999/10079

Table 7.1.2.1.3-10: Parameters for pyraclostrobin and its metabolites for derivation of trigger endpoints (BASF DocID 1999/10079)

Parameter	Pyraclostrobin	BF 500-3	BF 500-4	500M74/75 (R_t 16min)	500M74/75 (R_t 18min)
Kinetic model	DFOP	SFO	SFO	SFO	SFO
χ^2 [%]	0.2	8.9	39.4	14.3	11.1
k-rate [d^{-1}]	k1: 0.5261 k2: 0.0800 g: 0.8820	0.0122	0.0611	0.0189	0.0451
$DegT_{50}$ [d]	1.5	56.9	11.3	36.6	15.4
$DegT_{90}$ [d]	6.5	189.0	37.7	121.5	51.0

Kinetic evaluation of BASF DocID 1999/10079 - derivation of modeling endpoints

The same degradation pathway derived from the derivation of trigger endpoints was considered. All metabolites were included simultaneously into the SFO model for the parent.

The complete parameter estimation for derivation of trigger endpoints of pyraclostrobin and its metabolites BF 500-3, BF 500-4, 500M74/75 (R_t 16min) and 500M74/75 (R_t 18min) is given in Table 7.1.2.1.3-11.

Table 7.1.2.1.3-11: Parameters for pyraclostrobin and its metabolites for derivation of modeling endpoints (BASF DocID 1999/10079)

Parameter	Pyraclostrobin	BF 500-3	BF 500-4	500M74/75 (R_t 16min)	500M74/75 (R_t 18min)
Kinetic model	SFO	SFO	SFO	SFO	SFO
χ^2 [%]	5.2	8.8	39.2	13.9	10.7
k-rate [d^{-1}]	0.3413	0.0124	0.0608	0.0189	0.0448
$DegT_{50}$ [d]	2.0	55.9	11.4	36.7	15.5
ff [-]	-	1	0.692	0.540	0.450
ff sig.	-	<0.01	<0.01	<0.05	<0.01

III. CONCLUSION

Trigger and modeling endpoints were derived for pyraclostrobin and its metabolites in two anaerobic laboratory degradation studies. The kinetic evaluation of the data showed that the DFOP model is the best-fit model for pyraclostrobin, while SFO can be used to derive modeling endpoints. For the metabolites the SFO model was appropriate to derive trigger and modeling endpoints.

The derived trigger endpoints for pyraclostrobin and its metabolites are summarized in Table 7.1.2.1.3-12, and modeling endpoints are summarized in Table 7.1.2.1.3-13.

Table 7.1.2.1.3-12: Trigger endpoints for pyraclostrobin and its metabolites in anaerobic soil

Study (BASF DocID)	Compound	Kinetic model	$DegT_{50}$ [d]	$DegT_{90}$ [d]
1999/11103	Pyraclostrobin	DFOP	2.3	8.4
	BF 500-3	SFO	70.1	232.9
	BF 500-4	SFO	7.8	25.9
	BF 500-5	SFO	32.2	107.1
	500M74/75 (R_t 16min)	SFO	64.6	214.7
	500M74/75 (R_t 18min)	SFO	23.5	78.0
1999/10079	Pyraclostrobin	DFOP	1.5	6.5
	BF 500-3	SFO	56.9	189.0
	BF 500-4	SFO	11.3	37.7
	500M74/75 (R_t 16min)	SFO	36.6	121.5
	500M74/75 (R_t 18min)	SFO	15.4	51.0

Table 7.1.2.1.3-13: Modeling endpoints for pyraclostrobin and its metabolites in anaerobic soil

Study (BASF DocID)	Compound	Kinetic model	$DegT_{50}$ [d]	Formation fraction [-]
1999/11103	Pyraclostrobin	SFO	2.4	-
	BF 500-3	SFO	72.6	0.867
	BF 500-4	SFO	8.1	1.0 [a]
	BF 500-5	SFO	31.0	0.409 [b]
	500M74/75 (R_t 16min)	SFO	62.1	- [c]
	500M74/75 (R_t 18min)	SFO	22.7	- [c]
1999/10079	Pyraclostrobin	SFO	2.0	-
	BF 500-3	SFO	55.9	1.0
	BF 500-4	SFO	11.4	0.692 [a]
	500M74/75 (R_t 18min)	SFO	15.5	0.450 [b]
	500M74/75 (R_t 16min)	SFO	36.7	0.540 [b]

[a] from BF 500-3
[b] from BF 500-4
[c] No reliable formation fraction could be determined.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

Pyraclostrobin converted under anaerobic conditions within few days to the des-methoxy metabolite BF 500-3, which is further transformed to BF 500-4 (and its isomers). Binding to the organic matrix in soil results then in the formation of non-extractable residues which reached 37 - 61% TAR after 120 days. Low amounts of the cleavage product BF 500-5 could also be detected.

New kinetic evaluations of the already peer-reviewed anaerobic soil metabolism studies were performed according to current FOCUS guidelines. The new best-fit $DegT_{50}$ values of the anaerobic metabolites are listed together with the parent data in Table 7.1.2.1.3-10.

During data gap analysis for the renewal of approval, it became clear that the isomer structures 500M74 and 500M75 of BF 500-4 (500M73) were never unambiguously assigned to the two peaks at 16 and 18 min in the chromatograms of the anaerobic soil metabolism studies with pyraclostrobin and that the structures originally proposed are rather unlikely from a chemical point of view. Therefore, further investigations of the isomerization behaviour of BF 500-4 were performed. The experiments were included in the BF 500-4 aerobic soil degradation report. The soil degradation rate results of BF 500-4 are already described under *CA7.1.2.1.2/3*. The results of the isomer investigations are described below.

Report:	CA 7.1.2.1.4/1 Ebert D.,Dalkmann P., 2014a Rate of degradation of BF 500-4 (Reg. No. 358672) in soil under aerobic conditions 2013/1294779
Guidelines:	OECD 307, EPA 835.4100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This study was erroneously not listed in the application submitted for renewal of approval. The reason for submission is described below.

Executive summary

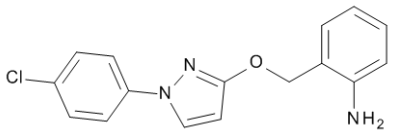
In order to get more information on the isomer structures of BF 500-4, 50 µg of ^{14}C-labeled test item was incubated in 20 mL of various aqueous and organic solvent solutions, respectively, and the isomer formation was followed over a period of 27 days by HPLC analysis. In addition, the interconversion of isomers was checked by fractionation of an isomeric peak and re-injection into HPLC.

For NMR analysis, larger amounts of isomers were produced by incubating non-labeled BF 500-4 in acetonitrile/H_2O mixture (20:80, v:v). The isomers were then fractionated by preparative HPLC and the fractions analysed by NMR for structure elucidation.

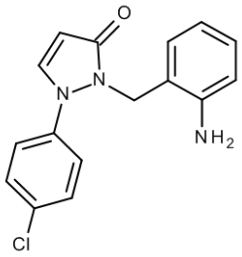
The results showed that the isomerization of BF 500-4 to 500M74 and 500M75 takes place preferably in pure water and buffer pH 9, as well as in acetonitrile water mixtures up to a maximum acetonitrile content of 50%. In acetonitrile/water 80:20 (v:v) and pure acetonitrile, the parent compound BF 500-4 was rather stable and the isomerization negligible.

When fractionating one isomer and reinjecting into HPLC, the other two isomers were formed again, proving that the isomers are interconvertible.

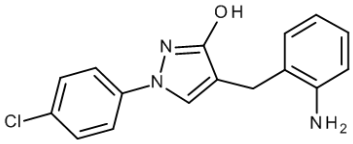
By NMR analysis, structures could be assigned to the two observed isomer peaks (500M74 and 500M75). The structure of BF 500-4 (500M73) was confirmed.



BF 500-4 (500M73)
m/z 299



500M74
m/z 299



500M75
m/z 299

The overall environmental significance of these isomers is considered rather low, since BF 500-4 occurs in detectable amounts only under strong anaerobic conditions in nitrogen-flushed, water-logged soil.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

^{14}C-labelled material

BAS code:	^{14}C-BF 500-4
Synonym:	500M73
Reg. No.:	358672
Chemical name (IUPAC):	2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenylamine
Molar mass:	299.76 g mol^{-1} (unlabeled)
Position of radiolabel:	Tolyl-U-C^{14}
Specific radioactivity of a.s.:	8.49 MBq mg^{-1}
Batch No.:	1072-1004
Radiochemical purity:	≥ 96.0%

non-labelled material

Batch No.:	01586-68
Purity:	99.8%

2. Test Design

Isomerization in different aqueous media / solvents

In order to investigate in which test medium the isomerization takes preferably place, a volume of 20 mL of each test solution (see Table 7.1.2.1.4-1) was treated with ~50 µg ^{14}C-BF 500-4 (200 µL of application solution in acetone). After application, a sample of 250 µL was taken from each test vessel. The amount of dissolved radioactivity was checked by LSC and subaliquots were analysed by HPLC.

Table 7.1.2.1.4-1: Test solutions used to investigate isomerization of BF 500-4

Test medium	test volume [mL]	measured amount ^{14}C-equivalents in 250 µL [µg]	actual dissolved amount of ^{14}C-equivalents in 20 mL [µg]
dest. H_2O, acidified to pH 1 with 100 µL HCOOH	20	1.290	51.6
dest. H_2O	20	1.072	42.9
buffer pH 9 *	20	0.930	37.2
ACN/H_2O 20:80 (v:v)	20	0.831	33.2
ACN/H_2O 50:50 (v:v)	20	1.294	51.8
ACN/H_2O 80:20 (v:v)	20	1.298	51.9
pure ACN	20	1.265	50.6

* Fa. Merck, Darmstadt, Germany (Order #109889)

As expected, the solubility in aqueous media proved to be significantly lower than in solution with a high acetonitrile content. The seemingly good solubility observed in dest. H_2O pH1 was attributed to the fact that BF 500-4 hydrolyzed almost immediately and formed polar substances.

The glass test vessels (20 mL vials) were closed with a screw cap and placed on a magnetic stirrer in a 20 ± 2°C incubator. After 7, 14 and 27 days, 250 µL aliquots were taken from each test solution and subjected to HPLC analysis. The isomer formation (peaks at ~21 and ~24 min) could be observed in pure dest. H_2O, buffer pH9, and ACN/H_2O 20:80 (v:v).

Interconversion of BF 500-4 isomers

During HPLC analysis of the 14 day sample of the dest. H_2O solution, the 24 min peak (representing an isomer of metabolite BF 500-4), was fractionated by hand (about 1 mL) and immediately re-injected into HPLC without further workup.

Sample preparation for MS and NMR analysis of BF 500-4 isomers

Parallel to the ^{14}C-test solutions, 25 mL of test solutions, in which formation of larger amounts of isomers was expected (see Table 7.1.2.1.4-2), were treated with non-labeled BF 500-4 in order to produce larger substance amounts for NMR analysis.

First, 20 mL of the test solution was filled in a 25 mL volumetric flask, then non-labeled BF 500-4 was carefully weighed into the flask until the solubility was (visibly) reached. Then the flask was filled to the calibration mark, closed with a glass stopper and placed on magnetic stirrers in a 20°C incubator.

Table 7.1.2.1.4-2: Test solutions used for production of isomers for NMR analysis

Test medium	test volume [mL]	added amounts of non-labeled BF 500-4 [mg]
buffer pH 9	25	32.8
ACN/H_2O 20:80 (v:v)	25	34.5
ACN/H_2O 50:50 (v:v)	25	164.1
ACN/H_2O 80:20 (v:v)	25	521.4

At day 0 as well as after 12 and 19 days, a 250 µL sample of each solution was taken and subjected to HPLC.

After 20/21 days, 10 x 2 mL aliquots from the ACN/H_2O 20:80 (v:v) solution were fractionated via preparative HPLC. The fractions 7 - 9.5 min, 16.5 - 19.5 min, and 27.5 - 29.5 min contained the compounds of interest, each with the mass 299 (500M74, 500M75 and BF 500-4 (500M73)). The fractions were separately collected in round bottom flasks. The water from the elution solvent was immediately evaporated by rotary evaporation. After 4 fractionation cycles, the dry residues were dissolved in 1 mL acetonitrile, respectively, and 200 µL subsampled for MS analysis. Then the fractionation was continued for another 6 fractionation cycles.

The collected fractions were dried by rotary evaporation and transferred in 1 mL volumetric flasks. The acetonitrile was evaporated under a stream of nitrogen and two times redissolved with few amounts of deuterated acetonitrile (to remove residual non-deuterated solvent). Finally, the flasks were made to volume with deuterated acetonitrile. These samples were subjected to NMR analysis.

II. RESULTS & DISCUSSION

Isomerization in different test solutions

The results of the isomerization experiment in various solutions are shown in Table 7.1.2.1.4-3. The isomerization of BF 500-4 can be observed in all aqueous media except in water pH 1, where the substance immediately hydrolysed to polar substances.

Table 7.1.2.1.4-3: Isomerization behaviour of ^{14}C-BF 500-4 in various solutions [%peak area in chromatogram]

solution	days after treatment	uk	uk	uk	uk	uk	BF 500-4 isomer	BF 500-4 isomer	BF 500-4	others *
		t_{Ret} 3.4	4.5	7.0	8.5	16.1	**21.3**	**24.3**	**34.6**	
H_2O, pH 1	0	39.5	7.9			0.9	**0.5**		**40.5**	10.7
	7	78.7	16.2			1.4			**2.3**	1.5
	14	76.5	17.5			1.5	**0.6**		**0.0**	3.9
	27	74.0	15.1			1.2			**8.2**	1.5
H_2O dest.	0	14.0					**8.6**	**9.0**	**65.2**	3.2
	7	28.2			2.8	17.3	**13.9**	**33.8**		3.9
	14	6.6		9.4	17.7	7.8	**14.7**	**36.5**	**0.0**	7.2
	27	5.5			19.0	2.8	**18.4**	**43.9**	**1.9**	8.4
H_2O, pH 9	0	17.0				0.7	**5.5**	**10.1**	**62.3**	4.5
	7	44.2				1.9	**12.7**	**33.4**	**0.0**	7.9
	14	43.8				1.8	**12.5**	**32.2**	**0.0**	9.7
	27	41.4				2.1	**14.2**	**34.4**	**0.5**	7.3
ACN/H_2O 20:80	0	5.9				0.4	**2.6**	**2.7**	**83.9**	4.4
	7	45.4				2.1	**14.1**	**22.3**	**10.8**	5.4
	14	47.9				1.6	**17.0**	**24.7**	**1.7**	7.1
	27	47.9				1.9	**18.1**	**24.1**	**0.3**	7.7
ACN/H_2O 50:50	0	0.8				0.6	**1.0**		**90.5**	7.1
	7	8.8				1.0	**3.8**	**1.6**	**80.4**	4.3
	14	15.8				0.7	**6.5**	**3.5**	**67.7**	5.8
	27	25.4				0.8	**11.2**	**5.3**	**51.2**	6.0
ACN/H_2O 80/20	0					0.6	**0.9**		**91.8**	6.7
	7	0.9					**1.7**		**93.5**	4.0
	14	1.7				0.5	**2.0**		**88.6**	7.2
	27	3.3					**2.5**		**89.2**	5.1
pure ACN	0					0.7	**0.8**		**90.4**	8.0
	7						**1.2**		**96.0**	2.8
	14						**1.0**		**93.1**	5.9
	27					0.9			**97.3**	2.7

uk = unknown tRet = retention time [min] * sum of several peaks; each single peak < 5% area in chromatogram

The isomerization seemed to be clearly dependent on the water content in the solution. The higher the water content, the higher the percentage of isomers visible in the chromatogram. In pure ACN and ACN/H_2O 80:20 (v:v), BF 500-4 was rather stable. However, it has to be pointed out that the total amount of isomers formed is dependent on the solubility of BF 500-4 in the respective solution.

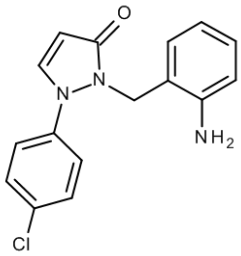
Interconversion of isomers

The reinjection of the fractionated BF 500-4 isomer (peak 24 min) showed that the isomers are easily interconvertible. Immediately, the fractionated peak transformed back into BF 500-4. Also small amounts of the isomer peak 21 min became visible.

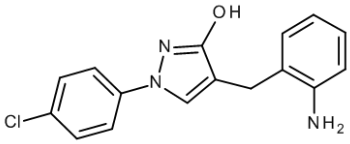
MS and NMR analysis

This interconversion was also observed during sample preparation for MS and NMR analysis. The immediate evaporation of water from the mobile phase of the preparative HPLC was still not fast enough to prevent conversion into another isomeric form within a fractionated sample. HPLC-MS/MS as well as NMR revealed that a second isomer was always present .

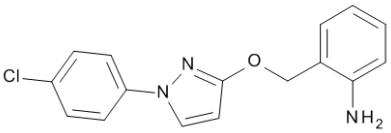
Nevertheless, the following isomeric structures could be concluded from NMR analysis:



500M74
m/z 299
ret. time 21 min



500M75
m/z 299
ret. time 24 min



BF 500-4 (500M73)
m/z 299
ret. time 34 min

Since it can be assumed that the sequence of peak elution from reversed phase columns will be kept, especially when using the same H_2O/acetonitrile HPLC solvent system, it can be concluded that the 21 min isomer peak from the 20:80 ACN/H_2O solution corresponds to the 16 min isomer peak in the ACN/H_2O extract of the anaerobic soil metabolism, and the 24 min isomer peak from the 20:80 ACN/H_2O solution corresponds to the 18 min isomer peak in the ACN/H_2O extract of the anaerobic soil metabolism.

Thus, the compound codes and structures of the BF 500-4 isomers found during pyraclostrobin anaerobic soil metabolism could finally be determined.

III. CONCLUSION

As a results of these investigations, the compound codes and isomeric structures of BF 500-4 are clearly assigned. The environmental significance of these isomers is however considered low since BF 500-4 itself is a very short lived intermediate within the pyraclostrobin degradation route in soil. It could only be formed when pyraclstrobin is sprayed into an anoxic water compartment. And only then the isomerization could take place. This scenario is very unlikely to occur under realistic agricultural field conditions. Furthermore, the isomers proved to be interconvertible and can thus be considered as one metabolite entity.

Summary of the maximum metabolite percentages reached during anaerobic soil metabolism of pyraclostrobin

Table 7.1.2.1.4-4: Maximum occurrence of pyraclostrobin soil metabolites in anaerobic soil studies

metabolite	BASF DocID	study	label	soil	maximum % of applied radioactivity observed
BF 500-3	1999/10079 [2]	anaerobic soil	tolyl	Bruch West	96
	1999/11103 [2]		chlorophenyl	Bruch West	80
BF 500-4	1999/10079 [2]	anaerobic soil	tolyl	Bruch West	11
	1999/11103 [2]		chlorophenyl	Bruch West	7
500M74 [1]	1999/10079 [2]	anaerobic soil	tolyl	Bruch West	11
	1999/11103 [2]		chlorophenyl	Bruch West	4
500M75 [1]	1999/10079 [2]	anaerobic soil	tolyl	Bruch West	5
	1999/11103 [2]		chlorophenyl	Bruch West	7
BF 500-5	1999/11103 [2]	anaerobic soil	chlorophenyl	Bruch West	7.7

[1] isomers of BF 500-4 (500M74 = peak 16 min; 500M75 = peak 18 min)
[2] already peer-reviewed during previous Annex I listing process

The new best-fit $DegT_{50}$ values are listed together with the parent data in Table 7.1.2.1.3-12.

CA 7.1.2.2 Field studies

CA 7.1.2.2.1 Soil dissipation studies

The already peer-reviewed field soil dissipation studies from 1999 [*old EU Dossier, A II M 7.1.1.2.2/1, Kellner O., Zangmeister W. - BASF DocID 1999/11301; old EU Dossier, A II M 7.1.1.2.2/2, Kellner O., Zangmeister W. - BASF DocID 1999/11292*] with results from six locations (3 in Germany, 2 in Spain and 1 in Sweden) are still considered valid. However, they were performed under test conditions where surface processes were not excluded. Nevertheless, the study results are considered suitable for derivation of persistence endpoints. Therefore, new kinetic evaluations were performed according to current FOCUS and EFSA guidelines.

Report: CA 7.1.2.2.1/1
Eickler B., 2014b
Kinetic evaluation of two field dissipation studies with BAS 500 F - Pyraclostrobin conducted in Germany, Sweden and Spain: Determination of trigger and modeling endpoints according to FOCUS Degradation Kinetics and EFSA
2014/1093423

Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0, EFSA Guidance to obtain DegT50 values in soil (2013)

GLP: no

Note: This study, which was not listed in the application submitted for renewal of approval, is replacing BASF DocID 2011/1142490, which was listed in the application. The reason for submission is provided below.

Executive Summary

The dissipation behavior of pyraclostrobin in soil has been investigated in two field dissipation studies [*BASF DocID 1999/11301; BASF DocID 1999/11292*]. The purpose of the evaluation was to analyze the dissipation behavior of pyraclostrobin in six soils under different climatic conditions and to derive non-normalized trigger and normalized modeling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA guidance on estimating $DegT_{50}$ values in soil for modeling purposes.

The field trials were situated in different regions of Europe (Germany, Spain and Sweden), considering a range of different soils and climatic conditions. The two field dissipation trials conducted in Spain are potentially sufficient for the calculation of dissipation rates, but both are not representative with respect to EU climate conditions (following CTB criteria), and the estimated half-lives are not sufficient for modeling purposes. Hence, data from the Spanish trials were excluded from the evaluation for derivation of modeling endpoints. However, best-fit endpoints were estimated as field trials can be assumed to represent worst-case conditions.

Kinetic evaluation of pyraclostrobin dissipation observed in the six field trials was performed in order to derive field dissipation parameters that are adequate to be used as trigger endpoints. The non-normalized field half-lives ($DisT_{50}$) for pyraclostrobin ranged from 5.2 to 55.8 days. The corresponding $DisT_{90}$ values ranged from 80.3 to 347.8 days.

Kinetic evaluation of pyraclostrobin dissipation observed in the four field trials from Germany and Sweden was performed to derive appropriate field half-lives for environmental fate modeling. Prior to kinetic evaluation, the studies were normalized to reference conditions (pF2, 20°C) by time-step normalization and kinetic evaluation was performed on the normalized dataset. Normalized (pF2, 20°C) field half-lives ($DegT_{50}$) for pyraclostrobin ranged from 15.5 to 32.2 days.

I. MATERIAL AND METHODS

The kinetic evaluation was conducted for six field trials with pyraclostrobin from the data of two field dissipation studies [*Kellner O., Zangmeister W. – BASF DocID 1999/11301; Kellner O., Zangmeister W. – BASF DocID 1999/11292*]. The trials were situated in different regions of Europe (Germany, Spain, Sweden) considering a range of different soils and climatic conditions. Pyraclostrobin was applied as EC formulation BAS 500 01 F to bare soil. The nominal application rate was 250 g a.s. ha^{-1} at all trial sites. Soil samples were collected at several post-application intervals to a depth of 50 cm and analyzed for pyraclostrobin.

Characteristics of the soils from the trial sites were determined from soil samples taken before application to a depth of 25 cm. Soil characteristics are summarized in Table 7.1.2.2.1-1.

Table 7.1.2.2.1-1: Characteristics of the field dissipation studies

	Trial					
	D05/02/97, Germany	**D08/01/97, Germany**	**DU2/02/97, Germany**	**ALO/01/98, Spain**	**ALO/02/98, Spain**	**HUS/02/98, Sweden**
pH ($CaCl_2$)	6.2	6.8	5.6	7.6	7.6	5.8
Organic carbon [%]	1.1	2.48	0.8	0.6	0.9	1.4
Maximum water holding capacity MWHC [g H_2O /100 g dry weight]	39.2	50	41	40	42	36
Particle size distribution [%]						
< 2 µm (clay)	13.6	25.7	11	17	20	11
2 - 63 µm (silt)	80.8	71.0	22	24	37	9
63 - 2000 µm (sand)	5.6	3.3	67	59	43	80

For the kinetic fits, the measured concentrations were scaled to %-values in order to minimize the effect of scale in the fitting procedure. Measured soil residues after modification of LOQ data according to FOCUS kinetics [*FOCUS (2006)*] used for kinetic analysis are presented in Table 7.1.2.2.1-2.

Table 7.1.2.2.1-2: Concentration of pyraclostrobin in soil of the field trials after modification of LOQ data for kinetic modeling following FOCUS

D05/02/97							
DAT[a]	**0**	**14**	**26**	**53**	**96**	**173**	**350**
Residue [g ha^{-1}]	155	140	107	40	18	7.5[b]	-
Residue [%][c]	100	90.3	69.0	25.8	11.6	4.8	-
D08/01/97							
DAT[a]	**0**	**12**	**26**	**64**	**98**	**182**	**362**
Residue [g ha^{-1}]	269	152	123	54	30	-[d]	7.5[b]
Residue [%][c]	100	56.5	45.7	20.1	11.2	-[d]	2.8
DU2/02/97							
DAT[a]	**0**	**12**	**29**	**57**	**96**	**174**	**347**
Residue [g ha^{-1}]	223	92	105	59	7.5[b]	-	-
Residue [%][c]	100	41.3	47.1	26.5	3.4	-	-
ALO/01/98							
DAT[a]	**0**	**14**	**30**	**60**	**98**	**182**	**349**
Residue [g ha^{-1}]	250	61	36	37	24	7.5[b]	-
Residue [%][c]	100	24.4	14.4	14.8	9.6	3.0	-
ALO/02/98							
DAT[a]	**0**	**15**	**30**	**63**	**99**	**182**	**356**
Residue [g ha^{-1}]	123	53	60	53	48	23	7.5[b]
Residue [%][c]	100	43.1	48.8	43.1	39.0	18.7	6.1
HUS/02/98							
DAT[a]	**0**	**16**	**31**	**59**	**100**	**177**	**351**
Residue [g ha^{-1}]	122	88	102	56	34	7.5[b]	-
Residue [%][c]	100	72.1	83.6	45.9	27.9	6.1	-

[a] days after treatment
[b] 0.5 x LOQ
[c] % of concentration at day 0
[d] samples lost accidentally

Normalization procedure

Evaluation of the suitability of field dissipation data for normalization was performed according to the evaluation criteria for normalization compiled by the Dutch regulatory authority (CTB criteria).

For the four field dissipation trials conducted in Germany and Sweden, all criteria were fulfilled. The Spanish field trials did not fulfil the crucial evaluation criteria 5 on representative climate for European conditions. The locations were potentially sufficient for the performance of dissipation trials, but had a long period without a noteworthy degradation. This phenomenon, which is not characteristic for pyraclostrobin, can be attributed to the late application dates (7th May for Manzanilla, 26th May for Alcala del Rio), that were followed by a long dry period that reduced microbial activity and thus microbial degradation in soil. Such climatic conditions are not typical for use of fungicides such as pyraclostrobin. Hence, these trials are not representative and the estimated half-lives would not be sufficient for modeling purposes. As climate conditions were not representative for the EU, data from the Spanish trials were excluded from the evaluation for derivation of modeling endpoints. However, the evaluation was conducted for best-fit endpoints, as the trial sites are assumed to represent worst-case conditions.

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily temperatures as calculated by FOCUS-PEARL 3.3.3 and a reference temperature of 20°C using the Q_{10} approach as described in the report of the FOCUS soil modeling working group [*FOCUS (1997): Soil persistence models and EU registration. The final report of the work of the Soil Modeling Work group of FOCUS (Forum for the Co-ordination of pesticide fate models and their Use)*]. The Q_{10} response function was applied for temperatures above 0°C. For field temperatures below 0°C, it was assumed that no degradation occurs (Equation 7.1.2.2-1 a). For the evaluation, the EFSA opinion on the default Q_{10} value [*EFSA (2007): Scientific Opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. The EFSA Journal (2007) 622, 1-32*] was followed and a Q_{10} value of 2.58 was included in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 3.3.3 and the reference soil moisture (θ_{ref}) (Equation 7.1.2.2-1 b).

Equation 7.1.2.2-1: Calculation of normalized day length based on combination of soil moisture and soil temperature correction factors

a)

$$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact-Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

b)

$$f_{moist} = \begin{cases} \left(\frac{\theta_{act}}{\theta_{ref}}\right)^{B} & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:

D_{norm} =	normalized day length (temperature and moisture)	
f_{temp} =	temperature correction factor	[-]
f_{moist} =	moisture correction factor	[-]
D =	1 d	[days]
T_{act} =	actual soil temperature	[C°]
T_{ref} =	reference temperature (20°C)	[C°]
Q_{10} =	factor of increase of degradation rate with an increase in temperature of 10°C (Q_{10} = 2.58)	[-]
θ_{act} =	actual soil moisture (vol. water content)	[m^3 m^{-3}]
θ_{ref} =	reference soil moisture at pF2	[m^3 m^{-3}]
B =	exponent of the moisture response function, B = 0.7	[-]

The trial data were normalized by time-step correction (varying the 'day length' virtually by applying the correction factors to the time given in days). The corrected day lengths for the time-step normalization are shown in Table 7.1.2.2.1-3.

Table 7.1.2.2.1-3: Corrected time from the time-step normalization and concentration of pyraclostrobin in soil of the field trials after modification of LOQ data for kinetic modeling following FOCUS

D05/02/97							
DAT[a]	**0**	**14**	**26**	**53**	**96**	**173**	**350**
Corr. DAT[b]	*0*	*5.5*	*10.6*	*25.1*	*54.9*	*106.9*	*145.4*
D08/01/97							
DAT[a]	**0**	**12**	**26**	**64**	**98**	**182**	**362**
Corr. DAT[b]	*0*	*7.7*	*16.2*	*43.3*	*74.0*	*119.7*	*172.7*
DU2/02/97							
DAT[a]	**0**	**12**	**29**	**57**	**96**	**174**	**347**
Corr. DAT[b]	*0*	*5.5*	*14.6*	*30.6*	*56.3*	*100.7*	*141.7*
HUS/02/98							
DAT[a]	**0**	**16**	**31**	**59**	**100**	**177**	**351**
Corr. DAT[b]	*0*	*7.9*	*16.3*	*33.3*	*62.2*	*95.5*	*126.5*

[a] days after treatment
[b] corrected time (days after treatment) for time-step normalization

To derive appropriate field half-lives for environmental fate modeling, the recommendations provided in the EFSA guidance to obtain DT_{50} values in soil [*EFSA (2010): Guidance for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ values of plant protection products in soil. EFSA Journal 2010;8(12):1936, 67 pp.*] were considered, i.e. splitting of field dissipation trials into two parts, i.e. before and after 10 mm of rain has fallen since application.

The day when 10 mm of rainfall were reached in the field studies is given in Table 7.1.2.2.1-4 for the original sampling days and normalized days after treatment, respectively.

Table 7.1.2.2.1-4: Day at which 10 mm of rainfall was reached

Trial site	**Day when 10 mm rainfall was reached**	
	DAT	**DAT_{norm}**
D05/02/97	3	1.1
D08/01/97	7	5.4
DU2/02/97	10	4.4
HUS/02/98	9	3.9

Kinetic modeling

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting [*BASF DocID 2007/1062781*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

Datasets were prepared for kinetic evaluation as follows:

- Values below the quantification or detection limit were treated as recommended by the FOCUS workgroup [*see FOCUS (2006), chapter 6.1.4 and chapter 8.3.1*]. As the LOD has not been reported, samples below LOQ (= 15 g ha^{-1}) were set to 0.5 x LOQ. Furthermore, all samples after the first non-detect (< LOQ) were omitted as no positive detections were made later. For the kinetic fits, the measured concentrations were scaled to %-values in order to minimize the effect of scale in the fitting procedure.
- For each sampling point, the residues of the single core segments given in mg kg^{-1} were transformed to residues given in g ha^{-1} considering the thickness of the respective segment and the default soil density of 1.5 g cm^{-3}.

Kinetic models included in the evaluations

The kinetic models which can be employed for these evaluations were described by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]:

- Single-First-Order (SFO); Box 5-1 in *FOCUS (2006)*
- Gustafson and Holden (FOMC); Box 5-2 in *FOCUS (2006)*
- Double first-order in parallel (DFOP); Box 5-4 in *FOCUS (2006)*
- Hockey-stick (HS); Box 5-3 in *FOCUS (2006)*

According to the EFSA guidance [*EFSA (2010)*], the kinetic models named above with exception of the FOMC model are proposed for the calculation of the DT_{50} for normalized decline curves.

The appropriateness of a distinct kinetic model to describe soil degradation can be tested according to the following criteria recommended by FOCUS [*FOCUS (2006), chapter 6.3.1*]:

- Visual assessment of goodness-of-fit
- Statistical evaluation of the goodness-of-fit by estimation of the error percentage at which the χ^2 test is passed [*Equation 6-2 in FOCUS (2006)*]
- Estimation of the reliability of parameter estimation using the t-test to evaluate whether estimated degradation parameters differ significantly from zero [*Equation 6-3 in FOCUS (2006)*]

A kinetic model is considered appropriate if the residuals are randomly distributed around zero, the χ^2 error value is <15% and the estimated degradation parameters differ from zero as outlined by *FOCUS (2006)*.

II. RESULTS AND DISCUSSION

A summary of the adequate $DisT_{50}$ and $DisT_{90}$ values to be used as trigger endpoints is given in Table 7.1.2.2.1-5. The visual fits are acceptable for all trials, moreover the χ^2 error is mostly < 15% and within range of acceptance for laboratory standards.

Table 7.1.2.2.1-5: Summary of best-fit endpoints of pyraclostrobin

Field trial	Soil type (DIN)	Best-fit kinetic model	$DisT_{50}$ [d]	$DisT_{90}$ [d]
D05/02/97, Germany	Loamy sand	SFO	33.3	110.5
D08/01/97, Germany	Loamy silt	FOMC	18.4	130.4
DU2/02/97, Germany	Loamy sand	SFO	24.2	80.3
ALO/01/98, Spain	Sandy loam / loamy sand	DFOP	5.2	86.9
ALO/02/98, Spain	Sandy loam	DFOP	14.2	347.8
HUS/02/98, Sweden	Loamy sand	SFO	55.8	185.3

The rate constants (*k*) and field half-lives ($DegT_{50}$) adequate to be used in environmental fate modeling are summarized in Table 7.1.2.2.1-6. The visual fits are acceptable for all trials; moreover the χ^2 error is < 15%.

Table 7.1.2.2.1-6: Summary of endpoints for use in modeling of pyraclostrobin

Field trial	Soil type (DIN)	Kinetic model	Rate k_{slow} [d^{-1}]	$DegT_{50}$* [d]
D05/02/97, Germany	Loamy sand	DFOP	0.04465	15.5
D08/01/97, Germany	Loamy silt	HS	0.02665	26.0
DU2/02/97, Germany	Loamy sand	HS	0.02797	24.8
HUS/02/98, Sweden	Loamy sand	DFOP	0.02154	32.2

* calculated from k_{slow}

III. CONCLUSION

Kinetic evaluation of six field trials with pyraclostrobin was conducted in order to derive trigger and modeling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA guidance on estimating $DegT_{50}$ values in soil for modeling purposes. The field trials were situated in different regions of Europe (Germany, Spain and Sweden), considering a range of different soils and climatic conditions. The two field dissipation trials conducted in Spain were found to be not representative and were excluded from the evaluation for derivation of modeling endpoints. However, best-fit endpoints were estimated as field trials can be assumed to represent worst-case conditions.

Kinetic evaluation of pyraclostrobin dissipation observed in the six field trials was performed in order to derive field dissipation parameters that are adequate to be used as trigger endpoints. The non-normalized field half-lives (DT_{50}) for pyraclostrobin ranged from 5.2 to 55.8 days. The corresponding DT_{90} values ranged from 80.3 to 347.8 days.

Kinetic evaluation of pyraclostrobin dissipation observed in the four field trials from Germany and Sweden was performed to derive appropriate field half-lives for environmental fate modeling. Normalized (pF2, 20°C) field half-lives (DT_{50}) for pyraclostrobin ranged from 15.5 to 32.2 days.

A **new field dissipation study** with pyraclostrobin was initiated in order to cover the requirements of the EPA/NAFTA guidelines (implemented in the new data requirements for EU registration) and in order to provide additional degradation rates in the soil matrix for modeling following the new EFSA guidance [*EFSA (2010)*], i.e. excluding soil surface processes.

Report:	CA 7.1.2.2.1/2 Bayer H.,Marwitz A., 2014a Field soil dissipation study of BAS 500 F (Pyraclostrobin) in the formulation BAS 500 14 F on bare soil at four different sites in Europe, 2011-2012 2013/1348661
Guidelines:	NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006), EPA 835.6100, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), EFSA Guidance to obtain DegT50 values in soil (2010), SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: This study was not correctly listed in the application submitted for renewal of approval. Authors have changed after submission of the application.

Executive Summary

The dissipation of pyraclostrobin (BAS 500 F) and its metabolites BF 500-6 (500M01), BF 500-7 (500M02) and BF 500-3 (500M07) under field conditions was investigated at four sites in Europe representative of Northern, Central and Southern EU conditions (Denmark, Germany, Italy and France). All sites represent typical regions of agricultural practice representative for the use of pyraclostrobin. The trial sites consisted of an untreated and a treated plot, the latter being subdivided into three subplots that were assigned for replicates.

The product BAS 500 14 F, formulated as an emulsifiable concentrate (EC), was applied to bare soil in a single application at a nominal rate of 250 g a.s. ha^{-1} using a target water volume of 300 L ha^{-1}. Applications were conducted in May 2011 using a calibrated boom sprayer. The actual application rates for each trial (determined by quantifying the amount of spray discharged) ranged from 245 to 256 g a.s. ha^{-1}, with an average of 252 g a.s. ha^{-1}. Results from spray broth analysis for the individual trial sites revealed mean concentrations between 106 and 114% of the nominal value with an average of 110% across all sites. Dose verification conducted via application monitors yielded mean recovery values for the individual trial sites ranging from 101 to 117% of the target rate and an average recovery of 109% across all sites.

Immediately after application of the test item, the plots were covered with a layer of sand of approximately 6 mm depth to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted until complete coverage of the soil surface was achieved. The layer of sand was controlled up to at least 27 days after application and was renewed when needed. It remained intact until at least 27 days. Within this time period, the individual fields received a cumulative precipitation (rain and irrigation) of 49 mm (Denmark), 71 mm (Germany), 48 mm (Italy) and 50 mm (Southern France), respectively.

No tillage or fertilization was performed during the course of the study and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of herbicides. Rainfall was supplemented with irrigation at sites in Denmark (91 mm), Germany (129 mm), Italy (439 mm) and Southern France (319 mm).

Soil samples were taken up to 540 days after application and down to a maximum soil depth of 50 cm. Soil cores were cut into 10 cm sections. Soil segments of the same depth and subplot from a defined sampling event were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis. All soil specimens were stored at about -18°C within a maximum of 8 hours 30 minutes after sampling and remained frozen until analysis.

In order to demonstrate stability of the residues in soil during storage and any shipments, shipment verification specimens were prepared at selected sampling occasions by fortifying untreated soil from the field sites with known amounts of pyraclostrobin. These specimens were stored and shipped under the same conditions as the actual residue specimens. Analysis of the shipping verification specimens on pyraclostrobin yielded an average recovery value of 103% across all sites confirming residue stability of pyraclostrobin during all storage and shipment procedures.

Soil specimens and shipping verification samples were analyzed for pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3 according to BASF method L0166/01. Application monitors (Petri dish specimens) were analyzed for pyraclostrobin according to BASF method L0161/01. The analytical methods involved extraction of the soil with acetonitrile and acetonitrile/water (80/20, v/v) or with acetonitrile/water (80/20, v/v) only. The final determination of the analytes was performed by LC-MS/MS with a limit of quantification (LOQ) of 0.001 mg kg^{-1} for each analyte. The limit of detection (LOD) was set at 0.0002 mg kg^{-1} (20% of LOQ).

Residue concentrations of the individual analytes in the untreated control samples were below the LOQ, proving that there were no interferences of the untreated soil material with the analytical procedures used. Procedural recovery experiments performed with untreated field soils spiked with the four analytes at concentration levels of 0.001, 0.01 and 0.1 mg kg^{-1} yielded mean recovery rates between 97.0 and 104.2% for the individual analytes, confirming the validity of the analytical method used in this study.

Residue values of pyraclostrobin and metabolites BF 500-6, BF 500-7 and BF 500-3 in mg kg^{-1} dry soil were converted to residue rates in g ha^{-1} taking into account the actual dry soil density of the individual field samples. Afterwardsb they were summed up for all depths between 0 and 50 cm that has been analyzed. Residue values were not corrected for procedural recoveries except for results obtained from Petri dish analysis.

Pyraclostrobin degraded under field conditions in soil at all four European field sites. The total amount of pyraclostrobin residues detected in the soil profiles decreased from an average of 196 g ha^{-1} at day 0 to an average of 17 g ha^{-1} after 1.5 years.

Pyraclostrobin residues were almost exclusively found in the top 0-10 cm soil layer, with only small amounts of the compound sporadically detected in the 10-20 cm layer. No residues above the LOQ were detected below 20 cm in any sample. It can be concluded that pyraclostrobin does not show any significant tendency to move into deeper soil layers, indicating low potential for pyraclostrobin residues to leach to groundwater.

Metabolites BF 500-6, BF 500-7, and BF 500-3 were detected at all sites reaching maximum amounts of 43 g ha^{-1}, 29 g ha^{-1}, and 2.4 g ha^{-1}, respectively. They were exclusively found in the top 0-20 cm soil layer, except for one single detection of BF 500-6 at the LOQ in the 30-40 cm layer at the site in Denmark. Apart from that, no residues of the 3 metabolites above the LOQ were observed in deeper soil layers in any sample at any site.

No calculation of dissipation times is provided in the summarized report. A detailed kinetic evaluation of the degradation behavior of pyraclostrobin and its metabolites in the four European field soils is provided in separate modeling reports.

I. MATERIAL AND METHODS

1. Test Material

Test item (formulation):	BAS 500 14 F
Active substance (a.s.):	Pyraclostrobin (BAS 500 F, Reg.No. 304428)
Type of formulation:	EC
Batch No.:	0003191314
Content of a.s.:	239.3 g L^{-1} (nominal 250.0 g L^{-1})
Expiration date:	June 30, 2011

2. Test sites

The dissipation of pyraclostrobin under field conditions was investigated at four sites in Europe representative of Northern, Central, and Southern EU conditions. Trials were performed in Denmark (L110057), Germany (L110058), Italy (L110059), and Southern France (L110060). The homogeneity of the upper soil layer was verified prior to the start of the trials. The site characteristics are presented in Table 7.1.2.2.1-7. Soil parameters were determined from untreated soil samples taken from the fields following segmentation according to the soil horizons. Soil taxonomy was determined on the basis of regional soil maps.

Table 7.1.2.2.1-7: Characteristics of the trial sites used to investigate the field dissipation of pyraclostrobin

Trial	**L110057**			**L110058**		
Location	**Middelfart, Denmark**			**Lentzke, Germany**		
Soil properties	0 - 30 cm	30 - 60 cm	60 - 90 cm	0 - 40 cm	40 – 65 cm	65 - 90 cm
Soil class (DIN 4220)	Poor silty sand (Su2)	Pure sand (Ss)	Pure sand (Ss)	Poor silty sand (Su2)	Poor silty sand (Su2)	Medium loamy sand (Sl3)
sand [%]	86.1	93.4	97.3	77.6	75.3	64.0
silt [%]	11.1	5.8	3.0	20.0	21.6	26.3
clay [%]	2.7	1.0	0.1	2.4	3.2	9.7
Soil class (USDA)	Sand	Sand	Sand	Loamy sand	Loamy sand	Sandy loam
sand [%]	89.6	93.7	97.1	80.1	78.2	69.4
silt [%]	9.4	5.0	2.0	16.7	17.9	17.9
clay [%]	1.1	1.2	0.8	3.1	3.8	12.6
Total organic C [%]	0.98	0.42	0.23	0.64	0.18	0.17
Organic matter [%] *	1.69	0.72	0.40	1.10	0.31	0.29
pH ($CaCl_2$)	6.08	6.14	6.00	6.44	6.66	6.86
pH (H_2O)	7.38	7.11	7.33	7.23	7.59	7.79
CEC [mval Ba/100g dry weight]	7.0	3.2	2.3	5.0	3.4	7.0
MWHC [g/100g dry weight]	36.1	34.8	28.4	33.9	39.3	30.3
pF 2.0 [g/100g dry weight]	10.3	5.7	4.5	10.0	14.4	20.9
pF 2.5 [g/100g dry weight]	8.2	5.3	3.9	10.0	10.4	14.5
Dry bulk density [g cm^{-3}]**	1.36	-	-	1.56	-	-
Soil taxonomy	Haplic Luvisol			Albic-Luvisols, Albeluvisol, Cambisol		

Trial	**L110059**			**L110060**	
Location	**Poggio Renatico, Italy**			**Barry d´Islemade, France**	
Soil properties	0 - 30 cm	30 - 60 cm	60 - 90 cm	0 - 40 cm	40 – 90 cm
Soil class (DIN 4220)	Sandy loamy silt (Uls)	Sandy loamy silt (Uls)	Poor silty sand (Su2)	Silty loamy sand (Slu)	Poor silty sand (Su2)
sand [%]	25.7	29.9	78.1	47.2	79.5
silt [%]	57.7	55.6	17.5	42.5	17.3
clay [%]	16.6	14.5	4.2	10.2	3.1
Soil class (USDA)	Silt loam	Loam	Loamy sand	Sandy loam	Loamy sand
sand [%]	33.2	36.8	82.4	54.1	83.0
silt [%]	50.2	48.1	13.8	35.4	13.0
clay [%]	16.6	15.0	3.9	10.5	3.8
Total organic C [%]	1.18	0.63	0.23	0.99	0.85
Organic matter [%] *	2.03	1.09	0.40	1.71	1.47
pH ($CaCl_2$)	7.66	7.83	7.98	7.64	7.88
pH (H_2O)	8.48	8.72	8.96	8.52	8.93
CEC [mval Ba/100g dry weight]	14.5	13.3	10.8	10.0	5.5
MWHC [g/100g dry weight]	34.2	32.3	30.3	43.1	38.1
pF 2.0 [g/100g dry weight]	32.6	26.1	15.6	24.0	13.7
pF 2.5 [g/100g dry weight]	23.9	21.9	15.2	15.1	9.6
Dry bulk density [g cm^{-3}]**	1.33	-	-	1.87	-
Soil taxonomy	Calcari Endostagnic Fluvisols			Endoeutric Albeluvisol	

The sum of particle size may slightly differ from 100% due to rounding tolerances
* organic matter = organic carbon x 1.724
** samples taken at 10-20 cm depth (mean of 3 replicates)
CEC = cation exchange capacity
MWHC = maximum water holding capacity

The selected fields represented typical regions of agricultural practice with soils representative for growing crops including cereals, and had been under cultivation for many years. The sites were flat without any significant slope. Before commencement of the first sampling, the soil at each trial site was prepared as for sowing and was rolled if considered necessary, but then was left fallow.

No product containing the active substance of the test item had been used on the test plots in the last three years.

3. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size: 30 - 108 m^2) and one treated plot (size: 306 - 648 m^2). The treated plot consisted of three equal sized subplots A, B, and C that were assigned for replicates.

The product formulated as an emulsifiable concentrate (EC), was broadcast applied to bare soil in a single application at a nominal rate of 250 g a.s. ha^{-1} using a target water volume of 300 L ha^{-1}. Applications were conducted in May 2011 using a calibrated boom sprayer. Treated plots were three-fold replicated with a subplot size ranging from 108 to 216 m^2. For each treated replicate, a separate spray mixture was prepared and the test item was applied to each subplot individually. Each spray mixture was visually checked for homogeneity, and small aliquots of the spray mixture were taken before and after application of each subplot for later analysis.

The actual application rates (determined by quantifying the amount of spray discharged) ranged from 245 to 256 g a.s. ha^{-1} averaged over the three replicates of each treated plot. In addition, the dose was verified by means of sampling Petri dishes filled with standard soil (approximately 50 g per dish, sieved to 2 mm). The Petri dishes with an inner diameter of 10.8 cm were placed on the treated plot (ten in each subplot) before application. On completion of the application, the petri dishes were closed with a lid, sealed with adhesive tape, chilled on blue ice or dry ice and placed in freezer storage within 3.5 hours. Further details of application are presented in Table 7.1.2.2.1-8.

Table 7.1.2.2.1-8: Application parameters of field trial sites treated with BAS 500 14 F (EC)

Trial Country	Application method	No. of appli-cations	Subplot (m^2)	Application rate per treatment				Application date
				nominal	actual*	dose verification**		
				[g a.s ha^{-1}]	[g a.s. ha^{-1}]	[g a.s ha^{-1}]	% of nominal	
L110057 Denmark	broadcast spray to bare soil	1	A (108)	250	248	293	117	04-May-2011
			B (108)	250	261	232	93	
			C (108)	250	258	291	116	
			Average	**250**	**256**	**272**	**109**	
L110058 Germany	broadcast spray to bare soil	1	A (102)	250	252	264	106	12-May-2011
			B (102)	250	264	316	126	
			C (102)	250	242	274	110	
			Average	**250**	**253**	**285**	**114**	
L110059 Italy	broadcast spray to bare soil	1	A (102)	250	243	201	80	02-May-2011
			B (102)	250	245	268	107	
			C (102)	250	248	289	116	
			Average	**250**	**245**	**253**	**101**	
L110060 France (South)	broadcast spray to bare soil	1	A (216)	250	253	296	118	18-May-2011
			B (216)	250	257	268	107	
			C (216)	250	253	315	126	
			Average	**250**	**254**	**293**	**117**	

* determined by calculation of spray liquid applied
** determined by means of Petri dishes filled with soil

Immediately after application of the test item and before subsequent soil sampling, the control plot and the treated replicates were covered with a thin layer of sand to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted manually or using a sand spreader until complete coverage of the soil surface was achieved. Fine or medium grained sand was used. The thickness of the sand layer necessary for complete coverage of the soil was approximately 6 mm (range: 4-10 mm) averaged across the sites. The layer of sand was controlled up to at least 27 days after application and was renewed when needed. It remained intact until at least 27 days. Within this time period of 27 days, the individual fields received a cumulative precipitation (rain and irrigation) of 49 mm (Denmark), 71 mm (Germany), 48 mm (Italy), and 50 mm (Southern France), respectively.

No tillage or fertilization was performed during the course of the study from first to last sampling and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of metribuzin, 2,4-D, MCPA, and/or glyphosate.

Rainfall was supplemented with irrigation at sites in Denmark (91 mm), Germany (129 mm), Italy (439 mm) and Southern France (319 mm).

Actual weather data are based on records of appropriate weather stations located on-site. Monthly summary results on temperature, precipitation and irrigation are presented in Table 7.1.2.2.1-9.

Table 7.1.2.2.1-9: Summary of climatic conditions at field trial sites used to investigate the dissipation of pyraclostrobin

Trial	L110057			L110058			L110059			L110060		
Location	Middelfart			Lentzke			Poggio Renatico			Barry d´Islemade		
	Denmark			Germany			Italy			France (South)		
Climatic conditions	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]
Month		∑	∑		∑	∑		∑	∑		∑	∑
May 11	12.1	48.8	0.0	14.9	36.0	0.0	18.7	28.7	19.4	18.5	2.2	0.0
Jun 11	15.2	67.0	0.0	17.1	80.0	22.4	22.0	75.4	43.4	18.6	49.8	25.0
Jul 11	15.6	124.1	11.3	17.0	232.0	0.0	23.3	49.3	21.6	19.3	81.8	25.0
Aug 11	16.1	129.6	0.0	17.9	76.2	0.0	25.0	0.0	40.4	21.8	0.4	49.0
Sep 11	13.9	85.6	0.0	15.0	46.4	0.0	21.8	30.0	41.9	19.7	14.2	56.0
Oct 11	9.9	48.6	0.0	9.4	49.2	0.0	12.8	24.8	30.3	13.7	17.2	37.0
Nov 11	6.5	26.2	0.0	3.7	7.8	0.0	7.3	32.4	0.0	11.2	19.2	38.0
Dec 11	4.1	98.0	0.0	4.1	77.6	0.0	3.4	14.4	0.0	7.4	66.4	0.0
Jan 12	2.5	73.2	0.0	1.4	66.0	0.0	1.1	9.4	0.0	5.7	33.2	0.0
Feb 12	0.0	19.8	0.0	-2.5	29.6	0.0	0.6	26.9	0.0	0.7	2.4	0.0
Mar 12	6.1	19.2	12.3	6.7	9.8	0.0	10.9	0.3	47.6	10.2	28.4	19.0
Apr 12	6.1	49.6	14.7	8.0	27.8	6.8	12.5	65.6	19.3	10.7	101.0	19.0
May 12	11.7	22.4	10.4	14.1	30.0	43.6	17.4	81.9	21.2	16.6	70.8	0.0
Jun 12	12.6	128.0	17.4	15.2	59.8	7.3	23.6	14.0	47.1	20.0	58.6	0.0
Jul 12	15.6	70.0	8.1	17.9	109.4	0.0	25.6	3.8	37.3	20.1	31.2	18.0
Aug 12	16.7	59.8	16.7	17.9	19.6	25.9	26.0	0.0	39.9	22.6	67.6	0.0
Sept 12	12.9	78.4	0.0	14.0	30.6	23.4	19.6	86.9	5.4	18.0	20.2	17.0
Oct 12	10.1	76.8	0.0	10.2	54.8	0.0	16.0	36.8	24.7	14.4	36.6	16.0
Nov 12	-	-	-	-	-	-	-	-	-	10.6	5.0	0.0

Weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling)

Historical (long-term) weather data on precipitation and average air temperature from at least 10 years were taken from official weather stations located nearby (8-34 km distance to trial site). The historical and actual data each averaged over the complete duration of the individual trials are presented in Table 7.1.2.2.1-10.

The actual air temperature recorded at the four field sites during the study period was slightly higher than the historic values. Whereas the sites in Northern Europe (Denmark and Germany) received more rain during the study period compared to the historic values, rainfall was less than the historic values in Southern Europe (Italy and Southern France). Due to additional irrigation, the Southern sites as well received a total water input sufficient to allow the cultivation of crops like cereals.

Table 7.1.2.2.1-10: Summary of historical and actual weather data at field trial sites averaged over entire trial duration (1.5 years)

Trial Country	T_{mean} Air [°C] (average over trial period)		Precipitation [mm] (sum over trial period)		Irrigation [mm]	Sum of actual precipitation and irrigation [mm]	% of historic precipitation
	Historic*	Actual	Historic*	Actual			
L110057 Denmark	9.9	10.4	982	1225	91	1316	134
L110058 Germany	10.5	11.2	798	1043	129	1172	147
L110059 Italy	15.3	16.0	931	581	439	1020	110
L110060 France (South)	14.4	14.7	1141	706	319	1025	90

* at least over ten years

4. Sampling

Replicate soil specimens (8 per treated subplot and 10 or 15 per control plot) were taken at intervals up to 540 days and down to a maximum soil depth of 50 cm. At day 0, immediately after application, the treated plots were sampled down to 10 cm only. The detailed sampling intervals are presented in Table 7.1.2.2.1-11.

Table 7.1.2.2.1-11: Summary of sampling intervals at each field trial site

Trial	Country	Sampling intervals [days after treatment]
L110057	Denmark	0, 6, 13, 28, 58, 85, 119, 148, 175, 230, 300, 363, 448, 539
L110058	Germany	-2, 0, 8, 13, 27, 62, 90, 117, 146, 174, 230, 298, 350, 446, 530
L110059	Italy	-3, 0, 7, 14, 28, 58, 92, 121, 147, 182, 234, 302, 353, 444, 539 (CTRL), 540 (TRT)
L110060	France (South)	-1, 0, 7, 14, 30, 63, 93, 127, 155, 174, 245, 308, 364, 449, 534

CTRL = Control; TRT = Treated

Untreated specimens were collected from the control plot on three occasions, between 3 days before the application and the day of application down to a depth of 50 cm, and after about one and 1.5 years to a depth of 10 cm. The specimens were taken randomly from one third of the untreated plot each time. The 15 cores collected at the first sampling interval were taken using a common soil probe equipped with a plastic liner of 4.5 to 5.0 cm diameter except for 30-50 cm samples taken at the site in Italy that were sampled using liners with 2.5 cm diameter. The cores were sectioned into 10 cm segments and pooled by depth. The 10 cores taken after about one year and 1.5 years were collected with a metal tube of minimum 7.5 and maximum 9.8 cm diameter.

Treated soil specimens were taken randomly (from eight points of each of the three treated subplots A – C) and were pooled according to subplot and depth. All soil specimens from 0-10 cm depth being collected from the treated plots, were taken separately using a metal tube of minimum 7.5 to maximum 9.8 cm diameter, which was pressed into the ground, and collecting the soil with a spoon or similar device. Soil specimens deeper than 10 cm were collected through the center of the excavation hole contained by the metal tube, using a common soil corer fitted with a plastic liner of diameter 4.5 to 5.0 cm, except for 30-50 cm samples taken at the site in Italy, which were sampled using liners with 2.5 cm diameter. Sampling of the 10-50 cm cores was conducted in one run or in two consecutive steps. All soil cores collected with the soil probe were sectioned into 10 cm segments and pooled by depth.

In addition to the main sampling described above, a second complete sampling (double sampling) was carried out for back-up purposes. The double samples were directly put into the freezers at the field test sites and were sectioned into 10 cm segments prior to processing.

All soil specimens intended for residue analysis were stored at about -18°C within a maximum of 8 hours and 30 minutes after sampling and remained frozen through storage, shipping and processing until final analysis. Sample processing was conducted in frozen state in a mill together with dry ice.

Shipment verification specimens were prepared to demonstrate stability of the residues in soil during storage and through any shipping processes. The samples were prepared at three occasions by fortification of soil with 0.15 mg/kg pyraclostrobin and were subsequently handled in the same manner as the actual residue samples.

5. Analytical procedure

Field soil specimens and shipping verification samples were analyzed for pyraclostrobin and metabolites BF 500-6, BF 500-7, and BF 500-3 according to the validated BASF method L0166/01 [*CA 4.1.2/1, BASF DocID 2013/1184817*]. The analytical method involved extraction of the soil with acetonitrile and acetonitrile/water (80/20, v/v) and final determination of the analytes by LC-MS/MS. In case of BF 500-7, the sum of E- and Z-isomers was used for calibration and calculation of residues (peak areas of E- and Z-isomer summarized). The limit of quantification (LOQ) was 0.001 mg kg^{-1} for each individual analyte. The limit of detection (LOD) was set at 0.0002 mg kg^{-1} (20% of LOQ). Analysis of field soil specimens originating from the treated plots was conducted down to 50 cm and was performed until a maximum of 540 days after treatment (DAT).

Petri dish specimens were analyzed for pyraclostrobin according to the validated BASF method L0161/01 [*CA 4.1.2/2, BASF DocID 2010/1075848*]. The analytical method involved extraction of the soil with acetonitrile/water (80/20, v/v) and final determination of the analyte by LC-MS/MS. The LOQ for pyraclostrobin was 0.001 mg kg^{-1}.

Spray broth specimens were analyzed for pyraclostrobin according to validated BASF analytical method L0184/01 (previously named APL0500/03) [*CA 4.1.2/3, BASF DocID 2008/1042150*]. Analysis was performed by diluting aliquots of the application solutions with acetonitrile/water, acidification with formic acid and subsequent HPLC-MS analysis. The LOQ was 1 µg L^{-1} for pyraclostrobin.

Generally, for soil samples, a second mass transition was monitored for each analyte.

The validity of the analytical methods for soil samples was proven within the present study by analysis of untreated control and fortified samples within each analytical sample set.

6. Storage stability experiments

Storage stability of pyraclostrobin and its metabolites BF 500-6, BF 500-7, and BF 500-3 in frozen soil was investigated in a separate study [*CA 7.1.2.2.1/5, Tilting N. et al., BASF DocID 2014/1000723*] with soils originating from the individual trial sites of the present terrestrial field dissipation study.

7. Calculation of dissipation times

No calculation of dissipation times is provided in the study report. A detailed kinetic evaluation of the degradation behavior of pyraclostrobin and its metabolites in the four European field soils is presented in two separate modeling reports [*CA 7.1.2.2.1/3, Pape L., BASF DocID 2014/1105763 and CA 7.1.2.2.1/4, Pape L., BASF DocID 2014/1105764*].

II. RESULTS AND DISCUSSION

1. Spray broth concentration and application verification

Spray broth homogeneity was confirmed by visual check for all trials. In addition, spray mixtures sampled before and after application of each subplot were analyzed for pyraclostrobin. Analyzed concentrations averaged across the individual trial sites were in the range of 0.887 to 0.949 g L^{-1} corresponding to 106-114% of the target concentration of 0.833 g L^{-1}. The analytical results were not corrected for procedural recoveries and confirm the integrity of the test item used in the trials.

Application verification was conducted by means of Petri dishes filled with standard soil. Untreated and fortified specimens of this soil were analyzed with the applied Petri dishes from the field. Fortification levels of pyraclostrobin were at 0.5 and 5.0 mg kg^{-1}. Mean recoveries for each analyzed set of samples ranged from 103.2 to 110.5% with an overall mean recovery of 106.5 ± 3.9% (mean ± RSD, n = 14). Residues in blank specimens were not detectable above the LOQ of 0.001 mg/kg.

Residue levels of pyraclostrobin achieved on extraction and analysis of the application monitors (Petri dishes filled with soil) were corrected for the mean recovery of the respective analytical set and converted into residue rates (in g ha^{-1}) taking into account the area of the Petri dishes (91.6 cm^2) used. The obtained application rates for the individual trials ranged from 253 to 293 g ha^{-1} representing 101-117% of the target rate (see Table 7.1.2.2.1-8 for individual figures). The applied amount determined by the application monitors in these trials is slightly above the nominal value of 250 g ha^{-1}, which is in agreement with the results obtained from the spray broth samples.

2. Residues in field soil samples

Untreated soil specimens (control samples) of the respective soil depths from each trial were analyzed for residues of pyraclostrobin, BF 500-6, BF 500-7, and BF 500-3. No residues above the LOQ of any analyte were detected in any of the untreated control samples proving that there were no interferences of the untreated soil material with the analytical procedures used. Procedural recovery experiments performed with untreated field soil specimens spiked with the four analytes at concentration levels of 0.001, 0.01, and 0.1 mg kg^{-1} yielded mean recovery rates for the individual analytes between 97.0 and 104.2%, confirming the validity of the analytical method used in this study. Detailed results are summarized in Table 7.1.2.2.1-12.

Table 7.1.2.2.1-12: Method procedural recoveries

Analyte	Trial	n	Mean recovery ± RSD* [%]
Pyraclostrobin	L110057	50	101.9 ± 7.5
	L110058	43	101.1 ± 6.9
	L110059	63	101.0 ± 6.3
	L110060	46	99.1 ± 6.0
BF 500-6	L110057	48	101.8 ± 11.1
	L110058	43	103.0 ± 11.8
	L110059	62	97.7 ± 9.3
	L110060	46	100.1 ± 10.0
BF 500-7	L110057	48	102.2 ± 9.2
	L110058	43	104.2 ± 8.8
	L110059	62	102.6 ± 7.7
	L110060	44	101.7 ± 9.2
BF 500-3	L110057	46	101.3 ± 8.3
	L110058	43	101.5 ± 7.7
	L110059	61	100.5 ± 6.9
	L110060	44	97.0 ± 8.6

* mean values are across all soil depths and fortification levels; RSD = relative standard deviation [%]

These data prove that the analytical method applied was suitable to accurately determine residues of pyraclostrobin and its metabolites in soil down to a concentration of 0.001 mg kg^{-1} for each analyte.

Field soil specimens from the treated plots were analyzed down to a depth of 50 cm and up to a sampling event of 540 days after treatment (DAT). For confirmatory purposes, selected samples were measured twice. If samples were analyzed in duplicate, the individual numbers were averaged to produce a mean for the respective soil sample. When one of the values was below the LOQ, it was averaged as half of LOQ. For all trials, the 0 DAT double samples of the 0-10 cm soil layer were analyzed as well, in order to account for the importance of the day 0 value, and the final data were obtained by averaging the values of the respective main and double samples. Additionally, selected double samples from other sampling occasions were analysed replacing individual main samples that had been contaminated during processing.

All residue values presented are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue levels of the four analytes in mg kg^{-1} dry soil were converted to residue rates in g ha^{-1} taking into account the actual dry soil density of the field samples, which was calculated based on the dry weight and the volume of the sampled soil specimens. The obtained residue rates in g ha^{-1} were summed up for all depths between 0 and 50 cm analyzed. Results are presented in Table 7.1.2.2.1-13 to Table 7.1.2.2.1-16.

Table 7.1.2.2.1-13: Total residues of pyraclostrobin under field conditions in soil calculated to g ha^{-1}* and summed up for all depths analyzed

Trial Country	L110057 Middelfart, Denmark			L110058 Lentzke, Germany		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]
0	**175**	**207**	**212**	**209**	**191**	**216**
6-8	**199**	**210**	**143**	**176**	**208**	**169**
13	**177**	**198**	**205**	**149**	**147**	**181**
27-28	**168**	**152**	**182**	**99**	**98**	**106**
58-62	**104**	**100**	**100**	**30**	**30**	**34**
85-90	**42**	**75**	**50**	**21**	**19**	**24**
117-119	**29**	**34**	**34**	**13**	**12**	**20**
146-148	**16**	**29**	**24**	**11**	**10**	**13**
174-175	**18**	**30**	**27**	**9.1**	**11**	**11**
230	**21**	**30**	**21**	**10**	**11**	**17**
298-300	**17**	**24**	**25**	**9.7**	**14**	**15**
350-363	**23**	**23**	**26**	**6.4**	**8.1**	**10**
446-448	**15**	**15**	**14**	**6.9**	**4.3**	**3.4**
530-539	**9.4**	**14**	**11**	**4.2**	**4.3**	**3.1**
Trial Country	**L110059 Poggio Renatico, Italy**			**L110060 Barry d'Islemade, France**		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]
0	**170**	**151**	**180**	**219**	**214**	**208**
7	**173**	**228**	**222**	**268**	**317**	**243**
14	**138**	**140**	**180**	**195**	**185**	**233**
28-30	**163**	**148**	**143**	**93**	**133**	**101**
58-63	**141**	**106**	**128**	**45**	**63**	**55**
92-93	**129**	**75**	**151**	**27**	**26**	**34**
121-127	**96**	**70**	**121**	**18**	**16**	**14**
147-155	**63**	**64**	**95**	**11**	**15**	**14**
174-182	**87**	**130**	**111**	**19**	**16**	**16**
234-245	**65**	**63**	**98**	**12**	**12**	**13**
302-308	**73**	**82**	**70**	**9.4**	**14**	**9.7**
353-364	**93**	**84**	**75**	**9.4**	**9.0**	**9.7**
444-449	**53**	**60**	**55**	**3.8**	**5.2**	**5.7**
534-540	**67**	**37**	**38**	**3.6**	**4.2**	**3.2**

* calculations are based on actual dry soil density for individual soil layers
Residue values < 1 µg kg^{-1} (<LOQ) were reported and treated as zero.
DAT = days after treatment

Table 7.1.2.2.1-14: Total residues of BF 500-6 under field conditions in soil calculated to g ha^{-1}* and summed up for all depths analyzed

Trial Country	L110057 Middelfart, Denmark			L110058 Lentzke, Germany		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]
0	0	0	0	0	0	0
6-8	0	0	0	**4.8**	**4.0**	**3.6**
13	**1.7**	**1.9**	**2.3**	**10**	**9.0**	**8.8**
27-28	**8.1**	**8.2**	**10**	**21**	**18**	**15**
58-62	**25**	**23**	**26**	**26**	**24**	**23**
85-90	**31**	**41**	**46**	**31**	**30**	**29**
117-119	**36**	**43**	**42**	**32**	**32**	**35**
146-148	**26**	**34**	**39**	**26**	**35**	**30**
174-175	**37**	**49**	**43**	**29**	**37**	**35**
230	**30**	**46**	**40**	**28**	**32**	**32**
298-300	**31**	**36**	**39**	**29**	**49**	**33**
350-363	**30**	**42**	**37**	**22**	**27**	**24**
446-448	**40**	**41**	**29**	**28**	**27**	**15**
530-539	**28**	**38**	**34**	**22**	**24**	**25**
Trial Country	**L110059 Poggio Renatico, Italy**			**L110060 Barry d'Islemade, France**		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]
0	0	0	0	0	0	0
7	**1.7**	**3.4**	0	0	0	0
14	**1.9**	**1.3**	**2.2**	**2.1**	**4.2**	**3.3**
28-30	0	0	**1.9**	**28**	**25**	**35**
58-63	**3.6**	**1.3**	**1.2**	**20**	**29**	**33**
92-93	**2.7**	0	**6.8**	**39**	**35**	**53**
121-127	**1.9**	0	**1.8**	**43**	**27**	**27**
147-155	**1.4**	**1.6**	**2.9**	**28**	**48**	**34**
174-182	**4.9**	**9.2**	**3.1**	**43**	**30**	**30**
234-245	**2.1**	**3.5**	**5.3**	**35**	**35**	**36**
302-308	**3.8**	**4.1**	**1.2**	**26**	**34**	**27**
353-364	**4.0**	**5.3**	**2.1**	**30**	**39**	**36**
444-449	**2.0**	**2.3**	0	**18**	**26**	**33**
534-540	**3.8**	**1.6**	**3.4**	**25**	**33**	**24**

* calculations are based on actual dry soil density for individual soil layers
Residue values < 1 µg kg^{-1} (<LOQ) were reported and treated as zero.
DAT = days after treatment

Table 7.1.2.2.1-15: Total residues of BF 500-7 under field conditions in soil calculated to g ha^{-1}* and summed up for all depths analyzed

Trial Country	L110057 Middelfart, Denmark			L110058 Lentzke, Germany		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]
0	0	0	0	0	0	0
6-8	0	0	0	**3.0**	**3.1**	**2.4**
13	**1.4**	**1.4**	**1.9**	**4.8**	**4.1**	**4.0**
27-28	**5.1**	**4.4**	**5.5**	**8.0**	**7.7**	**6.2**
58-62	**15**	**13**	**17**	**14**	**14**	**12**
85-90	**16**	**22**	**28**	**16**	**17**	**14**
117-119	**21**	**25**	**26**	**15**	**16**	**15**
146-148	**16**	**21**	**24**	**12**	**16**	**13**
174-175	**21**	**29**	**23**	**13**	**17**	**16**
230	**22**	**34**	**32**	**14**	**15**	**16**
298-300	**23**	**26**	**28**	**19**	**29**	**18**
350-363	**25**	**27**	**23**	**12**	**14**	**12**
446-448	**23**	**22**	**17**	**13**	**15**	**7.8**
530-539	**16**	**22**	**22**	**14**	**12**	**12**
Trial Country	**L110059 Poggio Renatico, Italy**			**L110060 Barry d'Islemade, France**		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]
0	0	0	0	0	0	0
7	0	**2.0**	0	0	0	0
14	0	0	0	0	**2.0**	**1.7**
28-30	0	0	0	**9.4**	**11**	**13**
58-63	0	0	0	**12**	**17**	**15**
92-93	0	0	0	**14**	**14**	**26**
121-127	0	0	0	**19**	**12**	**24**
147-155	0	0	**1.3**	**13**	**20**	**17**
174-182	**1.7**	**3.4**	**1.4**	**18**	**13**	**14**
234-245	0	**1.7**	**1.9**	**15**	**16**	**14**
302-308	**1.5**	**1.8**	0	**11**	**14**	**12**
353-364	**1.8**	**2.3**	0	**13**	**16**	**15**
444-449	0	0	0	**7.8**	**11**	**14**
534-540	0	**1.2**	**1.3**	**9.9**	**12**	**11**

* calculations are based on actual dry soil density for individual soil layers
Residue values < 1 µg kg^{-1} (<LOQ) were reported and treated as zero.
DAT = days after treatment

Table 7.1.2.2.1-16: Total residues of BF 500-3 under field conditions in soil calculated to g ha^{-1}* and summed up for all depths analyzed

Trial Country	L110057 Middelfart, Denmark			L110058 Lentzke, Germany		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]
0	0	0	0	0	0	0
6-8	**2.1**	**1.6**	**1.3**	0	0	0
13	**2.3**	**2.2**	**1.8**	**1.5**	0	**1.7**
27-28	**2.8**	**2.0**	**2.1**	**1.6**	0	0
58-62	**2.6**	**2.0**	**1.9**	0	0	0
85-90	**1.4**	**2.2**	0	0	0	0
117-119	0	**1.6**	**1.2**	0	0	0
146-148	0	**1.2**	0	0	0	0
174-175	0	0	0	0	0	0
230	0	0	0	0	0	0
298-300	0	0	0	0	0	0
350-363	0	0	0	0	0	0
446-448	0	0	0	0	0	0
530-539	0	0	0	0	0	0
Trial Country	**L110059 Poggio Renatico, Italy**			**L110060 Barry d'Islemade, France**		
	Replicate A	Replicate B	Replicate C	Replicate A	Replicate B	Replicate C
DAT	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]
0	0	0	0	0	0	0
7	0	**1.4**	**1.7**	0	0	**1.5**
14	0	0	**1.2**	0	0	0
28-30	**1.4**	**1.6**	0	0	0	0
58-63	**1.9**	0	**1.9**	0	0	0
92-93	**2.0**	**1.4**	**2.4**	0	0	0
121-127	**1.9**	**1.2**	**1.9**	0	0	0
147-155	**1.7**	**1.7**	**2.1**	0	0	0
174-182	**1.8**	**3.0**	**2.3**	0	0	0
234-245	**2.0**	**2.0**	**2.0**	0	0	0
302-308	**1.9**	**2.5**	**2.0**	0	0	0
353-364	**2.0**	**2.9**	**2.0**	0	0	0
444-449	**1.9**	**1.7**	**1.7**	0	0	0
534-540	**2.2**	**1.8**	**1.5**	0	0	0

* calculations are based on actual dry soil density for individual soil layers
Residue values < 1 µg kg^{-1} (<LOQ) were reported and treated as zero.
DAT = days after treatment

Pyraclostrobin degraded at all four European field sites. The total amount of pyraclostrobin residues detected in the soil profiles decreased from an average of 196 g ha^{-1} at day 0 to an average of 17 g ha^{-1} after 1.5 years.

Pyraclostrobin was almost exclusively found in the top 0-10 cm soil layer, with only small amounts of the compound sporadically detected in the 10-20 cm layer (≤ 0.004 mg kg^{-1}). No residues above the LOQ were detected below 20 cm in any sample. It can be concluded that pyraclostrobin does not show any significant tendency to move into deeper soil layers indicating low potential for pyraclostrobin residues to leach to groundwater.

Metabolite BF 500-6 was found in significant amounts at all sites. It was detected earliest from 7 DAT onwards reaching maximum values (mean of 3 replicates) of 43 g ha^{-1} (Denmark), 37 g ha^{-1} (Germany), 5.7 g ha^{-1} (Italy), and 42 g ha^{-1} (Southern France). Thereafter, residues declined to values between 3.0 and 33 g ha^{-1} by the end of the study. The maximum occurrence of BF 500-6 corrected for the molar mass in the field trials was observed at the site in Denmark accounting for 28% of the maximum amount of pyraclostrobin measured in this trial.

Metabolite BF 500-7 was also found in significant amounts at all sites. It was detected earliest from 7 DAT reaching maximum values (mean of 3 replicates) of 29 g ha^{-1} (Denmark), 22 g ha^{-1} (Germany), 2.2 g ha^{-1} (Italy), and 18 g ha^{-1} (Southern France). Thereafter, residues declined to values between 0.8 and 20 g ha^{-1} by the end of the study. The maximum occurrence of BF 500-7 corrected for the molar mass in the field trials was observed at the site in Denmark accounting for 19% of the maximum amount of pyraclostrobin measured in this trial.

Metabolite BF 500-3 was detected only in trace amounts at or close to the LOQ. It was found earliest from 6 DAT, but never reached values above 2.4 g ha^{-1} (mean of 3 replicates). At sites in Germany and Southern France, BF 500-3 was only detected sporadically. The maximum occurrence of BF 500-3 corrected for the molar mass in the field trials was 1% of the maximum amount of pyraclostrobin at trials in Denmark, Germany, and Italy.

Metabolites BF 500-6, BF 500-7, and BF 500-3 were exclusively found in the top 0-20 cm soil layer, except for one single detection of BF 500-6 at the LOQ in the 30-40 cm layer at the site in Denmark. Apart from that, no residues of the three metabolites above the LOQ were observed in deeper soil layers in any sample at any site.

3. Shipment verification specimens

Shipping verification specimens spiked with pyraclostrobin were analyzed to check stability of the residues in soil during storage at the test site and through any shipping processes. Concentrations of pyraclostrobin were not corrected for procedural recoveries.

The analytical results demonstrated no losses from the shipping verification samples. The average recovery of pyraclostrobin from the soil samples spiked at the field test sites was 103% across all trials. The results prove that pyraclostrobin residues were stable in all soils under the shipping and storage conditions used.

4. Time of storage

The predominant part of the samples was analyzed within 2 years (720 days). Very few individual samples typically foreseen for re-analysis were stored for a longer time period prior to analysis. From sampling till analysis, the maximum storage period for any soil sample from the present study was 763, 569, 763 and 748 days for trials L110057, L110058, L110059, and L110060, respectively.

III. CONCLUSION

Pyraclostrobin properly degraded under field conditions in soil at all four European field sites. The total amount of pyraclostrobin residues detected in the soil profiles decreased from an average of 196 g ha^{-1} at day 0 to an average of 17 g ha^{-1} after 1.5 years.

Pyraclostrobin residues were almost exclusively found in the top 0-10 cm soil layer, with only small amounts of the compound sporadically detected in the 10-20 cm layer. No residues above the LOQ were detected below 20 cm in any sample. It can be concluded that pyraclostrobin does not show any significant tendency to move into deeper soil layers, indicating low potential for pyraclostrobin residues to leach to groundwater.

Metabolites BF 500-6, BF 500-7, and BF 500-3 were detected at all sites reaching maximum amounts of 43 g ha^{-1}, 29 g ha^{-1}, and 2.4 g ha^{-1}, respectively. They were exclusively found in the top 0-20 cm soil layer, except for one single detection of BF 500-6 at the LOQ in the 30-40 cm layer at the site in Denmark. Apart from that, no residues of the 3 metabolites above the LOQ were observed in deeper soil layers in any sample at any site.

Report: CA 7.1.2.2.1/3
Pape L., 2014a
Kinetic evaluation of a field dissipation study with BAS 500 F – pyraclostrobin conducted in 2011 and 2012: Determination of best-fit endpoints according to FOCUS
2014/1105763
Guidelines: none
GLP: no

Note: This study was not listed in the application submitted for renewal of approval. The reason for submission is described below.

Executive Summary

Kinetic evaluation of the field dissipation of pyraclostrobin (BAS 500 F) was conducted on the data from a field dissipation study, in which four field trials with pyraclostrobin were included. The purpose of this evaluation was to analyze the degradation kinetics of pyraclostrobin and its metabolites BF 500-3, BF 500-6 and BF 500-7 observed in the four soils according to the current guidance of the FOCUS workgroup on degradation kinetics in order to derive reliable best-fit $DegT_{50}$ and $DegT_{90}$ values.

The field trials were situated in different regions of Europe (Denmark, Germany, Italy and Southern France), considering a range of different soils and climatic conditions [*BASF DocID 2013/1348661*].

For each trial, the best-fit model was selected based on a visual and statistical assessment. Kinetic evaluation showed that the dissipation behavior of pyraclostrobin and its metabolites BF 500-6 and BF 500-7 was best described using single first-order (SFO) kinetics for three trials (Denmark, Germany and Southern France). For the metabolite BF 500-3 no reliable endpoints could be derived from these trials. For the trial in Italy, kinetic evaluation showed that the dissipation behavior of pyraclostrobin was best described using double first-order in parallel (DFOP) kinetics, while for the metabolites BF 500-3, BF 500-6 and BF 500-7 no reliable endpoints could be derived. The best-fit field half-lives ($DegT_{50}$) for pyraclostrobin ranged from 27.5 to 161.0 days, for metabolite BF 500-6 from 553.1 to 944.1 days and for metabolite BF 500-7 from 412.8 to >1000 days. The corresponding $DegT_{90}$ values ranged from 91.3 to >1000 days and were >1000 days for pyraclostrobin and for metabolites BF 500-6 and BF 500-7, respectively.

As degradation caused by surface processes like photolysis or volatilization was excluded by covering all plots with a sand layer the reported best-fit endpoints represent a conservative estimate of the dissipation behavior of pyraclostrobin and its metabolites in soil. Therefore, the derived best-fit endpoints should not be used as triggers for additional work (trigger endpoints).

I. MATERIAL AND METHODS

The kinetic evaluation was conducted for four field trials with pyraclostrobin from the data of one field dissipation study, which can be found in *CA 7.1.2.2.1/2* [*Bayer, H., Marwitz, A. – BASF DocID 2013/1348661*]. The trials were situated in different regions of Europe (Denmark, Germany, Italy and Southern France) considering a range of different soils and climatic conditions. Detailed soil characteristics in each trial are reported in the cited study. Applications were made to bare soil in May 2011 using a calibrated boom sprayer. Immediately after application and before subsequent soil sampling all plots were covered with a thin layer of sand to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. Soil samples were taken at 14 sampling dates up to 540 days after application and down to a soil depth of up to 50 cm from three individual subplots.

Pyraclostrobin residues were almost exclusively found in the top 0-10 cm soil layer, with only small amounts of the compound sporadically detected in the 10-20 cm layer (≤ 0.004 mg/kg). No residues above the LOQ were detected below 20 cm in any sample.
Metabolites BF 500-6, BF 500-7 and BF 500-3 were detected at all sites. They were exclusively found in the top 0-20 cm soil layer, except for 1 single detection of BF 500-6 at the LOQ in the 30-40 cm layer at the site in Denmark. Apart from that, no residues of the 3 metabolites above the LOQ were observed in deeper soil layers in any sample at any site.
The samples were extracted with acetonitrile and acetonitrile/water (80/20, v/v) and extracts were analyzed for pyraclostrobin and its metabolites BF 500-3, BF 500-6 and BF 500-7 by means of LC-MS/MS. The limit of quantification (LOQ) was 0.001 mg kg^{-1}. The limit of detection (LOD) was set at 0.0002 mg kg^{-1} (20% of LOQ).

Kinetic modeling

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting [*Schäfer et al. (2007)*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

Datasets were prepared for kinetic evaluation as follows:

- Values below LOQ (0.001 mg kg^{-1}) or LOD (0.0002 mg kg^{-1}) were treated as recommended by the FOCUS workgroup [*FOCUS (2006), chapter 6.1.4*]. According to FOCUS, values between LOQ and LOD were set to:
  $0.5 \times (LOQ + LOD) = 0.0006$ mg kg^{-1}
  and values below LOD were set to:
  $0.5 \times LOD = 0.0001$ mg kg^{-1}.
- For each sampling point, the concentration of a compound in the single soil layer given in mg kg^{-1} was transformed to its residue given in g ha^{-1} considering the height of the respective layer and the respective undisturbed soil bulk density for each sample as given in the study report. The total residues in the sampled subplot were calculated as the sum of residues of the single soil layers.
- For the metabolites, the residues in g ha^{-1} were transformed into parent equivalents in g a.s. ha^{-1}. The metabolites BF 500-6 and BF 500-7 are formed by a dimeric reaction, i.e. one metabolite molecule is formed out of two parent molecules. Hence, the molar mass of the parent had to be considered twice.

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation report.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested. The recommended kinetic models, i.e. single-first order (SFO), double first-order in parallel (DFOP) and first-order multi-compartment (FOMC), were applied to the pyraclostrobin data. The respective model descriptions and corresponding equations for calculating endpoints ($DegT_{50}$, $DegT_{90}$) are shown in the FOCUS Kinetics guidance [*FOCUS (2006), Box 5-1, Box 5-4 and Box 5-2*].

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*]. A kinetic model is considered appropriate if the residuals are randomly distributed around zero, the χ^2 error value is ideally <15% and the estimated degradation parameters differ significantly from zero.

The metabolites were subsequently added to the selected model for the parent considering the SFO kinetic model. Based on the known degradation pathway of pyraclostrobin, the metabolite BF 500-3 was included as transient metabolite where appropriate which is further transformed to BF 500-6 and BF 500-7. Simultaneous formation of the degradation products was assumed for the kinetic evaluation.

II. RESULTS AND DISCUSSION

Kinetic evaluation showed that the dissipation behavior of pyraclostrobin and its metabolites BF 500-6 and BF 500-7 was best described using SFO kinetics for three trials (Denmark, Germany and Southern France). For the metabolite BF 500-3 no reliable endpoints could be derived. For one trial (Italy) kinetic evaluation showed that the dissipation behavior of pyraclostrobin was best described using DFOP kinetics. For the metabolites BF 500-3, BF 500-6 and BF 500-7 no reliable endpoints could be derived. A summary of the adequate $DegT_{50}$ and $DegT_{90}$ values is given in Table 7.1.2.2.1-17 to Table 7.1.2.2.1-19.

Table 7.1.2.2.1-17: Summary of best-fit $DegT_{50}$ and $DegT_{90}$ values of pyraclostrobin

Field trial (Trial no)	Best-fit kinetic model	Rate k [d^{-1}]	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Middelfart, Denmark (L110057)	SFO	0.0113	61.6	204.7
Lentzke, Germany (L110058)	SFO	0.0252	27.5	91.3
Poggio Renatico, Italy (L110059)	DFOP	k1: 0.0197 k2: 0.0014	161.0	>1000
Barry d'Islemade, France (L110060)	SFO	0.0216	32.1	106.5

Table 7.1.2.2.1-18: Summary of best-fit $DegT_{50}$ and $DegT_{90}$ values of BF 500-6

Field trial (Trial no)	Best-fit kinetic model	Rate k [d^{-1}]	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Middelfart, Denmark (L110057)	SFO	0.0013	553.1	>1000
Lentzke, Germany (L110058)	SFO	0.0007	944.1	>1000
Poggio Renatico, Italy (L110059)	no reliable endpoints could be derived			
Barry d'Islemade, France (L110060)	SFO	0.0012	558.8	>1000

Table 7.1.2.2.1-19: Summary of best-fit $DegT_{50}$ and $DegT_{90}$ values of BF 500-7

Field trial (Trial no)	Best-fit kinetic model	Rate k [d^{-1}]	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Middelfart, Denmark (L110057)	SFO	0.0011	630.4	>1000
Lentzke, Germany (L110058)	SFO	0.0005	>1000	>1000
Poggio Renatico, Italy (L110059)	no reliable endpoints could be derived			
Barry d'Islemade, France (L110060)	SFO	0.0017	412.8	>1000

III. CONCLUSION

Kinetic evaluation of four field trials with pyraclostrobin was conducted in order to derive reliable best-fit $DegT_{50}$ and $DegT_{90}$ values according to the current guidance of the FOCUS workgroup on degradation kinetics.

The best-fit field half-lives ($DegT_{50}$) for pyraclostrobin ranged from 27.5 to 161.0 days, for metabolite BF 500-6 from 553.1 to 944.1 days and for metabolite BF 500-7 from 412.8 to >1000 days. The corresponding $DegT_{90}$ values for pyraclostrobin ranged from 91.3 to >1000 days. For metabolites BF 500-6 and BF 500-7 the corresponding $DegT_{90}$ values were >1000 days.

Surface processes had been excluded by covering the soil with sand in the field study. Therefore, the derived best-fit endpoints should not be used as trigger endpoints.

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Pape L., 2014b
Kinetic evaluation of a field dissipation study with BAS 500 F – pyraclostrobin conducted in 2011 and 2012: Determination of modeling endpoints according to FOCUS
2014/1105764
Guidelines: none
GLP: no

Note: This study was not listed in the application submitted for renewal of approval. The reason for submission is described below.

Executive Summary

The dissipation behavior of the pyraclostrobin (BAS 500 F) in soil has been investigated in a field dissipation study including four field trials located in Denmark, Germany, Italy and Southern France. The purpose of this evaluation was to analyze the degradation kinetics of pyraclostrobin and its metabolites BF 500-3, BF 500-6 and BF 500-7 observed in the four soils according to the current guidance of the FOCUS workgroup on degradation kinetics in order to derive reliable normalized modeling endpoints.

The field trials were situated in different regions of Europe (Denmark, Germany, Italy and Southern France), considering a range of different soils and climatic conditions [*BASF DocID 2013/1348661*].

As the study design was tailored to exclude surface processes, kinetic evaluation was performed according to FOCUS kinetics as recommended by *EFSA (2010)*.

Prior to kinetic evaluation, sampling intervals of the field studies were normalized to reference conditions (20°C, pF2) by time-step normalization. The kinetic evaluation was performed on the normalized dataset. Modeling endpoints were derived based on a visual and statistical assessment. Kinetic evaluation of the time-step normalized dataset (20°C, pF2) resulted in normalized field half-lives (SFO-DegT_{50}) for pyraclostrobin between 15.0 and 181.2 days, for BF 500-6 between 163.5 and 361.4 days and for BF 500-7 between 183.7 and 460.1 days. For the metabolite BF 500-3 no reliable endpoints could be derived.

I. MATERIAL AND METHODS

The kinetic evaluation was conducted for four field trials with pyraclostrobin from the data of one field dissipation study, which can be found in *CA 7.1.2.2.1/2* [*Bayer, H., Marwitz, A. – BASF DocID 2013/1348661*].

The data set has already been evaluated for best-fit endpoints; a summary of the material and methods including data handling and kinetic models considered can be found in *CA 7.1.2.2.1/3* [*Pape, L. – BASF DocID 2014/1105763*].

Surface processes had been excluded by covering the soil with sand in the field study. Therefore, kinetic evaluation was performed according to FOCUS kinetics as recommended by EFSA [*EFSA (2010)*].

In order to derive modeling endpoints, kinetic evaluation was performed on the time-step normalized data set.

Normalization procedure

Evaluation of the suitability of field dissipation data for normalization was performed according to the evaluation criteria for normalization compiled by the Dutch regulatory authority (CTGB criteria).

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily soil temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20°C using the Q_{10} approach as described in the report of the FOCUS soil modeling working group [*FOCUS (2012)*]. The Q_{10} response function was applied for temperatures above 0°C and below field temperatures of 0°C it was assumed that no degradation occurs (Equation 7.1.2.2-2 c). For the evaluation, the EFSA opinion on the default Q_{10} value [*EFSA (2007)*] was followed and a Q_{10} value of 2.58 was included in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture at field capacity (pF 2) (Equation 7.1.2.2-2 d).

The normalized day lengths were derived according to Equation 7.1.2.2-2 a. For DAT 0, no normalization was considered and application was assumed to occur at the time point zero. Normalized sampling days (DAT_{norm}) after application were calculated by cumulatively summing up normalized day lengths according to Equation 7.1.2.2-2 b.

Equation 7.1.2.2-2: Calculation of normalized day length based on combination of soil moisture and soil temperature correction factors

a) $$D_{norm} = D * f_{temp} * f_{moisture}$$

b) $$t_i = \sum_{t=1}^{i-1} D_{norm}$$

with:

t_i =	Time from application till sampling at day i	[d]
D_{norm} =	Normalized day length (20°C, pF2)	[d]
i =	Time span between application and sampling	[d]

c) $$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact-Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

d) $$f_{moist} = \begin{cases} (\frac{\theta_{act}}{\theta_{ref}})^B & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:

D_{norm} =	normalized day length (temperature and moisture)	
D =	actual day length (1 d)	[days]
f_{temp} =	temperature correction factor	[-]
f_{moist} =	moisture correction factor	[-]
T_{act} =	actual soil temperature (°C)	[C°]
T_{ref} =	reference temperature (20°C)	[C°]
Q_{10}=	factor of increase of degradation rate with an increase in temperature of 10°C (Q_{10} = 2.58 [EFSA 2007])	[-]
θ_{act} =	actual soil moisture (vol. water content)	[m^3 m^{-3}]
θ_{ref} =	reference soil moisture at pF2	[m^3 m^{-3}]
B =	exponent of the moisture response function, B = 0.7	[-]

Table 7.1.2.2.1-20 shows the field sampling dates for the trial locations and the normalized (20°C, pF2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 4.4.4.

Table 7.1.2.2.1-20: Time-step normalized (temperature and moisture) sampling days

Middelfart, Denmark (L110057)		Lentzke, Germany (L110058)	
DAT	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0
6	1.8	8	3.3
13	4.1	13	5.8
28	10.2	27	13.3
58	24.1	62	36.2
85	39.6	90	57.2
119	60.0	117	76.9
148	73.9	146	92.3
175	82.8	174	100.9
230	94.9	230	110.8
300	104.2	298	119.8
363	117.6	350	130.9
448	153.6	446	185.5
539	196.3	530	230.4
Poggio Renatico, Italy (L110059)		**Barry d' Islemade, France (L110060)**	
DAT	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0
7	3.9	7	4.1
14	8.7	14	7.8
28	19.8	30	18.1
58	51.2	63	45.3
92	91.2	93	71.5
121	129.4	127	103.7
147	158.3	155	121.5
182	176.5	174	130.5
234	190.4	245	153.0
302	197.8	308	165.2
353	216.3	364	191.8
444	306.6	449	259.0
540	407.7	534	323.4

DAT = days after treatment
D_{norm} = normalized day (20°C, pF2)

II. RESULTS AND DISCUSSION

The dissipation behavior of pyraclostrobin and its metabolites BF 500-6 and BF 500-7 was best described using SFO kinetics for all trials. For the metabolites BF 500-6 and BF 500-7 no reliable endpoints could be derived for one trial (Italy). For the metabolite BF 500-3 no reliable endpoints could be derived for any trial. A summary of the adequate $DegT_{50}$ and $DegT_{90}$ values, as well as of the formation fractions for the metabolites, is given in Table 7.1.2.2.1-21 to Table 7.1.2.2.1-23.

Table 7.1.2.2.1-21: Summary of modeling endpoints of pyraclostrobin

Field trial	Soil type (USDA)	Best-fit kinetic model	Rate k [d^{-1}]	$DegT_{50}$ [d]
L110057 (DK)	Sand	SFO	0.0240	28.9
L110058 (DE)	Loamy sand	SFO	0.0461	15.0
L110059 (IT)	Silt loam	SFO	0.0038	181.2
L110060 (FR)	Sandy loam	SFO	0.0317	21.9

Table 7.1.2.2.1-22: Summary of modeling endpoints of BF 500-6

Field trial	Soil type (USDA)	Kinetic model	Rate k [d^{-1}]	$DegT_{50}$ [d]	Formation fraction [-]
L110057 (DK)	Sand	SFO	0.0042	163.5	0.380*
L110058 (DE)	Loamy sand	SFO	0.0019	361.4	0.223**
L110059 (IT)	Silt loam	no reliable endpoints could be derived			
L110060 (FR)	Sandy loam	SFO	0.0025	282.9	0.232**

* from BF 500-3 (formation fraction of BF 500-3 from pyraclostrobin was fixed to 1)
** from pyraclostrobin

Table 7.1.2.2.1-23: Summary of modeling endpoints of BF 500-7

Field trial	Soil type (USDA)	Kinetic model	Rate k [d^{-1}]	$DegT_{50}$ [d]	Formation fraction [-]
L110057 (DK)	Sand	SFO	0.0038	183.7	0.242*
L110058 (DE)	Loamy sand	SFO	0.0015	460.1	0.112**
L110059 (IT)	Silt loam	no reliable endpoints could be derived			
L110060 (FR)	Sandy loam	SFO	0.0032	216.4	0.116**

* from BF 500-3 (formation fraction of BF 500-3 from pyraclostrobin was fixed to 1)
** from pyraclostrobin

III. CONCLUSION

Kinetic evaluation of four field trials with pyraclostrobin was conducted in order to derive reliable normalized modeling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics. The dissipation behavior of pyraclostrobin and its metabolites BF 500-6 and BF 500-7 was best described using SFO kinetics for all trials. For the metabolites BF 500-6 and BF 500-7 no reliable endpoints could be derived for one trial (Italy). For the metabolite BF 500-3 no reliable endpoints could be derived for any trial.

Kinetic evaluation of the time-step normalized dataset (20°C, pF2) resulted in normalized field half-lives ($DegT_{50}$) for pyraclostrobin between 15.0 and 181.2 days, for BF 500-6 between 163.5 and 361.4 days and for BF 500-7 between 183.7 and 460.1 days.

Summary of degradation rates for pyraclostrobin and its metabolites in field dissipation studies

Table 7.1.2.2.1-24: Summary table on degradation rates of pyraclostrobin obtained in field soil studies

Study	Trial site	pH ($CaCl_2$)	Org. C [%]	Best-fit DT_{50} / DT_{90} [d]	Method of calculation	Modeling DT_{50} 20°C, pF2 [d]	Method of calculation
BASF DocID 2014/1093423 [1]	D05/02/97 (DE)	6.2	1.1	33.3 / 110.5 [1]	SFO	15.5	DFOP
	D08/01/97 (DE)	6.8	2.5	18.4 / 130.4 [1]	FOMC	26.0 [2]	HS
	DU2/02/97 (DE)	5.6	0.8	24.2 / 80.3 [1]	SFO	24.8 [2]	HS
	ALO/01/98 (ES)	7.6	0.6	5.2 / 86.9 [1]	DFOP	not calculated [3]	-
	ALO/02/98 (ES)	7.6	0.9	14.2 / 347.8 [1]	DFOP	not calculated [3]	-
	HUS/02/98 (SE)	5.8	1.4	55.8 / 185.3 [1]	SFO	32.2 [2]	DFOP
BASF DocID 2014/1105763 [4] 2014/1105764 [4]	L110057 (DK)	6.08	0.98	61.6 / 204.7 [5]	SFO	28.9	SFO
	L110058 (DE)	6.44	0.64	27.5 / 91.3 [5]	SFO	15.0	SFO
	L110059 (IT)	7.66	1.18	161 / >1000 [5]	DFOP	181.2	SFO
	L110060 (FR)	7.64	0.99	32.1 / 106.5 [5]	SFO	21.9	SFO

[1] field soil study including surface loss processes; best-fit endpoints serve as trigger endpoints for additional work
[2] calculated as $\ln(2)/k_{slow}$
[3] climatic conditions at trial sites not appropriate for derivation of modeling endpoints
[4] covered field soil study; only to derive modeling endpoints in the soil matrix, excluding surface loss processes [*EFSA, 2010*]
[5] best-fit endpoints should not be used as triggers for additional work due to exclusion of surface loss processes [*EFSA, 2010*]

Table 7.1.2.2.1-25: Degradation rates of pyraclostrobin metabolites in aerobic field dissipation studies

Metabolite	Study	Trial site	pH ($CaCl_2$)	Org. C [%]	Best-fit DT_{50} [d]	Method of calculation [1]	Modeling DT_{50} [1] SFO, 20°C, pF2 [d]	Formation fraction
BF 500-6	BASF DocID 2014/1105763 [2] 2014/1105764 [2]	L110057 (DK)	6.08	0.98	553.1 [3]	SFO	163.5	0.380
		L110058 (DE)	6.44	0.64	944.1 [3]	SFO	361.4	0.223
		L110059 (IT)	7.66	1.18	not calculated [3,4]	-	not calculated [4]	-
		L110060 (FR)	7.64	0.99	558.8 [3]	SFO	282.9	0.232
BF 500-7	BASF DocID 2014/1105763 [2] 2014/1105764 [2]	L110057 (DK)	6.08	0.98	630.4 [3]	SFO	183.7	0.242
		L110058 (DE)	6.44	0.64	>1000 [3]	SFO	460.1	0.112
		L110059 (IT)	7.66	1.18	not calculated [3,4]	-	not calculated [4]	-
		L110060 (FR)	7.64	0.99	412.8 [3]	SFO	216.4	0.116

[1] SFO kinetics for the parent
[2] covered field soil study; only to derive modeling endpoints in the soil matrix, excluding surface loss processes [*EFSA, 2010*]
[3] best-fit endpoints should not be used as triggers for additional work due to exclusion of surface loss processes [*EFSA, 2010*]
[4] no reliable endpoints derived in kinetic evaluation

Report: CA 7.1.2.2.1/5
Tilting N. et al., 2014a
Stability of residues of BAS 500 F (Pyraclostrobin, Reg.No 304428) and its metabolites 500M01 (Reg.No. 364380), 500M02 (Reg.No. 369315), and 500M07 (Reg.No. 340266) in various soils under frozen conditions
2014/1000723
Guidelines: OECD 506 (Oct. 2007)
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The storage stability of pyraclostrobin (BAS 500 F) and its metabolites BF 500-6 (500M01), BF 500-7 (500M02), and BF 500-3 (500M07) in four soils was investigated under frozen conditions (approx. -18°C) over a period of two years. The soils were derived from four field dissipation trials of a parallel field study [*BASF DocID 2013/1348661*]. Trials were conducted in Denmark, Germany, Italy and France.

The soil samples were fortified at a concentration level of 0.01 mg kg^{-1} (fortified samples). Each soil aliquot was only spiked with a single test item (two replicates per soil type and analyzed time point). Soil samples without test item were used for control measurements and stored as treated samples (one sample per soil type and time point). At different intervals (approximately 0, 30, 60, 120, 240, 365, 540 and 720 days) soil samples were analyzed using BASF method L0166/01. The limit of quantitation of the method is 0.001 mg kg^{-1}. Procedural recoveries analyzed within each analytical series proved the validity of the analytical method and were used for recovery correction of stored samples.

Results from the present study showed that the field soil samples from the trials in Denmark, Germany, Italy and France, containing pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3, can be stored under frozen conditions (approx. -18°C) without any significant loss of analytes over a period of two years.

I. MATERIAL AND METHODS

A. MATERIALS

Test materials:	BAS 500 F	BF 500-6	BF 500-7	BF 500-3
Synonyms		500M01	500M02	500M07
Batch No.:	01815-65	01311-142	01586-173	L74-118
Purity:	99.9%	99.2	99.5%	99.9%
CAS #:	175013-18-0	-	-	-

B. STUDY DESIGN

1. Experimental Conditions

Four soils from a related field soil dissipation study [*CA 7.1.2.2.1/2, Bayer H., Marwitz A. – BASF DocID 2013/1348661*] were used in the storage stability study for pyraclostrobin and its metabolites. The analytes were dosed at 0.01 mg kg^{-1} to soil samples (5 g) and then stored frozen for 0, 30 (± 2), 60 (± 2), 120 (± 5), 240 (± 7), 365 (± 7), 540 (± 14) and 720 (± 14) days. All samples were stored in a freezer throughout the entire period of the experiment at ≤-18°C. Plastic tubes (50 mL) were used as storage containers.

2. Description of analytical procedures

Determination of pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3

For determination of pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3, soil samples were extracted with acetonitrile. Soils were separated from the liquid phase by centrifugation. The supernatants were taken to dryness while the solid remainder was re-extracted with acetonitrile : water (80/20, v/v). After centrifugation, the liquid phase was separated and used to dissolve the dried residue from the first extraction. Afterwards, samples were analyzed by LC-MS/MS. The method achieved a limit of quantitation (LOQ) of 0.001 mg kg^{-1} and a limit of detection (LOD) of 0.05 pg (concentration on the LC column) for the individual compounds in soil.

LC-MS/MS measurements were performed as soon as possible after sample extraction and extract clean-up. If the sample extracts had to be stored prior to final measurement due to instrument shortage, they were kept refrigerated.

The validation of the method is described in *CA 4.1.2/1* [*Tilting N., Sopeña-Vazquez F. - BASF DocID* 2013/*1184817*].

The method was concurrently validated with analyses of stored soil samples within the summarized study, processing dose level fortifications (0.01 mg kg^{-1}), which resulted in overall average recoveries of 98 to 106% (RSD ≤ 10%).

II. RESULTS AND DISCUSSION

Frozen-storage stability of pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3 in four soils over approximately 0, 30, 60, 120, 240, 365, 540 and 720 days of storage was investigated. Soil extracts and freshly prepared standard dilutions were examined by LC-MS/MS. The results for pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3 at 0.01 mg kg^{-1} in soil samples stored frozen for up to 720 days prior to extraction are given in Table 7.1.2.2.1-26.

Overall results revealed that concentrations of BAS 500 F and its three metabolites BF 500-6, BF 500-7, and BF 500-3 remained stable in the treated soil samples from all trials when stored at -18°C over the tested period of 720 days.

According to the respective guidelines, the degradation of all analytes was not significant (≤ 30%) within the storage period of two years.

Table 7.1.2.2.1-26: Frozen storage stability of pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3 in four soils (mean of two replicates)

Soil	Storage period [days]	Recovery [%]			
		Pyraclostrobin	BF 500-6	BF 500-7	BF 500-3
L110057 (Denmark)	0	96	91	89	102
	30	108	102	97	92
	60	102	103	91	95
	120	104	94	93	97
	240	103	95	93	98
	365	101	94	92	102
	540	99	98	87	88
	720	96	92	105	93
L110058 (Germany)	0	99	92	87	95
	30	97	98	95	87
	60	103	94	86	80
	120	106	103	93	101
	240	98	93	87	100
	365	114	98	96	107
	540	111	105	85	90
	720	104	118	106	94
L110059 (Italy)	0	99	94	93	101
	30	101	86	90	86
	60	100	89	93	96
	120	102	97	102	109
	240	102	89	89	103
	365	102	98	95	108
	540	104	98	98	102
	720	104	90	98	96
L110060 (France)	0	114	124	92	95
	30	109	101	95	102
	60	111	99	96	97
	120	115	n/a [A)]	n/a [A)]	93
	240	120	120	89	102
	365	122	118	86	96
	540	125	120	97	94
	720	125	125	99	95

[A)] sample set rejected; mean of two procedural recoveries in the analytical series was > 120%

III. CONCLUSION

The results obtained from the storage stability study demonstrated that pyraclostrobin and its metabolites BF 500-6, BF 500-7 and BF 500-3 are stable in soil when stored frozen for up to 720 days, if, as a stability criterion, a threshold value of at least 85% recovery of the initial rate is applied.

CA 7.1.2.2.2 Soil accumulation studies

No experimental data available. Since all best-fit pyraclostrobin DT_{90} values from the field soil dissipation study used for derivation of trigger endpoints are < 1 year (Table 7.1.2.2.1-5), accumulation data are not needed for the active substance. The soil accumulation risk of metabolites BF 500-6 and BF 500-7 is addressed via PECsoil calculations.

CA 7.1.3 Adsorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

CA 7.1.3.1.1 Adsorption and desorption of the active substance

No new studies on adsorption/desorption behavior of the active substance pyraclostrobin were performed. The study as peer-reviewed during the previous Annex I listing process is considered to be still valid. The adsorption values as listed in the EU Review Report (SANCO/1420/2001) are shown in Table 7.1.3.1.1-1.

Table 7.1.3.1.1-1: Adsorption of pyraclostrobin on different soils (SANCO/1420/2001)

Soil	Soil type	OC [%]	pH [-]	K_f [mL g^{-1}]	K_{foc} [mL g^{-1}]	K_{fom} [mL g^{-1}]	1/n [-]
Li 35 b	sandy loam	0.8	6.4	60	7500	4350	0.896
LUFA 2.2	loamy sand	1.9	5.6	304	16000	9281	1.025
Bruch West	sandy loam	1.8	7.3	142	7889	4576	1.012
USA 538-30-5	loamy sand	0.5	5.9	30	6000	3480	0.861
USA 538-31-2	sandy loam	0.6	5.3	54	9000	5220	0.873
CAN-95024	sandy loam	3.9	7.6	368	9436	5473	1.005
Arithmetic mean					**9304**	**5397**	**0.945**

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

The adsorption/desorption behavior of the pyraclostrobin metabolites BF 500-3, BF 500-6 and BF 500-7 was already evaluated during the previous Annex I listing process.

The study performed with **BF 500-3** is considered to be still valid, and the adsorption values as given in the Monograph 12945/ECCO/BBA/01 are presented in Table 7.1.3.1.2-1.

Table 7.1.3.1.2-1: Adsorption of BF 500-3 on different soils as listed in the Monograph 12945/ECCO/ BBA/01

Soil	Soil type	OC [%]	pH [-]	K_f [-]	1/n	K_{foc} [mL g^{-1}]
Li 35 b	Loamy sand	1.1	6.5	74.3	0.802	6750
LUFA 2.2	Sand / loamy sand	2.5	5.8	268	0.942	10700
Bruch West	Sandy loam	1.5	7.5	63.5	0.688	4240
USA 538-30-5	Loamy sand	0.4	5.8	47.3	0.942	11800
USA 538-31-2	Loam	0.5	5.2	60.1	0.773	12000
CAN-95012	Sandy clay loam	3.4	7.5	354	0.831	10400
Arithmetic mean				**144.5**		**9315**

Metabolites **BF 500-6 and BF 500-7** showed very high adsorption values in the already peer-reviewed studies. Due to the very low water solubilities of both compounds and thus difficult analytics, the recoveries reported in those old studies were quite low and do not meet the requirements of guideline OECD 106. Although both compounds can be considered immobile in soil, new studies were initiated to confirm the high adsorption values under the provision of an acceptable material balance.

The old adsorption values for **BF 500-6** as given in the Monograph 12945/ECCO/BBA/01 are presented in Table 7.1.3.1.2-2, followed by the study summary of the new adsorption study performed with non-labeled BF 500-6.

Due to the very low water solubility, the compound could be tested only at one concentration.

Table 7.1.3.1.2-2: Adsorption of BF 500-6 on different soils as listed in the Monograph 12945/ECCO/BBA/01

Soil	Soil type	OC [%]	pH [-]	K_d [-]	K_{oc} [mL g^{-1}]
Li 35 b	Loamy sand	1.1	6.5	350	31830
LUFA 2.2	Sand / loamy sand	2.5	5.8	84	3360
Bruch West	Sandy loam	1.5	7.5	248	16550
USA 538-30-5	Loamy sand	0.4	5.8	365.5	91650
USA 538-31-2	Loam	0.5	5.2	634	126800
CAN-95024	Sandy clay loam	3.4	7.5	629.5	18500

Report: CA 7.1.3.1.2/1
Ebert D. et al., 2014a
Study of the adsorption behaviour of BF 500-6 (Reg.No. 364380) on different soils
2014/1000624
Guidelines: OECD 106, EPA 835.1230
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In laboratory experiments the adsorption behavior of the pyraclostrobin metabolite BF 500-6 was investigated on five European soils. The five tested soils covered a range of pH ($CaCl_2$) from 5.6 to 7.4, a range of organic carbon content from 0.60 to 1.85%, and four different USDA textural classes: one sand, two sandy loams, one silt loam and one sandy clay loam. Due to the low water solubility of BF 500-6, adsorption was only tested for one concentration level. Hence, no adsorption or desorption isotherm was determined. The application solution was prepared in 0.01 M $CaCl_2$ solution with a nominal concentration of 1 ng mL^{-1} of the test item. The ratio of soil versus test solution was 1 : 20, and the measurements were performed at the adsorption equilibrium time of one hour.

Sorption of BF 500-6 to soil proceeded fast, expressed by solution concentrations < LOD after one hour of shaking. Calculated mean distribution coefficients (K_d) differed only slightly between all soils ranging from 1732 mL g^{-1} (LUFA 2.3) to 1985 mL g^{-1} (Nierswalde). As almost the entire applied dose of BAS 500-6 was sorbed to the soil phase, distribution coefficients normalized to the fraction of organic carbon in the soils (K_{OC}) increased from the soil exhibiting the highest (Nierswalde; K_{OC}: 107301 mL g^{-1}) to the soil exhibiting the lowest organic carbon content (LUFA 2.1; K_{OC}: 311704 mL g^{-1}).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item	BF 500-6
Synonym:	500M01
Reg. No.:	364380
Chemical name (IUPAC):	N,N'-bis-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl} phenyl)diazene N-oxide
Batch No.:	01311-142
Purity:	99.2%
Molar mass:	611.5 g mol^{-1}

2. Soils

The study was conducted with five different soils originating from Europe. The physico-chemical properties of the soils are provided in Table 7.1.3.1.2-3.

Table 7.1.3.1.2-3: Characterization of soils used to determine the adsorption behavior of metabolite BF 500-6

Soil designation Origin	LUFA 2.1 Germany	LUFA 2.3 Germany	Bruch West Germany	Nierswalde Germany	La Gironda Spain
Textural class (DIN 4220)	Sand	Loamy sand	Loamy sand	Clay silt	Sandy clay loam
Soil texture [%], (ISO 11277)					
Sand	89.5	66.9	61.1	15.1	48.0
Silt	8.2	24.8	27.6	76.2	24.3
Clay	2.3	8.3	11.3	8.8	27.7
Textural class (USDA scheme)	Sand	Sandy loam	Sandy loam	Silt loam	Sandy clay loam
Soil texture [%], (USDA)					
Sand	90.8	68.6	63.7	17.7	49.2
Silt	6.9	23.1	25.1	73.5	23.0
Clay	2.3	8.3	11.3	8.8	27.7
Organic carbon [%] (ISO 10694)	0.60	0.99	1.63	1.85	1.22
Cation exchange capacitiy [$cmol^{+}$ kg^{-1}]	-0.7	7.5	11.9	3.1	26.3
pH ($CaCl_2$)	5.6	6.7	7.3	5.7	7.4
pH (water)	6.5	7.4	8.0	6.5	8.3
Max. water holding capacity [g/100g dry soil]	23.1	28.2	29.2	36.1	39.2
Bulk density [g L^{-1}]	1381	1226	1273	1236	1308

B. STUDY DESIGN

1. Experimental conditions

Preliminary tests: adsorption kinetics and soil/solution ratio

A preliminary experiment was run with two soils to determine the time needed to establish equilibrium conditions. The experiments were run with a soil / solution ratio of 1/20, using the soils LUFA 2.1 and La Gironda. The test was performed in 50 mL glass centrifuge vials containing 2 g soil and 40 mL application solution (1 ng mL^{-1} BF 500-6 in 0.01 M aqueous $CaCl_2$). The vials were protected from light and shaken for 0, 2, 4, 6 and 24 hours (225 rpm). The soil / solution suspension was then centrifuged and the mass of the test vessel including contents was determined before and after decanting the supernatant. An aliquot of the supernatant was sampled and the concentration of test item in the $CaCl_2$ solution was determined by LC-MS/MS.

Results of this first trial revealed that the adsorption equilibrium was reached fast (< 1 h). Therefore, a second trial was performed applying shorter incubation times (5, 15, 30, 60 and 90 minutes).

To find the optimal soil / solution ratio for the adsorption tests, another experiment was run with LUFA 2.1 and La Gironda soil. Three different soil / solution ratios were tested: 1/10, 1/20, and 1/40 applying an application solution with a concentration of 1 ng mL^{-1}. Samples were incubated for five minutes by mechanical shaking.

The adsorption experiments were carried out in duplicate at room temperature (22 ± 2°C). Due to the low solubility of the test item, the soils were not pre-equilibrated with $CaCl_2$ solution, but the $CaCl_2$ solution containing the dissolved test item was added in one step.

Stability and adsorption of the test substance

Due to the low solubility of the test item, no adsorption or desorption isotherms could be determined in this study. Hence, the stability test, which is typically performed during the pre-tests, corresponds to the adsorption test.

The test was performed as described for the equilibrium time test, additionally analyzing the soil by extraction with 25 mL of acetonitrile and then with 25 mL of acetonitrile / water (80/20; v/v), followed by quantification using LC-MS/MS.

2. Description of analytical procedures

The method for determining metabolite BF 500-6 in $CaCl_2$ solution was newly developed and validated within this study (method L0211/01). For determination of BF 500-6 in soil, the technical procedure of method L0166/1 was used as basis, adapted to the expected concentration range, and then also validated in two soils within this study.

Principle of the methods:

To analyze BF 500-6 in $CaCl_2$ solution, 5 mL of 0.01 M $CaCl_2$ solution was extracted into cyclohexane by mechanical shaking for 30 min at 225 rpm. A 2.5 mL aliquot of the sample was evaporated to dryness using an N-Evap at 40°C. Subsequently, the sample was reconstituted in 1 mL of acetonitrile/water (80/20; v/v) and analyzed by LC-MS/MS. The method has a limit of quantification (LOQ) of 0.02 µg L^{-1} and a limit of detection (LOD) of 0.004 µg L^{-1}.

For soil analysis, a 2 g soil aliquot was extracted with 25 mL acetonitrile on a mechanical shaker (30 min at 225 rpm). After extraction, the tube was centrifuged at 3000 rpm (5 min, 20°C). The liquid phase was separated and taken to dryness. The solid remainder was re-extracted with 25 mL acetonitrile / water 80/20 (v/v). After centrifugation, the liquid phase was separated and used to dissolve the dried residue from the first extraction. For HPLC-MS/MS measurement the extract was further diluted.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Analytical methods L0211/01 ($CaCl_2$ solution) and L0166/01 (soil) were validated within this study. Both methods revealed good average recoveries (91.5 – 116.9%) and relative standard deviations (< 7%) for the dose levels of 0.02 and 0.2 µg L^{-1} for $CaCl_2$ solution as well as 2 and 20 µg kg^{-1} for soil, respectively.

In the adsorption test, the average recovery values ($CaCl_2$ solution plus soil extract) ranged from 102.5 to 118.1%.

B. FINDINGS

Based on the results obtained, methods L0211/01 and L0166/01 are considered valid for the determination of BF 500-6 in $CaCl_2$ solution and in soil with a validated LOQ of 0.02 µg L^{-1} and 0.002 mg kg^{-1}, respectively.

Results of the adsorption equilibrium test carried out with BF 500-6 indicated that the adsorption equilibrium was reached after five minutes.

Results of control samples, where the test item was applied to $CaCl_2$ solution incubated without soil, revealed that BF 500-6 was sorbed to the wall of the test vessels, as only about 59% of the applied BF 500-6 could still be detected in solution after 1 hour. However, it is assumed that in experiments with soil, the presence of soil will generally reduce such adsorption.

No matrix interferences were detected.

After one hour of shaking, solution concentrations (C_{ads-aq}) were < LOQ for all soils. Hence, almost the entire applied amount of BF 500-6 was accounted to the fraction sorbed to the soil phase. Calculated mean distribution coefficients (K_d) differed only slightly between all soils ranging from 1732 mL g^{-1} (LUFA 2.3) to 1985 mL g^{-1} (Nierswalde). Distribution coefficients normalized to the fraction of organic carbon in the soils (K_{OC}) increased from the soil exhibiting the highest (Nierswalde; K_{OC}: 107301 mL g^{-1}) to the soil exhibiting the lowest organic carbon content (LUFA 2.1; K_{OC}: 311704 mL g^{-1}). Calculated K_d as well as K_{OC} values are presented in Table 7.1.3.1.2-4.

Table 7.1.3.1.2-4: Measured equilibrium concentrations and calculated distribution coefficients K_d and K_{OC} of BF 500-6

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Measured concentration			Adsorption	
				C_0 [µg L^{-1}]	C_{ads-aq} (eq) [µg mL^{-1}]	C_{ads-s} (eq) [µg g^{-1}]	K_d [mL g^{-1}]	K_{OC} [mL g^{-1}]
LUFA 2.1	Sand	0.60	5.6	0.91	0.00001	0.01870	1870	311704
LUFA 2.3	Sandy loam	0.99	6.7	0.84	0.00001	0.01732	1732	174921
Bruch West	Sandy loam	1.63	7.3	0.79	0.00001	0.01882	1882	115430
Nierswalde	Silt loam	1.85	5.7	0.85	0.00001	0.01985	1985	107301
La Gironda	Sandy clay loam	1.22	7.4	0.93	0.00001	0.01885	1885	154525

III. CONCLUSION

The adsorption behavior of the test item BF 500-6, metabolite of pyraclostrobin, was determined on five European soils. The soils covered a range of pH from 5.6 to 7.4 and a range of organic carbon content from 0.60 to 1.85%. The soils were classified by USDA scheme into four different textural classes: one sand, two sandy loams, one silt loam, and one sandy clay loam.

The test item BF 500-6 was fast and nearly completely adsorbed to the soil phase. This was expressed by high distribution coefficients with K_d values of 1732 – 1985 mL g^{-1} as well as high K_{OC} values of 107301 – 311704 mL g^{-1}.

Overview adsorption values of metabolite BF 500-6

Table 7.1.3.1.2-5: Adsorption of BF 500-6 on different soils

Soil	Soil type	OC [%]	pH [-]	K_d [-]	K_{oc} [mL g^{-1}]	Reference (BASF DocID)
Li 35 b	Loamy sand	1.1	6.5	350.0	31830	1999/10686
LUFA 2.2	Sand/ loamy sand	2.5	5.8	84.0	3360	
Bruch West	Sandy loam	1.5	7.5	248.0	16550	
USA 538-30-5	Loamy sand	0.4	5.8	365.5	91650	
USA 538-31-2	Loam	0.5	5.2	634.0	126800	
CAN-95024	Sandy clay loam	3.4	7.5	629.5	18500	
LUFA 2.1	Sand	0.60	5.6	1870.0	311704	2014/1000624
LUFA 2.3	Sandy loam	0.99	6.7	1732.0	174921	
Bruch West	Sandy loam	1.63	7.3	1882.0	115430	
Nierswalde	Silt loam	1.85	5.7	1985.0	107301	
La Gironda	Sandy clay loam	1.22	7.4	1885.0	154525	
Median				**634**	**107301**	

The old adsorption values for **BF 500-7** as given in the Monograph 12945/ECCO/BBA/01 are presented in Table 7.1.3.1.2-6, followed by the study summary of the new adsorption study performed with non-labeled BF 500-7.

Due to the very low water solubility, the compound could be tested only at one concentration.

Table 7.1.3.1.2-6: Adsorption of BF 500-7 on different soils as listed in the Monograph 12945/ECCO/BBA/01

Soil	Soil type	OC [%]	pH [-]	K_d [-]	K_{oc} [mL g^{-1}]
Li 35 b	Loamy sand	1.1	6.5	417.5	37950
LUFA 2.2	Sand / loamy sand	2.5	5.8	100.5	4020
Bruch West	Sandy loam	1.5	7.5	449.5	29950
USA 538-30-5	Loamy sand	0.4	5.8	543.5	135900
USA 538-31-2	Loam	0.5	5.2	749.5	149900
CAN-95024	Sandy clay loam	3.4	7.5	542.5	15950

Report: CA 7.1.3.1.2/2
Ebert D. et al., 2014a
Study of the adsorption behaviour of BF 500-7 (Reg.No. 369315) on different soils
2014/1000625
Guidelines: OECD 106, EPA 835.1230
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In laboratory experiments the adsorption behavior of the pyraclostrobin metabolite BF 500-7 was investigated on five European soils. The five tested soils covered a range of pH ($CaCl_2$) from 5.6 to 7.4, a range of organic carbon content from 0.60 to 2.01%, and four different USDA textural classes: one sand, two sandy loams, one sandy clay loam and one silt loam. Due to the low water solubility of BF 500-7, adsorption was only tested for one concentration level. Hence, no adsorption or desorption isotherm was determined. The application solution was prepared in 0.01 M $CaCl_2$ solution with a nominal concentration of 2.5 ng mL^{-1} of the test item. The ratio of soil versus test solution was 1 : 20, and the measurements were performed at the adsorption equilibrium time of one hour for the five soils.

Sorption of BF 500-7 to soil proceeded fast, expressed by solution concentrations < LOD after one hour of shaking. Calculated mean distribution coefficients (K_d) differed only slightly between all soils ranging from 4732 mL g^{-1} (LUFA 2.3) to 5025 mL g^{-1} (La Gironda). As almost the entire applied dose of BF 500-7 was sorbed to the soil phase, distribution coefficients normalized to the fraction of organic carbon in the soils (K_{OC}) increased from the soil exhibiting a large (Nierswalde; K_{OC}: 242564 mL g^{-1}) to the soil exhibiting a low organic carbon content (LUFA 2.1; K_{OC}: 801927 mL g^{-1}).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item:	BF 500-7
Synonym:	500M02
Reg. No.	369315
Chemical name (IUPAC):	N,N'-bis-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl} phenyl)diazene
Batch No.:	01586-173
Purity:	99.5%
Molar mass:	595.5 g mol^{-1}

2. Soils

The study was conducted with five different soils originating from Europe. The physico-chemical properties of the soils are provided in Table 7.1.3.1.2-7.

Table 7.1.3.1.2-7: Characterization of soils used to determine the adsorption behavior of pyraclostrobin metabolite BF 500-7

Soil designation Origin	LUFA 2.1 Germany	LUFA 2.3 Germany	LUFA 5M Germany	Nierswalde Germany	La Gironda Spain
Textural class (DIN 4220)	Sand	Loamy sand	Loamy sand	Clay silt	Sandy clay loam
Soil texture [%], (ISO 11277)					
Sand	89.5	66.9	55.5	17.7	48.0
Silt	8.2	24.8	33.6	72.9	24.3
Clay	2.3	8.3	10.9	9.4	27.7
Textural class (USDA scheme)	Sand	Sandy loam	Sandy loam	Silt loam	Sandy clay loam
Soil texture [%], (USDA)					
Sand	90.8	68.6	60.5	21.4	49.2
Silt	6.9	23.1	28.5	69.2	23.0
Clay	2.3	8.3	10.9	9.4	27.7
Organic carbon [%] (ISO 10694)	0.60	0.99	2.01	1.97	1.22
Cation exchange capacity [$cmol^{+}$ kg^{-1}]	-0.7	7.5	9.0	7.6	26.3
pH ($CaCl_2$)	5.6	6.7	7.2	5.8	7.4
pH (water)	6.5	7.4	8.1	6.8	8.3
max. water holding capacity [g/100g dry soil]	23.1	28.2	29.2	34.5	39.2
Bulk density [g L^{-1}]	1381	1226	1218	1234	1308

B. STUDY DESIGN

1. Experimental conditions

Preliminary tests: adsorption kinetics and soil/solution ratio

A preliminary experiment was run with two soils to determine the time needed to establish equilibrium conditions. The experiments were run with a soil / solution ratio of 1/20, using the soils LUFA 2.1 and La Gironda. The test was performed in 50 mL glass centrifuge vials, containing 2 g soil and 40 mL application solution (2.5 ng mL^{-1} BF 500-7 in 0.01 M aqueous $CaCl_2$). The vials were protected from light and shaken on a mechanical shaker for 0, 5, 15, 30, 60, 90 min, 2, 4, 6 and 24 hours (225 rpm). The soil / solution suspension was then centrifuged and the mass of the test vessel including contents was determined before and after decanting of the supernatant. An aliquot of the supernatant was sampled and the concentration of test item in the $CaCl_2$ solution was determined by LC-MS/MS.

To find the optimal soil / solution ratio for the adsorption tests, another experiment was run with LUFA 2.1 and La Gironda soil. Three different soil / solution ratios were tested: 1/10, 1/20, and 1/40 applying an application solution with a concentration of 2.5 ng mL^{-1}. Samples were incubated for five minutes by mechanical shaking.

The adsorption experiments were carried out in duplicate at room temperature (22 ± 2°C). Due to the low solubility of the test item, the soils were not pre-equilibrated with $CaCl_2$ solution, but the $CaCl_2$ solution containing the dissolved test item was added in one step.

Stability and adsorption of the test substance

Due to the low solubility of the test item (BF 500-7), no adsorption or desorption isotherms could be determined in this study. Hence, the stability test, which is typically performed during the pre-tests, corresponds to the adsorption test.

The test was performed as described for the equilibrium time test, additionally analyzing the soil by extraction with 25 mL of acetonitrile and then with 25 mL of acetonitrile / water (80/20; v/v), followed by quantification using LC-MS/MS.

2. Description of analytical procedures

The method for determining metabolite BF 500-7 in $CaCl_2$ solution was newly developed and validated within this study (method L0221/01). For determination of BF 500-7 in soil, the technical procedure of method L0166/1 was used as basis, adapted to the expected concentration range, and then also validated in two soils within this study.

Principle of the methods:

To analyze BF 500-7 in $CaCl_2$ solution, 5 mL of 0.01 M $CaCl_2$ solution were extracted into cyclohexane by mechanical shaking for 30 min at 225 rpm. A 2.5 mL aliquot of the sample was evaporated to dryness using an N-Evap at 40°C. Subsequently, the sample was reconstituted in 1 mL of acetonitrile / water (80/20; v/v) and analyzed by LC-MS/MS.

For soil analysis, a 2 g soil aliquot was extracted with 25 mL acetonitrile on a mechanical shaker (30 min at 225 rpm). After extraction, the tube was centrifuged at 3000 rpm (5 min, 20°C). The liquid phase was separated and taken to dryness. The solid remainder was re-extracted with 25 mL acetonitrile / water 80/20 (v/v). After centrifugation, the liquid phase was separated and used to dissolve the dried residue from the first extraction. For HPLC-MS/MS measurement the extract was further diluted.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

Analytical methods L0221/01 ($CaCl_2$ solution) and L0166/01 (soil) were validated within this study. Both methods revealed average recoveries of 83.1 – 117.9% and relative standard deviations < 12% for the dose levels of 0.02 and 0.2 µg L^{-1} for $CaCl_2$ solution as well as 5 and 50 µg kg^{-1} for soil, respectively.

In the adsorption test, the average recovery values ($CaCl_2$ solution plus soil extract) ranged from 100.4 to 112.4%.

B. FINDINGS

Based on the results obtained, methods L0221/01 and L0166/01 are considered valid for the determination of BF 500-7 in $CaCl_2$ solution and in soil with a validated LOQ of 0.02 µg L^{-1} and 0.005 mg kg^{-1}, respectively.

Results of the adsorption equilibrium test carried out with BF 500-7 indicated that the adsorption equilibrium was reached after five minutes (0.083 h).

Results of control samples, where the test item was applied to $CaCl_2$ solution incubated without soil, revealed that BF 500-7 was sorbed to the wall of the test vessels, as only about 56% of the applied BF 500-7 could still be detected in solution after 1 hour. However, it is assumed that in experiments with soil, the presence of soil will generally reduce such adsorption.

No matrix interferences were detected.

After one hour of shaking, solution concentrations ($C_{ads\text{-}aq}$) were < LOQ for all soils. Hence, almost the entire applied amount of BF 500-7 was accounted to the fraction sorbed to the soil phase. Calculated mean distribution coefficients (K_d; mean) differed only slightly between all soils ranging from 4732 mL g^{-1} (LUFA 2.3) to 5025 mL g^{-1} (La Gironda). Distribution coefficients normalized to the fraction of organic carbon in the soils (K_{OC}) increased from the soil exhibiting a large (Nierswalde; K_{OC}: 242564 mL g^{-1}) to the soil exhibiting a low organic carbon content (LUFA 2.1; K_{OC}: 801927 mL g^{-1}). Calculated K_d as well as K_{OC} values are presented in Table 7.1.3.1.2-8.

Table 7.1.3.1.2-8: Measured equilibrium concentrations and calculated distribution coefficients K_d and K_{OC} of BF 500-7

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	Measured concentration			Adsorption	
				C_0 [µg L^{-1}]	$C_{ads\text{-}aq}$ (eq) [µg mL^{-1}]	$C_{ads\text{-}s}$ (eq) [µg g^{-1}]	K_d [mL g^{-1}]	K_{OC} [mL g^{-1}]
LUFA 2.1	Sand	0.60	5.6	2.13	0.00001	0.04812	4812	801927
LUFA 2.3	Sandy loam	0.99	6.7	2.15	0.00001	0.04732	4732	478005
LUFA 5M	Sandy loam	2.01	7.2	2.14	0.00001	0.04897	4897	243656
Nierswalde	Silt loam	1.97	5.8	2.33	0.00001	0.04779	4779	242564
La Gironda	Sandy clay loam	1.22	7.4	2.33	0.00001	0.05025	5025	411866

III. CONCLUSION

The adsorption behavior of test item BF 500-7, a metabolite of pyraclostrobin, was determined on five European soils. The soils covered a range of pH from 5.6 to 7.4 and of organic carbon content from 0.60 to 2.01%. The soils were classified by USDA scheme into four different textural classes: one sand, two sandy loams, one silt loam, and one sandy clay loam.

The test item BF 500-7 was fast and nearly completely adsorbed to the soil phase. This was expressed by high values of the distribution coefficients with K_d values of 4732 – 5025 mL g^{-1} as well as high K_{OC} values of 242564 – 801927 mL g^{-1}.

Overview adsorption values of metabolite BF 500-7

Table 7.1.3.1.2-9: Adsorption of BF 500-7 on different soils

Soil	Soil type	OC [%]	pH [-]	K_d [-]	K_{oc} [mL g^{-1}]	Reference (BASF DocID)
Li 35 b	Loamy sand	1.1	6.5	417.5	37950	1999/10684
LUFA 2.2	Sand/ loamy sand	2.5	5.8	100.5	4020	
Bruch West	Sandy loam	1.5	7.5	449.5	29950	
USA 538-30-5	Loamy sand	0.4	5.8	543.5	135900	
USA 538-31-2	Loam	0.5	5.2	749.5	149900	
CAN-95024	Sandy clay loam	3.4	7.5	542.5	15950	
LUFA 2.1	Sand	0.60	5.6	4812.0	801927	2014/1000625
LUFA 2.3	Sandy loam	0.99	6.7	4732.0	478005	
LUFA 5M	Sandy loam	2.01	7.2	4897.0	243656	
Nierswalde	Silt loam	1.97	5.8	4779.0	242564	
La Gironda	Sandy clay loam	1.22	7.4	5025.0	411866	
Median				**750**	**149900**	

Due to the new data requirements under EU Regulation 1107/2009, two additional metabolites appearing in amounts > 5% TAR in soil (although only under anaerobic conditions) are considered for leaching assessment. Therefore, adsorption/desorption studies with the anaerobic soil metabolites **BF 500-4** and **BF 500-5** were performed.

Report: CA 7.1.3.1.2/3
Tuffnail W., 2014b
Adsorption/desorption of BF 500-4 (Reg.No. 358672) on soil
2014/1000721
Guidelines: OECD 106 (2000)
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In laboratory experiments the adsorption behavior of the pyraclostrobin metabolite BF 500-4 was investigated on five European soils. The soils covered a range of pH from 5.6 to 7.4 and of organic carbon content from 0.60 to 1.85%. The soils were classified by USDA scheme into four different textural classes: one sand, two sandy loams, one silt loam, and one sandy clay loam.

Due to the observed instability and high adsorption rate of the test item and the limitation of solubility concentrations, no isotherms could be obtained and the main phase of the study was conducted at a single target concentration of 0.125 µg mL^{-1}. In addition, no desorption experiments were conducted as the test item was not sufficiently stable in the $CaCl_2$/soil test system.

The application solution was prepared in 0.01 M $CaCl_2$ solution with a nominal concentration of 0.125 µg mL^{-1} of the test item. The ratio of soil versus test solution was 1 : 25, and the measurements were performed at the adsorption equilibrium time of two hours.

Distribution coefficients K_d ranged from 64 mL g^{-1} (soil LUFA 2.1) to 291 mL g^{-1} in soil Nierswalde. Distribution coefficients normalized to the organic carbon content K_{OC} varied between 6871 mL g^{-1} (Bruch West) and 15748 mL g^{-1} (Nierswalde).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS code: BF 500-4
Synonym: 500M73
Reg. No. 358672
Chemical name (IUPAC): 2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenylamine
Molar mass: 299.8 g mol^{-1} (unlabeled)
Position of radiolabel: Tolyl-ring-U-^{14}C
Specific radioactivity of a.i.: 8.49 MBq mg^{-1}
Batch No.: 1072-1004
Radiochemical purity: 98.0%

2. Soils

The study was conducted with five different soils originating from Europe. The physico-chemical properties of the soils are provided in Table 7.1.3.1.2-10.

Table 7.1.3.1.2-10: Characterization of soils used to determine the adsorption behavior of pyraclostrobin metabolite BF 500-4

Soil designation Origin	Bruch West Germany	Nierswalde Germany	LUFA 2.1 Germany	LUFA 2.3 Germany	La Gironda Spain
Textural class (DIN 4220)	Loamy sand	Clay silt	Sand	Loamy sand	Sandy clay loam
Soil texture [%], (ISO 11277)					
Sand	61.1	15.1	89.5	66.9	48.0
Silt	27.6	76.2	8.2	24.8	24.3
Clay	11.3	8.8	2.3	8.3	27.7
Textural class (USDA scheme)	Sandy loam	Silt loam	Sand	Sandy loam	Sandy clay loam
Soil texture [%], (USDA)					
Sand	63.7	17.7	90.8	68.6	49.2
Silt	25.1	73.5	6.9	23.1	23.0
Clay	11.3	8.8	2.3	8.3	27.7
Organic carbon [%] (ISO 10694)	1.63	1.85	0.60	0.99	1.22
Cation exchange capacity [$cmol^{+}$ kg^{-1}]	11.9	3.1	-0.7	7.5	26.3
pH ($CaCl_2$)	7.3	5.7	5.6	6.7	7.4
pH (water)	8.0	6.5	6.5	7.4	8.3
Max. water holding capacity [g/100g dry soil]	29.2	36.1	23.1	28.2	39.2
Bulk density [g L^{-1}]	1273	1236	1381	1226	1308

B. STUDY DESIGN

1. Experimental conditions

Preliminary tests: adsorption kinetics and soil/solution ratio

The adsorption of the test item to glass, polypropylene, and Teflon vessel surfaces was investigated at a nominal concentration of 0.125 µg mL^{-1} in vessels with no soil present after an agitation period of 24 hours. Measurements of total radioactivity remaining in the aqueous solution were determined and demonstrated that there was significant adsorption to both glass and polypropylene test vessels (mean recoveries of 33 and 18%, respectively), but reduced adsorption to Teflon vessels (mean recovery of 78%). Therefore, Teflon vessels were used throughout further experiments.

The solution to soil ratio to be used for the main experiment was investigated using LUFA 2.1 (lowest pH) and La Gironda soil (highest pH) at a nominal/target test item solution concentration of 0.125 µg mL^{-1}. Adsorption experiments were conducted with duplicate vessels prepared at solution/soil ratios of 1:1, 5:1, and 25:1. The vessels were mixed continuously on a roller mixer at 20±2°C for approximately 24 hours in darkness. The solutions were then separated by centrifugation at 2000 rpm (1162 G) for 60 minutes and the solution radioactivity concentrations were measured by liquid scintillation counting (LSC). The percentage of applied radioactivity adsorbed to soil was calculated from these measurements by subtraction (indirect method). Due to the observed strong adsorption of the test item, the highest solution to soil ratio of 25:1 was chosen for the main experiment.

The equilibration time experiments were performed at a solution to soil ratio of 25:1 and at the proposed test substance concentration of 0.125 µg mL^{-1} using LUFA 2.1 and La Gironda soils. After intervals of 2, 4, 8 and 12 hours single tubes for the soil were removed and centrifuged at 3200 rpm (2977 G) for 15 minutes. The direct method was applied, in which both the aqueous and soil phases were quantified. The soil phase was quantified following solvent extraction and the extracts were analyzed by LSC; the soil residue was quantified by combustion in a sample oxidizer. The aqueous and pooled soil extracts were analyzed by radio-HPLC to identify the proportion of test item present.

It was noted that after 12 hours agitation up to 6 components were present in the aqueous phase indicating potential degradation of the test item within the system. In order to minimize degradation, the agitation time for the main experiment was reduced to 2 hours. A peak at ca. 3.2 min retention time by HPLC analysis, which was formed even within the short agitation time of 2 hours, was further investigated by LC-MS/MS.

Determination of distribution constants

For the adsorption experiment, a solution to soil ratio of 25:1 and equilibration time of 2 hours was chosen for all soils. Due to the observed instability and high adsorption rate of the test item and the limitation of solubility, concentrations below the half water solubility of 0.125 µg mL^{-1} were not possible due to detection sensitivity and thus no isotherms could be obtained. Therefore, the main phase of the study was conducted at a single target concentration of 0.125 µg mL^{-1}. In addition, no desorption experiments were conducted as the test item was not sufficiently stable in the $CaCl_2$/soil test system. Hence, no desorption isotherms were established.

Portions (1.2 g) of air dried soils were weighed into 50 mL Teflon centrifuge tubes, in triplicate. The soils were pre-conditioned with 30 mL of 0.01 M $CaCl_2$ solution overnight prior to the application. Then, the $CaCl_2$ solution in the test vessels was spiked with 30 µL of the [^{14}C]-BF 500-4 application solution forming an achieved test substance concentration of 0.124 µg mL^{-1} determined by comparison of dose check solutions produced with the theoretical dose calculation.

Following application, the vessels were agitated on a roller mixer at 20 ± 2°C in darkness for 2 hours. At the end of the equilibration period, each sample tube was centrifuged at 3200 rpm (2977 G) for 15 minutes to separate the soil and solution. The supernatant solution was carefully decanted into pre-weighed 50 mL Teflon tubes and the weight recorded.

2. Description of analytical procedures

The direct method was applied where both the aqueous and soil phases consisting of three solvent extractions were analyzed by LSC followed by soil residue combustions. The aqueous and extraction phases (pooled) were then further analyzed by radio-HPLC to identify the proportion of test item present within the system and subsequent distribution coefficient calculations adjusted accordingly. From this data the distribution coefficient K_d and organic carbon content adjusted K_{oc} values were generated for the test item for each soil.

After removal of the $CaCl_2$ phase, the soil samples were extracted to determine the adsorbed amount of [^{14}C]-BF 500-4 on the soil. The soil samples were extracted with 6 mL of acetonitrile by shaking on an orbital shaker for at least 15 minutes, followed by centrifugation at 3200 rpm (2977 G) for 15 minutes. The supernatant was carefully decanted into pre-weighed 20 mL glass vials and the weight was recorded. The concentration of the radioactivity in the extracts was determined in duplicate by LSC. The extraction procedure was repeated two more times and the corresponding extracts of each soil sample were then pooled and analyzed by radio-HPLC.

In order to obtain a material balance, 0.2 g aliquots of the remaining soil were air dried and combusted in a sample oxidizer and samples set for LSC. Aqueous solutions that were not analyzed immediately by HPLC were stored at -80°C until required. Soil extracts and aqueous solutions after HPLC analysis and residues were kept in a freezer at <-15°C.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The aqueous and pooled extract phases were analyzed using radio-HPLC analysis. The total mass balance of applied radioactivity of the aqueous phase, soil extracts, and soil combustion was between 83.6 - 99.6% for all soils.

B. FINDINGS

As the amount of test item in the aqueous solution of the sample containing no soil was less than 90%, the direct method of analyzing both the aqueous and soil phases (by extraction) and combustion of the residues was used throughout all further experiments.

LC-MS/MS analysis of the peak present at ca. 3.2 min retention time present after the short agitation time of 2 hours was performed. The absence of a peak in the three summary MRM (Multiple Reaction Monitoring) chromatograms corresponding to the peak observed at ca. 2.4 min in the [^{14}C] radiochromatogram (ca. 3.2 min in the profiling system) confirms that this peak has no relation to the test item BF 500-4.

After an adsorption time of 2 hours, between 70.6 and 84.3% were sorbed to the soils. The data were adjusted for the proportion of radioactivity recovery of test item in both the aqueous phase and soil extracts following analysis by HPLC.

Calculated distribution coefficients K_d ranged from 64 mL g^{-1} (soil LUFA 2.1) to 291 mL g^{-1} in soil Nierswalde. Distribution coefficients normalized to the organic carbon content K_{OC} varied between 6871 mL g^{-1} (Bruch West) and 15748 mL g^{-1} (Nierswalde). Calculated K_d as well as K_{OC} values are presented in Table 7.1.3.1.2-11.

Table 7.1.3.1.2-11: Calculated distribution coefficients K_d and K_{OC} of BF 500-4

Soil	Soil Type (USDA)	Organic C [%]	pH ($CaCl_2$)	Adsorption (2 h)	
				K_d [mL g^{-1}]	K_{OC} [mL g^{-1}]
Bruch West	Sandy Loam	1.63	7.3	112	6871
Nierswalde	Silt Loam	1.85	5.7	291	15748
LUFA 2.1	Sand	0.60	5.6	64	10667
LUFA 2.3	Sandy Loam	0.99	6.7	74	7475
La Gironda	Sandy Clay Loam	1.22	7.4	102	8333
Arithmetic mean				**129**	**9819**

III. CONCLUSION

The adsorption behavior of test item BF 500-4, metabolite of pyraclostrobin, was determined on five soils. The soils covered a range of pH from 5.6 to 7.4 and of organic carbon content from 0.60 to 1.85%. The soils were classified by USDA scheme into four different textural classes: one sand, two sandy loams, one silt loam, and one sandy clay loam.

The adsorption of BF 500-4 on five soils was high. Calculated distribution coefficients K_d values were lowest in soil LUFA 2.1 (64 mL g^{-1}) and highest in soil Nierswalde (291 g mL^{-1}). Distribution coefficients normalized to the organic carbon content K_{OC} varied between 6871 mL g^{-1} (Bruch West) and 15748 mL g^{-1} (Nierswalde).

Report:	CA 7.1.3.1.2/4 Tuffnail W., 2014a Adsorption/desorption of BF 500-5 (Reg.No. 298327) on soil 2014/1000722
Guidelines:	OECD 106 (2000)
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

In laboratory experiments the adsorption behavior of the pyraclostrobin metabolite BF 500-5 was investigated on five European soils. The soils covered a range of pH from 5.6 to 7.4 and of organic carbon content from 0.60 to 1.85%. The soils were classified by USDA scheme into four different textural classes: one sand, two sandy loams, one silt loam, and one sandy clay loam.

For the determination of the adsorption isotherm, the soils were treated at five concentrations; 0.001, 0.01, 0.025, 0.05 and 0.1 µg mL^{-1} in the adsorption solution. No desorption phases were required as the apparent instability of the test compound determined that the direct method of analyzing soil extracts was employed. The ratio of soil versus test solution was 1 : 25, and the measurements were performed at the adsorption equilibrium time of four hours.

The mass balance recoveries of total applied radioactivity for each soil type were in the range 80.1 – 96.5%. Recoveries related to percent radioactivity of test item were 68.5 - 88.5%.

Calculated K_f values ranged from 5 mL g^{-1} in soil LUFA 2.1 to 15 mL g^{-1} in soil Nierswalde. Carbon normalized adsorption coefficients $K_{f,oc}$ varied between 400 (Bruch West) and 831 mL g^{-1} (La Gironda). Freundlich exponents 1/n ranged from 0.7985 (LUFA 2.3) to 0.8798 (Bruch West).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS code: BF 500-5
Synonym: 500M04
Reg. No. 298327
Chemical name (IUPAC): 1-(4-chlorophenyl)-1H-pyrazol-3-ol
Molar mass: 194.6 g mol^{-1} (unlabeled)
Position of radiolabel: Phenyl-U-^{14}C
Specific radioactivity of a.i.: 8.99 MBq mg^{-1}
Batch No.: 724-2102
Radiochemical purity: 99.8%

2. Soils

The study was conducted with five different soils originating from Europe. The physico-chemical properties of the soils are provided in Table 7.1.3.1.2-12.

Table 7.1.3.1.2-12: Characterization of soils used to determine the adsorption behavior of pyraclostrobin metabolite BF 500-5

Soil designation Origin	Bruch West Germany	Nierswalde Germany	LUFA 2.1 Germany	LUFA 2.3 Germany	La Gironda Spain
Textural class (DIN 4220)	Loamy sand	Clay silt	Sand	Loamy sand	Sandy clay loam
Soil texture [%], (ISO 11277)					
Sand	61.1	15.1	89.5	66.9	48.0
Silt	27.6	76.2	8.2	24.8	24.3
Clay	11.3	8.8	2.3	8.3	27.7
Textural class (USDA scheme)	Sandy loam	Silt loam	Sand	Sandy loam	Sandy clay loam
Soil texture [%], (USDA)					
Sand	63.7	17.7	90.8	68.6	49.2
Silt	25.1	73.5	6.9	23.1	23.0
Clay	11.3	8.8	2.3	8.3	27.7
Organic carbon [%] (ISO 10694)	1.63	1.85	0.60	0.99	1.22
Cation exchange capacity [$cmol^{+}$ kg^{-1}]	11.9	3.1	-0.7	7.5	26.3
pH ($CaCl_2$)	7.3	5.7	5.6	6.7	7.4
pH (water)	8.0	6.5	6.5	7.4	8.3
Max. water holding capacity [g/100g dry soil]	29.2	36.1	23.1	28.2	39.2
Bulk density [g L^{-1}]	1273	1236	1381	1226	1308

B. STUDY DESIGN

1. Experimental conditions

Preliminary tests: adsorption kinetics and soil/solution ratio

The adsorption of the test substance to glass and polypropylene vessel surfaces was investigated at the highest concentration in vessels (0.1 µg mL^{-1}) with no soil present.

The solution to soil ratio to be used for the main experiment was investigated using LUFA 2.1 (lowest pH) and La Gironda (highest pH) at the highest test substance solution concentration (0.1 µg mL^{-1}). Adsorption experiments were conducted with duplicate vessels prepared at solution/soil ratios of 1:1, 5:1 and 25:1. The vessels were mixed continuously on a roller mixer, at approximately 20°C for approximately 24 hours in darkness. The solutions were then separated by centrifugation at 8500 rpm (7027 G) for 15 minutes and the solution radioactivity concentration measured by liquid scintillation counting (LSC). The percentage of applied test compound adsorbed to soil was calculated from these measurements with a target percentage adsorbed to the soil above 20% and preferably <80%. A solution to soil ratio of 25:1 was chosen for the main experiment.

Adsorption experiments to determine the time needed to establish equilibrium conditions were set up using the solution/soil ratio determined above, with LUFA 2.1 and La Gironda soils and a test substance concentration of 0.1 µg mL^{-1}. After intervals of 2, 4, 8 and 24 hours single tubes for the soil were removed for separation by centrifuging and LSC as described above. The direct method was employed where both the aqueous phase and soil extracts were analyzed by HPLC for test item at the highest concentration. Results indicated that an equilibrium time of 4 hours agitation was required for all the soil types to give a high degree of determinable test item at or near equilibrium.

Determination of Freundlich adsorption isotherms

Since BF 500-5 could not be regarded as stable only adsorption but no desorption isotherms were established.

Portions of air dried soils (ca. 1.2 g) were weighed into 50 mL polypropylene centrifuge tubes. The soils were conditioned for use by mixing with 0.01 M calcium chloride overnight prior to the application. Application solutions of the test substance, prepared in acetonitrile, were applied by spiking to the test vessels. The achieved test substance concentrations of BF 500-5 were 0.001, 0.01, 0.025, 0.05 and 0.1 µg mL^{-1}. The test was conducted in duplicate at each test substance concentration, except for the highest concentration which was performed in triplicate. Single blank tubes at each concentration excluding soil were also treated to confirm that the test material did not adsorb to the test vessels.

Following application, the vessels were mixed at approximately 20°C, in a controlled temperature room in darkness, for the equilibration time of 4 hours.

2. Description of analytical procedures

At the end of the equilibration period, each sample tube was centrifuged (3200 rpm (2977 G) for 30 minutes) to separate the soil and solution. As much as reasonably possible of the supernatant solution was removed into pre-weighed 50 mL centrifuge tubes and the weight was recorded with the concentration of radioactivity in the solution determined in duplicate by LSC for all samples and radio-HPLC at the highest concentration only.

Following adsorption in the main study, all soil samples for each soil type were taken for extraction. The soil residues were extracted with 5 mL of acetonitrile by shaking for 10 minutes, followed by centrifugation at 8500 rpm (7027 G) for 15 minutes. As much as reasonably possible the extract solution was removed into pre-weighed 50 mL centrifuge tubes and the weight recorded with the concentration of radioactivity in the solution determined in duplicate by LSC. The procedure repeated two more times and selected samples at the highest concentration were analyzed by radio-HPLC.

Extracts of soil, adsorption solution, soil extracts and soil residues following extraction were stored at <-15°C.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The mass balances of the test item in the adsorption and extraction phases were between 68.52 - 88.48%. These recovery values are from both phases (preliminary and adsorption phase) and were adjusted for test item using the HPLC analysis from selected samples from the highest concentration. All soils after combustion gave a total mass balance of applied radioactivity of between 80.05 – 96.46%. It is suggested that the low recoveries may be due to compound becoming bound to the vessel surface, although this was not apparent when tested in tier 1.

B. FINDINGS

Following treatment at rates ranging from 0.1 to 0.001 µg mL^{-1} and an adsorption time of 4 hours, the Freundlich adsorption coefficients were calculated from the corresponding $CaCl_2$-solution and soil concentration data. The data were adjusted for recovery of test item in both the aqueous phase and soil extracts.

Adsorption coefficients K_f ranged from 5 (LUFA 2.1) to 15 mL g^{-1} (Nierswalde), while the coefficients adjusted for the organic carbon content ($K_{f,oc}$) was highest in soil La Gironda (831 mL g^{-1}) and lowest in soil Bruch West (400 mL g^{-1}). Freundlich exponents 1/n ranged from 0.7985 (LUFA 2.3) to 0.8798 (Bruch West). Results are summarized in Table 7.1.3.1.2-13.

Table 7.1.3.1.2-13: Calculated adsorption coefficients K_f, carbon normalized adsorption coefficients $K_{f,oc}$ and Freundlich exponents 1/n of BF 500-5

Soil	Soil Type (USDA)	Organic C [%]	pH ($CaCl_2$)	Adsorption (4 h)		
				K_f [mL g^{-1}]	1/n [-]	$K_{f,oc}$ [mL g^{-1}]
Bruch West	Sandy Loam	1.63	7.3	7	0.8798	400
Nierswalde	Silt Loam	1.85	5.7	15	0.8311	830
LUFA 2.1	Sand	0.60	5.6	5	0.8578	785
LUFA 2.3	Sandy Loam	0.99	6.7	7	0.7985	678
La Gironda	Sandy Clay Loam	1.22	7.4	10	0.8774	831
Arithmetic mean				**8.8**	**0.85**	**705**

III. CONCLUSION

The adsorption behavior of test item BF 500-5 (500M04), metabolite of pyraclostrobin, was determined on five soils. The soils covered a range of pH from 5.6 to 7.4 and of organic carbon content from 0.60 to 1.85%. The soils were classified by USDA scheme into four different textural classes: one sand, two sandy loams, one silt loam, and one sandy clay loam.

Calculated K_f values ranged from 5 mL g^{-1} in soil LUFA 2.1 to 15 mL g^{-1} in soil Nierswalde. Carbon normalized adsorption coefficients $K_{f,oc}$ varied between 400 (Bruch West) and 831 mL g^{-1} (La Gironda).

CA 7.1.3.2 Aged sorption

No experimental data are available. Due to the high soil adsorption values of all pyraclostrobin metabolites, no aged sorption experiments are considered necessary.

CA 7.1.4 Mobility in soil

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

No new experimental data are available. The soil mobility of pyraclostrobin and its metabolites was evaluated during the previous Annex I inclusion process based on non-aged and aged column leaching studies. No residues were found in any of the leachates.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

Please see justification in M-CA 7.1.4.1.1 above.

CA 7.1.4.2 Lysimeter studies

The leaching risk of pyraclostrobin and its metabolites is addressed by PEC_{gw} calculations using results from degradation rate and adsorption/desorption studies. Neither the active substance nor its metabolites reveal any risk for groundwater contamination. Lysimeter studies are therefore considered to be not necessary.

CA 7.1.4.3 Field leaching studies

Neither the active substance nor its metabolites reveal any risk for groundwater contamination. Field leaching studies are therefore considered to be not necessary.

CA 7.2 Fate and behaviour in water and sediment

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

CA 7.2.1.1 Hydrolytic degradation

Report: CA 7.2.1.1/1
Scharf J., 1999b
Hydrolysis of BAS 500 F
1999/10060
Guidelines: EEC 94/37, EPA 161-1
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report: CA 7.2.1.1/2
Ebert D., 2011a
Hydrolysis of BAS 500 F (Report Amendment)
2011/1201705
Guidelines: EEC 94/37, EPA 161-1
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

No new experimental data were produced, however, a typing error occurred in the text of the original study in the results part of the 50°C experiment (page 20, chapter 4.2.1.1, tolyl label, first paragraph, third sentence) leading to a wrong designation of one of the identified peaks (BF 500-5 instead of BF 500-3). The text was corrected in the report amendment. The overall results as presented in the tables were unaffected.

Pyraclostrobin was stable at pH 4, 5, and 7. Only at pH 9 a slow hydrolysis was observed with formation of known derivatives (BF 500-6, BF 500-7 and BF 500-5).

CA 7.2.1.2 Direct photochemical degradation

The already peer-reviewed data are considered to be still valid. Therefore, no new experimental data are provided. Pyraclostrobin degraded under irradiated conditions with half-lives << 1 d.

CA 7.2.1.3 Indirect photochemical degradation

Since pyraclostrobin degraded very fast during the direct aqueous photolysis study and in the irradiated water/sediment studies, an indirect aqueous photolysis study is considered to be not necessary.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 "Ready biodegradability"

The already peer-reviewed data are considered to be still valid. Therefore, no new experimental data are provided. According to the previous EU evaluation pyraclostrobin has to be considered as "*not readily biodegradable*".

CA 7.2.2.2 Aerobic mineralisation in surface water

Report:	CA 7.2.2.2/1 Ebert D.,Possienke M., 2013a ^{14}C-BAS 500 F (Pyraclostrobin): Aerobic mineralisation in surface water 2013/1002741
Guidelines:	OECD 309 (April 2004)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The degradation of pyraclostrobin under aerobic aquatic conditions was investigated over a period of up to 63 days at 20°C in the dark. The study was performed according to OECD guideline 309 (Aerobic mineralization in surface water – Simulation biodegradation test). Two test variants, pelagic test and suspended solid test, were investigated in parallel. Both test variants were performed with two different pyraclostrobin concentrations (10 µg L^{-1} and 50 µg L^{-1}). Two differently ^{14}C-labeled test items were applied (chlorophenyl-label and tolyl-label). The chlorophenyl-labelled test item contained the metabolite BF 500-3 as impurity.

The test vessels were attached to a flow-through system for continuous aeration and incubated at a temperature of approximately 20 ± 2°C in the dark. Samples for the experiment were taken at 0, 3, 7, 14, 30, 44 and 63 days after treatment. Water and sediment of the suspended solid test were worked up separately. Water samples and sediment extracts were analyzed by liquid scintillation counting (LSC) and radio-HPLC. The amount of non-extractable residues was determined by combustion and LSC analysis. Volatiles were trapped in appropriate trapping solutions and also analyzed by LSC.

The obtained results showed that pyraclostrobin was degraded very slowly in a pure water environment as provided in the pelagic test. More than 78 – 97% TAR were still detectable as unchanged parent in the water phase 63 days after treatment under dark conditions. The results were overall very comparable to the sterile test vessels. The degradation of pyraclostrobin under the pelagic test conditions was thus characterized by slow hydrolysis and formation of low amounts of cleavage products, of which BF 500-5 occurred at 5.6 - 10.9% TAR. All other peaks never exceeded 2.1% TAR.

In the suspended solid test, pyraclostrobin dissipated at a fast rate from the water phase (< 45% TAR after 3 days) and adsorbed to the solid particles floating in the water. Despite the overall low amount of suspended solids, pyraclostrobin behaved as known from soil and sediment studies and converted quickly from extractable residues into non-extractable residues due to binding to the organic matrix.

In the water phase of the suspended solid test, the hydrolysis product BF 500-5 was detected in maximum amounts of 7.7% TAR with the chlorophenyl-label. One peak in the tolyl-label treated test vessels (low concentration) reached 5.8% TAR after 44 days (but only max. 2% TAR in the high concentration test). It declined again to 2.1% TAR after 63 days. Due to the low substance amounts, identification was not possible. All other peaks never exceeded 3.8% TAR.

In the sediment extracts of the suspended solid test, besides BF 500-3 which was present in the treatment solution of the chlorophenyl-labelled test item, also the known soil and sediment metabolites BF 500-6 (≤ 2.3% TAR) and BF 500-7 (≤ 2.6% TAR) were formed. Other components did not exceed 0.5% TAR at any sampling time.

Overall, the degradation of pyraclostrobin was characterized by a low mineralization rate in both test variants irrespective of test concentration or label position. The amount of $^{14}CO_2$ never exceeded 5% TAR within 63 days.

Kinetic analysis and calculations of DT_{50} and DT_{90} values for the pelagic and the suspended solid test were performed following the recommendations of the FOCUS Kinetics workgroup [FOCUS, 2006] to derive aquatic persistence and modeling endpoints. The analysis was done by a non-linear regression method (Iteratively Reweighted Least Squares) using the software package KinGUI version 2.

The $DegT_{50}$ values in the pelagic test ranged from 410 to 458 days for pyraclostrobin and from 11 to 29 days for BF 500-3. In the suspended solid test, the $DegT_{50}$ for pyraclostrobin for the whole system ranged from 26 to 28 days, while the $DisT_{50}$ for the water compartment was calculated to range from 7 to 10 days and for the suspended sediment from 44 to 47 days. The $DisT_{50}$ for BF 500-5 was 103 days when calculated with the high test concentration. For the low test concentration no adequate fit was achieved.

In general, pyraclostrobin hydrolyses only slowly under the pelagic test conditions, but adsorbs quickly to suspended solids, when available, and is then further degraded by formation of bound residues.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Pyraclostrobin (BAS 500 F)
Chemical name:	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3yl]oxymethyl} phenyl)-(N-methoxy)carbamate
Molecular formula:	$C_{19}H_{18}ClN_3O_4$
Molar mass:	387.82 g mol^{-1} (unlabeled)

Label 1 (chlorophenyl label)

Label:	chlorophenyl-U-^{14}C
Batch No.:	579-6103
Specific activity of a.s.:	6.57 MBq mg^{-1}
Radiochemical purity:	98.3%
Chemical purity:	97.6%

Label 2 (tolyl label)

Label:	tolyl-U-^{14}C
Batch No.:	566-4201
Specific activity of a.s.:	7.32 MBq mg^{-1}
Radiochemical purity:	99.4%
Chemical purity:	97.8%

2. Test system

Water and small amounts of sediment were collected from Berghäuser Altrhein, a pond-like side arm of the river Rhine south of Speyer (Rhineland-Palatinate, Germany).

The sediment was passed through a 2 mm sieve, and the water was filtered through an 0.2 mm sieve. Sieving and transfer of water and sediment into the test vessels was done at the same day after collection of the water/sediment systems. The physico-chemical properties of the system are summarized in Table 7.2.2.2-1.

Table 7.2.2.2-1: Characterization of the water/sediment system

Designation Origin		**Berghäuser Altrhein Rhineland-Palatinate, Germany**	
Water			
Temperature	[°C]	17.2	
pH water	-	7.84	
Oxygen concentration	[mg L^{-1}]	8.4	
Redox potential (Eh)	[mV]	266	
Hardness	[mmol L^{-1}]	0.08	
Total organic carbon	[mg L^{-1}]	1.3	
Total N	[mg L^{-1}]	0.32	
Total P	[mg L^{-1}]	0.06	
		Beginning	End
Bacteria	[cfu mL^{-1}]	17800	184
Fungi	[cfu mL^{-1}]	250	8
Actinomycetes	[cfu mL^{-1}]	92	0
Sediment			
Textural class		DIN 4220	USDA
Sand	[%]	12.4	15.2
Silt	[%]	55.2	52.4
Clay	[%]	32.4	32.4
Soil type	-	Silty clay (Tu3)	Silty clay loam
pH (H_2O)	-	7.5	
pH ($CaCl_2$)	-	7.1	
Redox potential (Eh)	[mV]	-195	
Cation exchange capacity	[$cmol^{+}$ kg^{-1}]	38.7	
Total organic carbon	[%]	10.96	
Total N	[%]	0.25	
Total P	[mg kg^{-1}]	740	
		Beginning	
Bacteria	[cfu g^{-1}]	2.16×10^8	
Fungi	[cfu g^{-1}]	2.44×10^6	
Actinomycetes	[cfu g^{-1}]	2.33×10^6	

B. STUDY DESIGN

1. Experimental conditions

A total of 72 flasks was prepared: 36 flasks for the pelagic test, 28 flasks for the suspended solid test, 6 flasks as system control with benzoic acid and 2 flasks as untreated control samples for the pelagic test.

The flasks were filled with about 450 mL water. For the suspended solid test, about 0.9 g wet sediment was added to the respective test vessels. Taking into account that the wet sediment still contained a high percentage of water (water content of 64.37% determined in a random sample), the overall suspended sediment concentration was finally about 0.9 - 1 g L^{-1}. Four test vessels (for the pelagic test) were sterilized in an autoclave.

Appropriate amounts (20 µL) of the respective application solutions were pipetted to the water surface to achieve a nominal application rate of 50 µg L^{-1} or 10 µg $L^{-1,}$ respectively.

The systems were incubated at 20 ± 2°C in a metabolism apparatus (incubator) with a gas flow system. The test vessels were placed on multiplate magnetic stirrers. For the pelagic test, the upper 1-2 cm water layer was slightly agitated to keep the oxygen saturation on sufficient high level, while for the suspended solid test the water was completely agitated to keep the sediment particles in suspension. However, the arrangement of the high number of test vessels in the incubator prevented optimal cooling, and the running magnetic stirrers also emitted low heat, so that a slightly increased temperature of 24°C was measured in the water of the vessels on the third day after treatment. Therefore, the incubator temperature was stepwise adjusted within the next days to decrease the temperature about 2-3°C to the desired range.

Each test vessel was connected to a volatile trapping system of two gas washing bottles containing different trapping solutions (ethylene glycol, NaOH) for the ^{14}C-volatiles to be expected. Sterilized test vessels were not connected to the air flow system to prevent contamination by airborne germs.

2. Sampling

Samples were taken at 0, 3, 7, 14, 30, 44 and 63 days after treatment (DAT). The sterile vessels (only pelagic test) were sampled after 28 and 63 days.

For sampling, the respective flasks were removed from the incubator. The following physico-chemical parameters were determined: oxygen content, pH and redox potential of the water and temperature.

3. Description of analytical procedures

Water
The radioactivity in the water samples was measured by Liquid Scintillation Counting (LSC). Aliquots of the water samples were analyzed by High Performance Liquid Chromatography (HPLC).

Sediment (only suspended solid test)
The sediment was extracted 3 times with acetonitrile (ACN; 9-10 mL) by shaking for 15 minutes. Due to the high water content of the sediment after separation from the water phase, the first extraction is referred to as ACN/H_2O extraction. The extracts were collected separately and analyzed for radioactivity by LSC. Subsequently, the extracts were combined and measured again for radioactivity by LSC to ensure that no material losses due to adsorption at glass walls occurred. Both extracts were finally subjected to HPLC analysis without further clean-up or concentration.

The extracted sediment residue was dried under nitrogen flow. The amount of sediment was just sufficient for one combustion sample to determine the non-extractable residues. Nevertheless, it enabled to establish a material balance for each test vessel.

Volatiles
Radioactivity in the trapping solutions was determined by LSC. In order to confirm mineralization to CO_2, selected NaOH traps from the suspended solid test (59/63 day samples) were further investigated by addition of 1 mL concentrated HCl and expelling CO_2 by sonication and purging with nitrogen. Then the samples were measured again by LSC. No significant amounts of radioactivity could be detected anymore (<0.2% TAR), confirming that the radioactivity found in the NaOH traps consisted originally of $^{14}CO_2$.

Benzoic acid (system control)
The volatile traps of the ^{14}C-benzoic acid treated test vessels were sampled at 0, 3, 8, 15, 29, 43 and 64 days after treatment, whereas water samples were taken after 15, 29 and 64 days. The parameters temperature, O_2 content, pH and redox potential of the water were measured on the days of water sampling. Since one test vessel showed no mineralization at all, it was defined as an outlier and excluded from further evaluation.

4. Calculation of the degradation/dissipation rates

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0*] in order to derive modeling endpoints. The analysis was done by non-linear regression methods (Iteratively Reweighted Least Squares) using the KinGUI software version 2.

The evaluation of the results of the pelagic test system for high and low test concentrations showed that degradation in the water phase was best described by SFO kinetics. Degradation of the metabolite BF 500-3 could also be sufficiently described with SFO kinetics. For the metabolite BF 500-5, no adequate fit could be achieved.

The evaluation of the results of the suspended sediment test system at P-I level showed that degradation in the total system and dissipation from the sediment phase could be described by SFO kinetics, while dissipation from the water phase could be adequately described by bi-phasic models (FOMC and DFOP). Kinetic evaluation at P-II level was not reliable and was not pursued further.

The evaluation of the results of the metabolites at M-I level provided adequate dissipation parameters for BF 500-5 in the high test concentration with the chlorophenyl label. For the low test concentration, no adequate fit could be achieved.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The distribution of radioactivity in the different compartments of the water/sediment system treated with ^{14}C-pyraclostrobin is presented in Table 7.2.2.2-2 and Table 7.2.2.2-3.

The actual applied amounts of test item per test vessel were 23.54 µg (high concentration, chlorophenyl label), 22.98 µg (high concentration, tolyl label), 4.81 µg (low concentration, chlorophenyl label) and 4.64 µg (low concentration, tolyl label). In general, no significant differences were found in pyraclostrobin behavior between the high and low test concentration. Therefore, results mentioned in the text are described always for both concentrations.

Pelagic test

The material balance for the pelagic test ranged from 92.0 - 98.2% of the total applied radioactivity (TAR). In the sterile vessels, the material balance ranged from 96.3 - 99.7% TAR.

The amount of radioactivity in the water was found to be very stable. At the end of the study (63 DAT) it ranged from 91.5 to 97.0% TAR. For all pelagic test samples and sampling time points, the radioactivity in the volatile traps never exceeded 0.7% TAR indicating a low rate of mineralization in this test variant.

Suspended solid test

For the suspended solid test the material balance ranged from 84.4 - 99.4% TAR. The values for the sampling time point directly after application (0 DAT) were taken from the pelagic test samples since similar conditions at the experiment start were assumed for both test variants.

The radioactivity dissolved freely in the water phase declined relatively fast for the suspended solid test. After 3 days, only 34.4 - 44.5% of the applied radioactivity was still detectable in the water phase and it declined further to 8.7 - 12.2% TAR at the end of incubation. Since pyraclostrobin quickly adsorbed to the sediment, the radioactivity in the sediment increased to maximum amounts of 73.0 – 80.2% TAR at the end of incubation.

Whereas at early samplings, most of the radioactivity was still extractable from the sediment (49.4 to 55.0% TAR after 3 days), the extractability declined significantly to 19.1 - 30.6% TAR after 63 days. Further degradation processes took place in or on the sediment particles, since the non-extractable residues increased constantly, reaching 47.3 - 57.5% TAR at the end of incubation.

Comparable to the pelagic test, the mineralization rate was rather low. In the NaOH traps, the $^{14}CO_2$ formation accumulated to 2.8 - 4.6% TAR over the test period. Other volatiles never exceeded 0.3% TAR at any sampling time.

Table 7.2.2.2-2: Material balance and distribution of radioactivity after application of ^{14}C-pyraclostrobin to pelagic test and incubation under dark conditions [%TAR]

DAT	Water	Volatiles			Material balance
		Ethylene-glycol	NaOH (CO_2)	Total	
High concentration, chlorophenyl-label					
0	98.2	n.p.	n.p.	n.p.	98.2
3	94.7	0.0	0.0	0.0	94.8
7	94.9	0.0	0.0	0.0	94.9
14	92.7	0.0	0.0	0.0	92.8
30	93.4	0.0	0.1	0.1	93.5
44	93.7	0.0	0.3	0.3	94.0
63	92.2	0.0	0.7	0.7	93.0
28 (sterile)	96.3	n.p.	n.p.	n.p.	96.3
63 (sterile)	97.2	n.p.	n.p.	n.p.	97.2
High concentration, tolyl-label					
0	95.7	n.p.	n.p.	n.p.	95.7
3	95.5	0.0	0.0	0.0	95.5
7	95.2	0.0	0.0	0.0	95.2
14	96.0	0.0	0.1	0.1	96.1
30	94.1	0.0	0.1	0.1	94.2
44	91.7	0.0	0.3	0.3	92.0
63	97.0	0.1	0.7	0.8	97.7
28 (sterile)	98.1	n.p.	n.p.	n.p.	98.1
63 (sterile)	99.7	n.p.	n.p.	n.p.	99.7
Low concentration, chlorophenyl-label					
0	96.4	n.p.	n.p.	n.p.	96.4
3	96.5	0.0	0.0	0.0	96.5
7	96.3	0.0	0.1	0.1	96.4
14	93.4	0.2	0.1	0.3	93.7
30	95.7	0.4	0.3	0.7	96.3
44	92.2	0.1	0.4	0.5	92.6
63	91.5	0.3	0.7	1.0	92.5
Low concentration, tolyl-label					
0	97.2	n.p.	n.p.	n.p.	97.2
3	97.7	0.0	0.0	0.0	97.8
7	96.7	0.1	0.1	0.2	96.8
14	95.3	0.0	0.0	0.0	95.3
30	94.6	0.1	0.2	0.3	94.9
44	92.6	0.0	0.0	0.0	92.7
63	93.4	0.0	0.4	0.4	93.9

TAR = total applied radioactivity
DAT = days after treatment
n.p. = not performed

Table 7.2.2.2-3: Material balance and distribution of radioactivity after application of ^{14}C-pyraclostrobin to suspended soil test and incubation under dark conditions [%TAR]

DAT	Water	Sediment						Volatiles			Material balance
		Extractable				NER	Total	Ethylene-glycol	NaOH (CO_2)	Total	
		ACN/ water	ACN 1	ACN 2	Total extractability						
High concentration, chlorophenyl-label											
0*	98.2	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	98.2
3	43.6	38.0	11.0	1.3	50.3	3.9	54.2	0.0	0.1	0.1	97.9
7	31.7	35.2	10.0	1.7	46.9	16.0	62.8	0.0	0.4	0.4	94.9
14	26.2	32.7	9.7	1.4	43.8	25.2	69.0	0.0	0.5	0.5	95.7
30	22.0	24.8	10.1	1.3	36.2	35.8	72.0	0.0	0.0	0.0	94.0
44	14.3	21.2	7.3	1.5	30.1	45.5	75.6	0.0	2.1	2.1	92.1
63	9.9	13.3	7.3	1.7	22.2	50.8	73.0	0.0	3.2	3.2	86.2
High concentration, tolyl-label											
0*	95.7	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	95.7
3	44.5	39.3	8.7	1.4	49.4	4.5	54.0	0.0	0.0	0.0	98.5
7	33.5	30.6	17.8	2.2	50.6	11.9	62.5	0.0	0.1	0.1	96.1
14	32.2	35.4	11.3	1.4	48.2	17.9	66.1	0.0	0.1	0.1	98.4
30	13.2	18.1	11.4	1.8	31.3	38.4	69.7	0.0	1.5	1.5	84.4
44	18.8	22.5	8.5	1.5	32.5	40.0	72.5	0.0	0.0	0.0	91.2
63	10.1	18.4	7.9	2.2	28.5	47.3	75.8	0.0	3.4	3.4	89.3
Low concentration, chlorophenyl-label											
0*	96.4	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	96.4
3	34.4	40.1	11.1	1.3	52.6	8.7	61.3	0.0	0.0	0.0	95.7
7	30.4	36.0	15.0	1.9	52.8	12.1	64.9	0.0	0.2	0.2	95.6
14	16.1	26.3	7.2	1.5	35.0	41.4	76.5	0.3	1.1	1.4	93.9
30	14.9	25.0	10.2	1.6	36.8	41.7	78.5	0.2	2.0	2.2	95.5
44	19.8	26.8	8.0	1.1	35.9	39.6	75.6	0.2	2.3	2.5	97.9
63	12.2	20.9	7.5	2.3	30.6	49.6	80.2	0.1	2.8	2.9	95.3
Low concentration, tolyl-label											
0*	97.2	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	97.2
3	39.5	38.5	15.1	1.4	55.0	4.9	59.9	0.0	0.0	0.0	99.4
7	24.0	37.7	10.4	1.5	49.6	21.7	71.3	0.0	0.2	0.2	95.5
14	16.6	28.3	8.7	1.7	38.7	39.3	77.9	0.1	0.6	0.7	95.3
30	11.8	17.0	8.2	1.5	26.7	51.0	77.7	0.0	1.6	1.6	91.1
44	13.7	16.9	5.3	1.2	23.4	44.8	68.2	0.0	4.3	4.3	86.2
63	8.7	13.0	4.7	1.4	19.1	57.5	76.6	0.1	4.6	4.7	90.0

TAR = total applied radioactivity
DAT = days after treatment
ACN = acetonitrile
NER = non-extractable residues
n.p. = not performed
* 0 day values taken from pelagic test samples

B. TRANSFORMATION OF PARENT COMPOUND

Characterization and identification of residues in water and sediment extracts

The summary of radio-HPLC analysis of the water samples and sediment extracts is presented in Table 7.2.2.2-4 to Table 7.2.2.2-6.

Water
In the **pelagic test**, pyraclostrobin degraded only slowly. After 63 days, between 78.3 and 97.0% TAR could still be recovered as unchanged parent for the different concentrations and radiolabels. The sterile controls showed in principle the same results as the viable test vessels. The occurrence of hydrolysis product BF 500-5 (max. 5.6 - 10.9% TAR) and the low formation of other metabolites (BF 500-3 was present already in the application solution of the chlorophenyl-labelled test item) indicated that only negligible microbial degradation took place.

In the **suspended solid test**, the behavior of pyraclostrobin was very different. It was quickly eliminated from the water phase by binding to the suspended solids. Already after 3 days, only 30 - 45% TAR of parent was dissolved in the water and the amount declined further to 1.5 - 6.1% TAR at the end of incubation. BF 500-3 (applied as impurity in the chlorophenyl-label treated vessels) also quickly disappeared from the water, which can be explained by fast adsorption to the suspended sediment due to the low water solubility and high K_{oc} values of this compound.

The formation of the hydrolysis product BF 500-5 was observed in the chlorophenyl-label treated test vessels, whereas several minor unknown degradation products were formed in the tolyl-label treated vessels. One of them reached 5.8% TAR after 44 days in the low concentration test and declined again to 2.1% TAR after 63 days. In the high concentration test it reached only 2% TAR. Identification of this peak was not possible due to the low total substance amount. All other peaks never exceeded 3.8% TAR.

Sediment (only suspended solid test)
Due to the fast adsorption, the highest amount of unchanged pyraclostrobin was detected in sediment extracts already after three days (47.2 - 55.0% TAR). During further incubation, it started to degrade to 17.1 - 27.5% TAR after 63 days. Again, the results were independent of test concentration or label-position. As in the water phase, metabolite BF 500-3 could be detected in the sediment extracts of the chlorophenyl-label treated test vessels. The HPLC retention time of two further peaks matched with the reference items BF 500-6 and BF 500-7. However, they never exceeded 2.3% TAR. All other peaks were found only sporadically in trace amounts ($\leq$ 0.5% TAR).

Table 7.2.2.2-4: Metabolite overview for the water phase after application of ^{14}C-pyraclostrobin to pelagic test [%TAR]

DAT	^{14}C total	BF 500-5 tR 52.1	BF 500-3 tR 94.4	Pyraclostrobin tR 95.5	BF 500-7 tR 106.2	Others*
High concentration, chlorophenyl-label						
0	98.2	n.d.	4.4	93.9	n.d.	n.d.
3	94.7	n.d.	5.0	89.8	n.d.	n.d.
7	94.9	1.3	6.0	87.6	n.d.	n.d.
14	92.7	2.3	6.7	82.8	n.d.	0.9
30	93.4	3.1	5.0	84.1	0.8	0.4
44	93.7	3.4	3.9	85.7	0.7	n.d.
63	92.2	5.6	3.5	78.3	3.7	1.2
28 (sterile)	96.3	1.6	7.1	87.7	n.d.	n.d.
63 (sterile)	97.2	4.0	4.7	88.5	n.d.	n.d.
High concentration, tolyl-label						
0	95.7	n.d.	n.d.	95.7	n.d.	n.d.
3	95.5	n.d.	0.8	94.7	n.d.	n.d.
7	95.2	n.d.	0.9	94.4	n.d.	n.d.
14	96.0	n.d.	0.6	95.4	n.d.	n.d.
30	94.1	n.d.	n.d.	92.2	1.2	0.8
44	91.7	n.d.	n.d.	89.9	n.d.	1.9
63	97.0	n.d.	n.d.	97.0	n.d.	n.d.
28 (sterile)	98.1	n.d.	n.d.	98.1	n.d.	n.d.
63 (sterile)	99.7	n.d.	0.7	99.0	n.d.	n.d.
Low concentration, chlorophenyl-label						
0	96.4	n.d.	3.5	92.9	n.d.	n.d.
3	96.5	n.d.	3.6	92.9	n.d.	n.d.
7	96.3	n.d.	6.3	90.0	n.d.	n.d.
14	93.4	2.5	6.0	85.0	n.d.	n.d.
30	95.7	6.0	3.7	86.0	n.d.	n.d.
44	92.2	1.1	0.8	88.1	n.d.	2.1
63	91.5	10.9	0.8	79.8	n.d.	n.d.
Low concentration, tolyl-label						
0	97.2	n.d.	n.d.	97.2	n.d.	n.d.
3	97.7	n.d.	n.d.	97.7	n.d.	n.d.
7	96.7	n.d.	n.d.	96.7	n.d.	n.d.
14	95.3	n.d.	n.d.	93.8	n.d.	1.5
30	94.6	n.d.	n.d.	94.6	n.d.	n.d.
44	92.6	n.d.	1.4	87.1	1.4	2.7
63	93.4	n.d.	n.d.	93.4	n.d.	n.d.

* sum of several peaks, each single peak ≤ 2.1% TAR
DAT = days after treatment
tR = retention time [min], approx. value
n.d. = not detected

Table 7.2.2.2-5: Metabolite overview for water phase after application of ^{14}C-pyraclostrobin to suspended solid test [%TAR]

DAT	^{14}C total	Unknown tR 7.3	Unknown tR 7.7	Unknown tR 8.1	Unknown tR 29.7	Unknown tR 44.8	BF 500-5 tR 52.1	BF 500-3 tR 94.4	Pyraclostrobin tR 95.5	Others*
High concentration, chlorophenyl-label										
0	98.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.4	93.9	n.d.
3	43.6	n.d.	n.d.	n.d.	n.d.	n.d.	2.5	1.2	39.2	0.7
7	31.7	n.d.	n.d.	n.d.	n.d.	n.d.	4.4	0.5	25.3	1.5
14	26.2	1.9	n.d.	n.d.	n.d.	n.d.	5.3	0.3	18.4	0.4
30	22.0	3.8	n.d.	n.d.	n.d.	n.d.	4.5	0.2	12.1	1.4
44	14.3	0.4	1.4	n.d.	n.d.	n.d.	4.8	n.d.	4.9	2.8
63	9.9	1.3	1.0	n.d.	n.d.	n.d.	3.7	n.d.	2.8	1.2
High concentration, tolyl-label										
0	95.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	95.7	n.d.
3	44.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	44.5	n.d.
7	33.5	n.d.	n.d.	n.d.	n.d.	0.9	n.d.	n.d.	32.6	n.d.
14	32.2	1.3	n.d.	n.d.	0.8	0.7	n.d.	n.d.	28.0	1.4
30	13.2	1.6	n.d.	1.6	0.3	2.0	n.d.	n.d.	7.8	n.d.
44	18.8	2.5	3.1	n.d.	1.0	2.0	n.d.	n.d.	9.1	1.0
63	10.1	1.9	n.d.	2.0	n.d.	1.5	n.d.	n.d.	4.2	0.4
Low concentration, chlorophenyl-label										
0	96.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.5	92.9	n.d.
3	34.4	n.d.	n.d.	n.d.	n.d.	n.d.	3.3	n.d.	29.9	1.2
7	30.4	n.d.	n.d.	n.d.	n.d.	n.d.	3.4	0.2	25.2	1.6
14	16.1	n.d.	2.0	n.d.	n.d.	n.d.	4.9	n.d.	8.7	0.5
30	14.9	n.d.	n.d.	n.d.	n.d.	n.d.	7.7	n.d.	7.2	n.d.
44	19.8	n.d.	n.d.	n.d.	n.d.	n.d.	7.6	n.d.	12.2	n.d.
63	12.2	n.d.	n.d.	n.d.	n.d.	n.d.	4.3	n.d.	6.1	1.8
Low concentration, tolyl-label										
0	97.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	97.2	n.d.
3	39.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	39.5	n.d.
7	24.0	n.d.	n.d.	n.d.	1.5	n.d.	n.d.	n.d.	22.5	n.d.
14	16.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	16.6	n.d.
30	11.8	n.d.	n.d.	n.d.	3.0	3.5	n.d.	n.d.	3.5	1.7
44	13.7	n.d.	n.d.	n.d.	1.5	5.8	n.d.	n.d.	4.3	2.0
63	8.7	n.d.	0.6	n.d.	0.2	2.1	n.d.	n.d.	1.5	4.3

* sum of several peaks, each single peak < 1.3% TAR
DAT = days after treatment
tR = retention time [min], approx. value
n.d. = not detected

Table 7.2.2.2-6: Metabolite overview for sediment extracts after application of ^{14}C-pyraclostrobin to suspended solid test [%TAR]

DAT	^{14}C total	BF 500-3 tR 87.1	Pyraclostrobin tR 88.3	BF 500-6 tR 109.5	BF 500-7 tR 115.7	Others*
High concentration, chlorophenyl-label						
3	50.3	2.3	47.2	0.4	0.4	n.d.
7	46.9	2.5	43.4	0.3	0.4	0.2
14	43.8	2.4	40.3	0.3	0.8	n.d.
30	36.2	1.4	32.2	1.2	1.4	n.d.
44	30.1	1.2	25.2	1.6	2.1	n.d.
63	22.2	1.0	17.1	2.3	1.5	0.3
High concentration, tolyl-label						
3	49.4	n.d.	49.4	n.d.	n.d.	n.d.
7	50.6	n.d.	49.1	0.6	0.9	n.d.
14	48.2	n.d.	45.4	1.2	1.5	n.d.
30	31.3	0.2	26.5	2.0	2.6	n.d.
44	32.5	n.d.	29.0	1.9	1.7	n.d.
63	28.5	n.d.	25.3	1.6	1.7	n.d.
Low concentration, chlorophenyl-label						
3	52.6	1.8	50.8	n.d.	n.d.	n.d.
7	52.8	2.9	49.9	n.d.	n.d.	n.d.
14	35.0	1.8	33.2	n.d.	n.d.	n.d.
30	36.8	1.4	34.1	0.4	0.4	0.5
44	35.9	1.3	32.7	1.2	0.7	n.d.
63	30.6	1.1	27.5	1.2	0.8	n.d.
Low concentration, tolyl-label						
3	55.0	n.d.	55.0	n.d.	n.d.	n.d.
7	49.6	n.d.	49.6	n.d.	n.d.	n.d.
14	38.7	n.d.	38.1	0.3	n.d.	0.2
30	26.7	n.d.	25.8	0.4	0.5	n.d.
44	23.4	n.d.	21.2	1.3	0.9	n.d.
63	19.1	n.d.	17.8	0.7	0.6	n.d.

* sum of several peaks, each single peak < 0.5% TAR
DAT = days after treatment
tR = retention time [min], approx. value
n.d. = not detected

Control samples
The control vessels treated with ^{14}C-benzoic acid (pelagic test) showed that the system Berghäuser Altrhein was microbially active. After 64 days, 77.9 - 83.0% TAR were evolved as $^{14}CO_2$.

Regarding the sterilized incubations, there was in principle no difference in pyraclostrobin concentration compared to the viable vessels. This shows that the major route of degradation of pyraclostrobin under the applied test conditions consisted of slow hydrolysis. Microbial degradation played only a minor role.

Degradation rates

A summary of the DT_{50} and DT_{90} values of pyraclostrobin and its metabolite BF 500-3 for the pelagic test are given in Table 7.2.2.2-7. A summary of the DT_{50} and the DT_{90} values of pyraclostrobin for total system as well as for water and sediment separately determined for the suspended solid test is presented in Table 7.2.2.2-8. DT_{50} and DT_{90} values of the metabolite BF 500-5 for the suspended solid test are shown in Table 7.2.2.2-9.

Table 7.2.2.2-7: Summary of the kinetic evaluation of pyraclostrobin and its metabolite BF 500-3 for the pelagic test

Component	Test system		Modeling endpoints	
	Pelagic test	Kinetic model	$DegT_{50}$ [d]	$DegT_{90}$ [d]
Pyraclostrobin	High test concentration	SFO	410.4	1363
Pyraclostrobin	Low test concentration	SFO	458.1	1522
BF 500-3	High test concentration	SFO	28.6	94.8
BF 500-3	Low test concentration	SFO	10.7	35.6

Table 7.2.2.2-8: Summary of the kinetic evaluation of pyraclostrobin for the suspended solid test (Level P-I)

Test system	Best-fit kinetics			Modeling endpoints	
Total system	Kinetic model	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Kinetic model	$DegT_{50}$ [d]
High test concentration	SFO	28.3	94.1	SFO	28.3
Low test concentration	SFO	26.4	87.7	SFO	26.4
Water compartment	Kinetic model	$DisT_{50}$ [d]	$DisT_{90}$ [d]	Kinetic model	$DisT_{50}$ [d]
High test concentration	DFOP	2.3	34.6	DFOP	10.4*
Low test concentration	FOMC	1.7	22.9	FOMC	6.9*
Suspended sediment compartment	Kinetic model	$DisT_{50}$ [d]	$DisT_{90}$ [d]	Kinetic model	$DisT_{50}$ [d]
High test concentration	SFO	46.8	155.5	SFO	46.8
Low test concentration	SFO	43.6	144.8	SFO	43.6

* Calculated according to FOCUS ($DT_{50} = DT_{90}/3.32$)

Table 7.2.2.2-9: Summary of the kinetic evaluation of the metabolite BF 500-5 for the suspended solid test (Level M-I, chlorophenyl label)

Test system		Modeling endpoints		
Suspended solid test	Kinetic model	$DisT_{50}$ [d]	$DisT_{90}$ [d]	Formation fraction [-]
High test concentration	SFO	103.4	343.5	0.07
Low test concentration	-	No adequate fit could be achieved		

III. CONCLUSION

The results showed that pyraclostrobin was degraded very slowly in a pure water environment as provided in the pelagic test. The major route of degradation under those conditions represented hydrolysis to cleavage products (BF 500-5 detected at max. 5.6 - 10.9% TAR with the chlorophenyl-label, and trace amounts of several unknown peaks). However, it adsorbed and degraded very fast as soon as dissolved particles were available as shown in the suspended solid test. Even with low amounts of suspended sediment particles, it was quickly converted from extractable residues into non-extractable residues and thus tightly bound to the organic matrix.

The formation of volatiles in both test variants was generally low (<5% TAR at any time point), irrespective of test concentration or label position.

The $DegT_{50}$ values in the pelagic test were calculated to range from 410 to 458 days for pyraclostrobin and from 11 to 29 days for BF 500-3.

In the suspended solid test, the $DegT_{50}$ for pyraclostrobin ranged for the whole system between 26 and 28 days, while the $DisT_{50}$ for the water compartment was calculated to range from 7 to 10 days and for the suspended sediment from 44 to 47 days. The $DisT_{50}$ for BF 500-5 was 103 days when calculated with the high test concentration. For the low test concentration no adequate fit was achieved.

CA 7.2.2.3 Water/sediment studies

The already peer-reviewed data are considered to be still valid. Therefore, no new experimental data are provided, however, the kinetic evaluation of the old dark water/sediment study was updated according to the newest guidelines and guidance documents.

Report: CA 7.2.2.3/1
Wiedemann G., 2013a
Kinetic evaluation of BAS 500 F - Pyraclostrobin in water/sediment systems under aerobic conditions
2012/1165029

Guidelines: FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.

GLP: no

Executive Summary

The aim of the study was to evaluate the dissipation and degradation kinetics of pyraclostrobin in two aerobic water/sediment systems with two different labels [*BASF DocID 1999/11241*] and to derive persistence and modeling endpoints according to the recommendations of FOCUS kinetics.

In the laboratory study the degradation of pyraclostrobin was investigated over a period of 100 days in two water/sediment systems called System A and B (a pond and a pond-like side arm of a river located in Southern Germany). Two radio-labels of the active substance were used in the study and were considered independently as replicates in the kinetic evaluation. The experimental data were evaluated using single first order (SFO), first-order multi-compartment (FOMC), double first-order in parallel (DFOP) and hockey stick (HS) kinetic models at the evaluation levels P-I and P-II.

The evaluation at the P-I level resulted in reliable persistence and modeling half-lives for System A for all compartments. The persistence and modeling $DegT_{50}$ for the whole system ranged from 23.3 to 26.8 days. Regarding System B the $DisT_{50}$ in the water compartment could reliably be estimated, however, the dissipation in sediment could not be characterized. Therefore, no endpoints could be derived for the whole system $DegT_{50}$ and the $DisT_{50}$ in sediment for System B. The P-II level did not yield any reliable fits.

I. MATERIAL AND METHODS

The kinetic evaluation of pyraclostrobin was conducted for an aerobic water/sediment study in the dark with two natural aerobic aquatic systems [*old EU Dossier, A II M 7.2.1.3.2/1, Staudenmaier H. - BASF DocID 1999/11241*]. One of them represented a pond (area of "Kastenbergheide" west of Schifferstadt, Germany) and the other a pond-like side arm of a river ("Berghäuser Althrein" south of Speyer, Germany). Kinetic evaluation was performed in order to derive persistence and modeling endpoints according to the recommendation of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*].

Two radio-labels of the active substance, referred to as chlorophenyl- and tolyl-label, respectively, were used in each system of the study. The test vessels were treated with 83 µg a.s. L^{-1} to represent the recommended field application rate of 250 g a.s. ha^{-1}. The test system was incubated for up to 100 days at 20°C in the dark.

Kinetic modeling

The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The best-fit model was selected based on visual and statistical assessment, and corresponding DT_{50} and DT_{90} values are reported as *persistence endpoints*. Appropriate DT_{50} values for use in environmental fate models were derived depending on the kinetic model and are reported as *modeling endpoints*.

Kinetic models included in the assessment

The kinetic models employed for this evaluation were described by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The analysis at P-I level (one-compartment approach) was done for degradation in the whole system as well as the respective dissipations from the water and sediment phases of the test system. At the P-II level (two-compartment approach) the kinetic analysis considered the degradation in water and sediment taking into account the partitioning between the two phases.

As the purpose of the study was to derive modeling and persistence endpoints, all four kinetic models proposed by FOCUS were used during the evaluation (SFO, FOMC, DFOP and HS). Details on the models are given in the FOCUS Kinetics guidance [*FOCUS (2006)*].

The appropriateness of a distinct kinetic model to describe degradation can be tested with the following checks recommended by *FOCUS (2006)*:

- Visual assessment of goodness-of-fit
- Estimation of the error percentage at which the χ^2 test is passed [*Equation 6-2 in FOCUS (2006)*]
- t-test to evaluate whether estimated degradation parameters differ from zero.

A kinetic model is considered appropriate if the residuals are randomly distributed, the χ^2 - error value is < 15% and the estimated degradation parameters differ from zero as outlined by *FOCUS (2006)*.

Data handling

Degradation kinetics were evaluated for the parent substance pyraclostrobin. Degradation in the whole system and dissipation from the water compartment (P-I level) were evaluated starting on the day of treatment (i.e. 0 days after treatment, DAT 0). The dissipation from the sediment phase (P-I level) was evaluated starting from the point of maximum occurrence. For the P-II level, the initial concentration of the sediment compartment was assumed to be zero. The FOCUS kinetics guidance recommends using the material balance at DAT 0 as the initial value in water for the parent substance. Thus, the total measured occurrence of the radioactive label in water and sediment (parent + metabolites + unknowns) was used as the initial value for the parent.

All measurements were used for the analysis; outlier tests were not performed since the visual evaluation did not reveal any deviating or inappropriate measurements. Concentrations in all analyzed samples were above the limit of quantification.

Software for kinetic evaluation

The software package KinGUII (version 2.2011) was used for parameter fitting [*Schäfer, D., Mikolasch, M., Rainbird, P., Harvey, B. (2007) KinGUII: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. In: Del Re, A.A.M. et al. (Eds.): Proceedings of the XIII Symposium on Pesticide Chemistry, Piacenza, 2007, p. 916-923. - BASF DocID 2007/1062781*].

Experimental data

The kinetic evaluation was based on the findings from a dark water/sediment study [*Staudenmaier H. - BASF DocID 1999/11241*]. The experimental data of the parent substance used as model input values for the kinetic evaluations are given in Table 7.2.2.3-1 and Table 7.2.2.3-2.

Table 7.2.2.3-1: Model input for pyraclostrobin – System A

Time [d]	Concentration [% Total Applied Radioactivity]					
	Chlorophenyl-label			Tolyl-label		
	Water	Sediment	Whole system	Water	Sediment	Whole system
AX_1 0	95.1[a]	0.0[b]	95.1	97.6[a]	0.0[b]	97.6
AX_2 0.25	72.9	19.5	92.5	75.6	18.5	94.0
AX_3 1	61.9	31.5	93.4	58.5	34.1	92.6
AX_4 2	49.6	34.4	84.0	50.5	29.8	80.3
AX_5 7	22.5	34.9	57.4	26.7	44.2	70.9
AX_6 14	16.0	51.3	67.3	14.9	53.7	68.7
AX_7 30	6.7	33.7	40.4	7.1	31.4	38.4
AX_8 61	not reported[c]	13.3	13.3	3.5	20.8	24.3
AX_9 100	not analyzed	6.5	6.5	not analyzed	6.4	6.4

[a] sum of radioactivity found in all compartments (sum of parent and metabolites)
[b] set to zero as recommended by FOCUS for the P II-level
[c] poor signal to noise ratio
Sediment values before day 14 (max. occurrence in sediment) were not used, when fitting only degradation in sediment at the P I-level.

Table 7.2.2.3-2: Model input for pyraclostrobin – System B

Time [d]	Concentration [% Total Applied Radioactivity]					
	Chlorophenyl-label			Tolyl-label		
	Water	Sediment	Whole system	Water	Sediment	Whole system
AX_100	95.0[a]	0.0[b]	95.0	95.0[a]	0.0[b]	95.0
AX_110.25	67.2	26.7	93.9	59.0	33.8	92.8
AX_121	39.6	53.7	93.2	37.4	57.0	94.4
AX_132	25.9	62.1	88.0	23.5	62.0	85.5
AX_147	8.1	25.4	33.4	9.5	70.0	79.5
AX_1514	1.8	15.0	16.8	1.6	14.5	16.2
AX_1630	1.0	8.1	9.1	0.6	9.7	10.3
AX_1761	not reported[c]	9.4	9.4	1.1	7.1	8.2
AX_18100	not analyzed	7.4	7.4	not analyzed	11.8	11.8

[a] sum of radioactivity found in all compartments (sum of parent and metabolites)
[b] set to zero as recommended by FOCUS for the P II-level
[c] poor signal to noise ratio
Sediment values before the maximum occurrence were not used, when fitting only degradation in sediment at the P I-level.

II. RESULTS AND DISCUSSION

For modeling endpoints, the initial fit was performed using SFO kinetics. If the fit was not satisfactory, FOMC, DFOP and HS kinetics were tested. For persistence endpoints, SFO and FOMC kinetics were tested in a first step; if SFO was not acceptable or worse than FOMC, DFOP and HS kinetics were tried in addition. Graphical presentations of the tested kinetic models and the results of the χ^2 - test and all other statistical endpoints used in the decision-making process are given in the original study report.

Level P-I

The evaluation of persistence and modeling endpoints of both test systems for both labels at P-I level showed that dissipation in the water phase could be well described by bi-phasic kinetics (FOMC: System A, chlorophenyl label; DFOP: System A, tolyl label, and System B, both labels), while dissipation from sediment and degradation in the whole system were best described by SFO kinetics (System A, both labels). For system B, none of the models was acceptable for dissipation from sediment and degradation in the whole system.

An overview of the estimated persistence and modeling endpoints for pyraclostrobin from both water/sediment systems is given in Table 7.2.2.3-3 and Table 7.2.2.3-4.

Table 7.2.2.3-3: Persistence endpoints at P-I level

System, Label	Water		Sediment		Whole system	
	DT_{50}/DT_{90} [d]	Kinetic model	DT_{50}/DT_{90} [d]	Kinetic model	DT_{50}/DT_{90} [d]	Kinetic model
System A, chlorophenyl-label	2.13 / 28.8	FOMC	25.7 / 85.5	SFO	23.3 / 77.4	SFO
System A, tolyl-label	2.30 / 17.5	DFOP	29.8 / 98.9	SFO	26.8 / 89.1	SFO
System B, chlorophenyl-label	0.636 / 6.19	DFOP	-*	-	-*	-
System B, tolyl-label	0.443 / 6.28	DFOP	-*	-	-*	-

* The data could not reliably be described by the models and should therefore not be considered for the assessment. Note, that in the study report a default of 1000 days was reported, which, however, is not in line with FOCUS and should therefore be corrected.

Table 7.2.2.3-4: Modeling endpoints at P-I level

System, Label	Water		Sediment		Whole system	
	DT_{50} [d]	Kinetic model	DT_{50} [d]	Kinetic model	$DegT_{50}$ [d]	Kinetic model
System A, chlorophenyl-label	8.67*	FOMC	25.7	SFO	23.3	SFO
System A, tolyl-label	5.27*	DFOP	29.8	SFO	26.8	SFO
System B, chlorophenyl-label	1.86*	DFOP	-**	-	-**	-
System B, tolyl-label	1.89*	DFOP	-**	-	-**	-

* Back-calculated from DT_{90} ($DT_{50} = DT_{90} / 3.32$) as required by FOCUS kinetics.

** The data could not reliably be described by the models and should therefore not be considered for the assessment. Note, that in the study report a default of 1000 days was reported, which, however, is not in line with FOCUS and should therefore be corrected.

Level P-II

Degradation of pyraclostrobin in water and sediment as well as partitioning between both phases was analyzed according to the P-II level kinetic concept (two-compartment approach) of the FOCUS guidance document. A compartment model was used and SFO kinetics were considered for the transfer and degradation rates.

P-II level analysis was performed for both systems and both labels, however, the results showed a poor fit to the measured data. For system A the fits were visually good for water and sediment, but statistically poor. While for system B (chlorophenyl label) the fits were visually good and statistically poor for water, it was the contrary for sediment. The fit in water and sediment did not reflect sufficiently the substance behavior in the system, probably because the degradation in sediment seemed to stop soon after the beginning of the study. Repeating the optimization with different starting values did not change the estimated values. The visual fit for water and sediment was moderate for system B (tolyl label), but statistically poor.

The F_{sed} test was performed for both systems and labels, but failed in all cases.

No reliable fits for the level P-II could be obtained.

III. CONCLUSION

The dissipation and degradation of pyraclostrobin in water/sediment systems under dark conditions was evaluated according to the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*] to determine persistence and modeling endpoints.

The experimental data on pyraclostrobin in both test systems (Systems A and B) and for both labels (chlorophenyl- and tolyl-labels) were evaluated at Level P-I and Level P-II. For system B no reliable endpoints could be derived for the sediment compartment and for the whole system, for both labels. As a consequence a "default" value was assumed, which, however, is not correct following the FOCUS kinetics guidance. According to FOCUS kinetics a case-by-case decision should be made if reliable endpoints cannot be derived with the recommended standard procedures. Moreover, for System B a $DegT_{90}$ in the whole system of approximately 30 days can be estimated visually, which is considerably shorter than the endpoint calculated for System A. Hence, the results of the kinetic evaluation of System A represent the worst-case of the two w/s systems investigated. Consequently, only the results of the kinetic evaluation of System A are to be considered as worst-case for further usage.

Reliable $DegT_{50}$ values for the whole system were derived for System A for both labels which range from 23.3 to 26.8 days. The analysis at the P-II level did not result in reliable fits.

CA 7.2.2.4 Irradiated water/sediment study

Since pyraclostrobin proved to be very susceptible for photolytic breakdown, information on the degradation in an irradiated water/sediment system was already provided in the old EU dossier submitted for the previous Annex I listing process. A new kinetic evaluation of this study was performed and summarized in *CA 7.2.2.4/1*.

Since no complete material balance was measured at that time during the study, a new water/sediment study under irradiated conditions was performed with an updated study design (including collection of volatiles). This study is summarized in *CA 7.2.2.4/2*.

An overview of metabolite occurrences in the different water/sediment studies (dark and irradiated) and of degradation rates are given in Table 7.2.2.4-30 and Table 7.2.2.4-31, respectively, at the end of this chapter.

Report: CA 7.2.2.4/1
Miles B., 2012b
Kinetic evaluation of BAS 500 F in water/sediment systems under aerobic conditions
2012/1021122
Guidelines: FOCUS Kinetics Report SANCO/10058/2005 ver. 2.0
GLP: no

Executive Summary

The aim of the study was to evaluate the dissipation and degradation kinetics of pyraclostrobin in aerobic water/sediment systems under irradiated conditions [*BASF DocID 1999/11791*] and to derive modeling endpoints to be used in FOCUS surface water modeling. The fate and behavior of pyraclostrobin was investigated over a period of up to 62 days in one natural water/sediment system (Kellmetschweiher pond, Germany). Two radio-labels of the active substance were used in the study and were treated as replicates for the kinetic evaluation. The experimental data were evaluated using single first order (SFO) kinetic models at levels P-I, P-II and M-I. In addition to the parent compound, the metabolites BF 500-3, BF 500-11, BF 500-13 and BF 500-14 were considered.

The best-fit $DegT_{50}$ was 7.2 days in the total system (Level P-I), and the best-fit $DegT_{50}$ were 7.5 and 6.5 days in the water and sediment phase, respectively (Level P-II).

For the metabolites BF 500-11 and BF 500-14, the best-fit total system $DisT_{50}$ values were 22.6 and 17.3 days, respectively. For the metabolite BF 500-3, a system $DisT_{50}$ of 92.5 days could be determined. For the metabolite BF 500-13, no reliable dissipation and degradation rates could be determined. Consequently, a conservative default value of 1000 days should be used for risk assessment.

I. MATERIAL AND METHODS

The kinetic evaluation was based on the results of an irradiated aerobic water/sediment study [*old EU Dossier, A II M 7.2.1.3.2/2, Ebert, D. - BASF DocID 1999/11791*] where the degradation of pyraclostrobin was investigated over a period of 62 days in one water/sediment system (Kellmetschweiher, a pond close to Schifferstadt, Germany). The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] in order to derive modeling aquatic degradation endpoints.

Two radio-labels of the active substance, referred to as chlorophenyl- and tolyl-label, respectively, were used in the study. The test vessels were treated with 250 µg L^{-1} representing an application rate of 500 g a.s. ha^{-1}. The test system was incubated for up to 62 days under a temperature and light regime simulating the climate of central Europe for the period mid-May to mid-July. The two radio-labels were tested separately and were therefore considered in the kinetic evaluation as replicates for each sampling time.

Kinetic modeling

Kinetic evaluations were performed for pyraclostrobin and four of its metabolites considering the different levels proposed by the FOCUS Kinetics Guidance [*FOCUS (2006)*]. For the parent substance, the analysis at P-I level (one compartment approach) was done for degradation in the whole system as well as the respective dissipations from the water and sediment phases of the test system. At the P-II level (two-compartment approach), the kinetic analysis considered the degradation in water and sediment taking into account the partitioning between the two phases.

For the metabolites, dissipation calculations were performed at level M-I, although the considered radio-labels and system compartments differed for each metabolite.

As the purpose of the study was to derive modeling endpoints, only the single first order (SFO) kinetic model was considered as long as acceptable results were obtained in line with the FOCUS Kinetics Guidance [*FOCUS (2006)*].

Evaluation at level P-I was carried out using the KinGUII software version 2.2, while the models at level P-II were implemented in ModelMaker [*Anonymous (1997): Model Maker User Manual, Version 3. Cherwell Scientific Publishing Limited*] by means of a compartment model.

Level M-I kinetic analysis was performed for dissipation and degradation of the metabolites BF 500-3, BF 500-11, BF 500-13 and BF 500-14 was carried out using the KinGUII software version 2.2. The metabolite kinetics were described with the SFO kinetic model.

The dissipation kinetics were evaluated for the metabolites BF 500-11 and BF 500-14 in the water compartment, for BF 500-3 in the sediment compartment, and for all three metabolites in the total system.

Degradation kinetics for the water/sediment system were evaluated for the metabolites BF 500-3, BF 500-11, and BF 500-14. The metabolite BF 500-13 could not be evaluated in either compartment.

The appropriateness of a distinct kinetic model to describe degradation can be tested with the following checks recommended by *FOCUS (2006)*:

- Visual assessment of goodness-of-fit
- Estimation of the error percentage at which the χ^2 test is passed [*Equation 6-2 in FOCUS (2006)*]
- t-test to evaluate whether estimated degradation parameters differ from zero.

A kinetic model is considered appropriate if the residuals are randomly distributed, the χ^2 - error value is < 15% and the estimated degradation parameters differ from zero as outlined by *FOCUS (2006)*.

Data handling

Where possible, replicate measurements (chlorophenyl- and tolyl-label) for each time point were used in the parameter estimation for the parent compound. While the sampling times can be equal for synchronous replicates in KinGUII, for technical reasons they were entered in ModelMaker with a small time offset (0.0001 days). The impact of this time offset on the calculated kinetics is however negligible.

Degradation in the whole system and dissipation from the water compartment of the parent compound (P-I level) were evaluated starting at DAT 0. The dissipation of the parent from the sediment phase (P-I level) was evaluated starting from the point of maximum occurrence.

For the P-II level, the initial concentration of the sediment compartment was assumed to be zero as recommended by the FOCUS Kinetics Guidance [*FOCUS (2006)*]. However in the degradation study the first measurement for the sediment was taken after 1 day, so a complete material balance for DAT 0 was not available. Instead the total measured occurrence of the radioactive labels in water (parent + metabolites + unknowns) was used as the initial value for the parent. It can be assumed that this will be lower than the actual mass balance at DAT 0, which means that using these values in the parameter estimation will result in conservative values for the degradation rates.

At M-I level, the dissipation calculations were conducted from the point of maximum occurrence of the considered metabolite, with the time of maximum occurrence set as the initial time (t=0) in the evaluation and the subsequent observation times corrected accordingly. For the degradation, the recovered amount of the parent substance at DAT 0 was set equal to the total measured occurrence of the radioactive labels in water (parent + metabolites + unknowns) and the initial concentration of the metabolites was fixed to zero.

Software for kinetic evaluation

The analysis was done by non-linear regression methods (Marquardt algorithm, ordinary least squares optimization) using the KinGUII (version 2.2011 [*Schäfer, D. et al. - BASF DocID 2007/1062781*]) and ModelMaker (version 3.0.4 [*Anonymous (1997): Model Maker User Manual, Version 3. Cherwell Scientific Publishing Limited*]) software packages.

Experimental data

The kinetic evaluation was based on the findings of *Ebert D.* [*BASF DocID 1999/11791*]. The experimental data of the parent substance used as model input values for the kinetic evaluations are given in Table 7.2.2.4-1.

Table 7.2.2.4-1: Model input for pyraclostrobin and metabolites

Time [d]	Concentration [% Total Applied Radioactivity]										
	Pyraclostrobin			BF 500-3		BF 500-11		BF 500-13		BF 500-14	
	Water	Sed.	Sys.	Sed.	Sys.	Water	Sys.	Water	Sys.	Water	Sys.
0 (c)	88.0*				2.2**					0.3	0.3**
0 (t)	89.4*				2.4**	0.2	0.2**		0.0	0.1	0.1**
0.125 (c)	80.7									1.5	
0.125 (t)	84.0					0.9				1.3	
0.25 (c)	79.3									1.5	
0.25 (t)	82.4					1.1				1.4	
0.375 (c)	75.0									2.2	
0.375 (t)	75.5					1.2				1.7	
1 (c)	69.1	9.5	78.6	0.7	3.6					3.9	4.0
1 (t)	68.7	8.9	77.6	0.6	3.2	2.1	2.1	0.4	0.4	2.7	2.8
2 (c)	58.2									5.9	
2 (t)	61.0					3.7		0.6		4.7	
3 (c)	46.2	15.6	61.8	1.6	4.5					9.0	9.4
3 (t)	51.0	15.0	66.0	1.4	4.4	5.7	5.7	1.2	1.3	6.6	6.9
7 (c)	28.3	17.5	45.8	4.1	6.6					10.4	11.1
7 (t)	34.2	18.3	52.5	4.0	6.7	7.8	7.9	2.2	2.6	8.5	9.0
10 (c)	14.9									11.1	
10 (t)	17.3					10.4		3.7		10.8	
14 (c)	12.5	9.7	22.2	10.0	11.9					11.4	12.1
14 (t)	14.0	6.4	20.4	12.4	14.8	10.3	10.5	4.1	4.9	9.7	10.3
21 (c)	3.8									8.1	
21 (t)	5.4					11.4		7.0		8.6	
30 (c)	0.7	0.8	1.5	15.9	19.2					4.2	4.7
30 (t)	2.1	0.9	3.0	16.9	21.9	10.5	10.8	10.5	12.3	5.6	6.0
45 (c)		0.4	0.4	13.2	16.3					2.8	3.5
45 (t)	0.8	0.5	1.3	14.3	19.0	5.5	6.0	14.0	16.1	2.3	2.9
62 (c)		0.3	0.3	12.2	15.7					1.6	2.1
62 (t)	0.9	0.3	1.2	12.7	16.8	3.9	4.5	15.7	17.6	1.7	2.2

Sed = sediment compartment
Sys = total system (water + sediment compartments)
(c), (t) chlorophenyl-, tolyl-label
* Total water concentration (parent + metabolites + unknowns)
** Set to zero for evaluation of $DegT_{50}$ at level M-I

II. RESULTS AND DISCUSSION

As the purpose of the study was to derive modeling endpoints, only the single first order (SFO) kinetic model was considered. Graphical presentations of the tested kinetic models and the results of the χ^2 - test and all other statistical endpoints used in the decision-making process are given in the original study report.

Level P-I

The dissipation in the water and sediment compartments and the total system degradation could be well described by the SFO kinetic model (Table 7.2.2.4-2) without apparent significant systematic deviations in the residual errors. The fitted parameter values with their associated statistical attributes are given in Table 7.2.2.4-3. An overview of the estimated modeling endpoints for pyraclostrobin at Level P-I is given in Table 7.2.2.4-4.

Table 7.2.2.4-2: Evaluation of SFO kinetic models for pyraclostrobin at P-I level

	Number of measurements used for fitting	χ^2 error	Visual fit
BAS 500 F Whole system	16	4.568	Excellent
BAS 500 F Water	24	5.346	Excellent
BAS 500 F Sediment	8	3.300	Excellent

Table 7.2.2.4-3: Fitted parameter values and statistical assessment for pyraclostrobin at P-I level

	Parameter	Estimated value	Std error	type I error rate
BAS 500 F Whole system	M(0)	87.65	1.42	<0.001
	k	9.60E-02	4.27E-03	<0.001
BAS 500 F Water	M(0)	83.18	0.94	<0.001
	k	0.155	6.89E-03	<0.001
BAS 500 F Sediment	M(0)	17.93	0.63	<0.001
	k	0.117	1.07E-02	<0.001

Table 7.2.2.4-4: Modeling endpoints for pyraclostrobin at P-I level

	DT_{50} [days]	$DegT_{50}$ [days]
BAS 500 F Whole system	-	7.22
BAS 500 F Water	4.47	-
BAS 500 F Sediment	5.93	-

Level P-II

Degradation of pyraclostrobin in water and sediment as well as partitioning between both phases was analyzed according to the P-II level kinetic concept (two-compartment approach) of the FOCUS guidance document [*FOCUS (2006)*]. A compartment model was used and SFO kinetics were considered for the transfer and degradation rates.

Good visual fits were obtained with SFO kinetics for both the water and sediment compartments. The standard errors were low for all of the estimated parameters and the t-test was passed in all cases (Table 7.2.2.4-5).

Table 7.2.2.4-5: Evaluation of SFO kinetic model for pyraclostrobin at P-II level with fitted parameter values

	χ^2 error	Visual fit	Parameter	Estimated value	Std error	type I error rate
BAS 500 F – Water	3.0	Good	M(0)	87.71	1.13	<0.001
			k_{water}	9.24E-02	1.64E-02	<0.001
			$r_{W\rightarrow S}$	0.143	1.45E-02	<0.001
BAS 500 F – Sediment	12.0	Good	M(0)	Fixed to 0		
			k_{sed}	1.07E-01	4.22E-02	0.011
			$r_{S\rightarrow W}$	0.227	4.68E-02	<0.001

The F_{sed} test was additionally performed and passed, showing that the estimated parameter values for the transfer rates are plausible (Table 7.2.2.4-6). As the K_{oc} value for pyraclostrobin with the Kellmetschweiher sediment was not determined in the study, the average value from two soils with comparable OC content and particle size distributions determined in an adsorption/desorption study for pyraclostrobin [*old EU Dossier, A II M 7.1.2/1, Ziegler, G. – BASF DocID 1998/10650*] was used. As a further plausibility check for the P-II evaluation, the total system degradation half-life ($DegT_{50}$) was estimated from the total calculated degradation for the two compartments. This crosscheck yielded a $DegT_{50}$ of 7.25 – 7.5 days, which is consistent with the value of 7.22 days for total system degradation obtained in the P-I evaluation.

Table 7.2.2.4-6: F_{sed} test for pyraclostrobin for transfer rates fitted at P-II level

Parameter	Value	Description
Koc [L kg^{-1}]	6750	K_{oc} for BAS 500 F in sandy sediment with low OC*
OC [%]	0.4	organic carbon of sediment
kd [L kg^{-1}]	30	sorption coefficient of sediment
rho_b [kg L^{-1}]	1.606	dry bulk density of sediment (derived from OC and clay content according to [*Beltman, W.H.J, Ter Horst, M.M.S, Adriaanse, P.I., De Jong, A. (2006) Manual of FOCUS_TOXSWA version 2.2.1. Wageningen, Alterra, Alterra-rapport 586. 198 pp.]*
theta [-]	0.25	saturated volumetric water content of sediment+
Zwc [cm]	15	height of water column
Zsed [cm]	1.5	height of sediment column
D_L [cm^2 d^{-1}]	0.432	recommended default value
f [-]	0.500	tortuosity factor (calculated)
t [d]	62	duration of experiment
Fsed theoretical range:		
Fsed min	0.36	
Fsed max	0.69	
Fsed model:	0.39	= r_{ws} / (r_{ws} + r_{sw})

* Mean value for sediments with comparable OC and particle size distributions [*BASF DocID 1998/10650*]
+ Estimated from sediment mass of 300 g and volume in container of ca. 150 ml, assuming mineral density 2.65 g/cm^3 for quartz sand grains

The results obtained for the SFO kinetic model at Level P-II can be considered acceptable and the estimated degradation rates can be used as modeling endpoints. The $DegT_{50}$ values are given in Table 7.2.2.4-7.

Table 7.2.2.4-7: Modeling endpoints for pyraclostrobin at P-II level

	$DegT_{50}$ [days]
Water	7.50
Sediment	6.48

Level M-I

Five metabolites were found in the water/sediment systems: BF 500-3, BF 500-11, BF 500-12, BF 500-13 and BF 500-14. Metabolite BF 500-12, however, was not evaluated because the total occurrence in water and sediment was below 5% TAR. Both dissipation and degradation of the metabolites were studied at the M-I level according to the FOCUS guidance. The metabolite BF 500-13 could not be evaluated on the basis of the experimental data.

Dissipation

The dissipation kinetics were evaluated for the metabolites BF 500-11 and BF 500-14 in the water compartment and for BF 500-3 in the sediment compartment, and for all three metabolites in the whole system. The dissipation in the water and sediment compartments for the metabolites could be adequately described by the SFO kinetic model without apparent significant systematic deviations in the residual errors and with the t-test passed in all cases (Table 7.2.2.4-8; Table 7.2.2.4-9). The resulting modeling endpoints are given in Table 7.2.2.4-10.

Table 7.2.2.4-8: Evaluation of SFO kinetic models for metabolite dissipation at M-I level

	Number of measurements used for fitting	χ^2 error	Visual fit
BF 500-3 Sediment	6	1.74	Good
BF 500-3 Whole System	6	1.56	Good
BF 500-11 Water	4	7.62	Medium
BF 500-11 Whole System	3	6.41	Good
BF 500-14 Water	10	5.16	Good
BF 500-14 Whole System	8	5.30	Good

Table 7.2.2.4-9: Fitted parameter values and statistical assessment for metabolite dissipation at M-I level

	Parameter	Estimated value	Std error	type I error rate
BF 500-3 Sediment	M(0)	16.20	0.44	<0.001
	k	8.82E-03	1.51E-03	0.002
BF 500-3 Whole System	M(0)	20.31	0.98	<0.001
	k	7.49E-03	2.59E-03	0.022
BF 500-11 Water	M(0)	11.96	0.90	0.003
	k	2.75E-02	5.38E-03	0.018
BF 500-11 Whole System	M(0)	10.57	0.86	0.026
	k	3.06E-02	6.81E-03	0.070
BF 500-14 Water	M(0)	10.69	0.44	<0.001
	k	4.37E-02	4.15E-03	<0.001
BF 500-14 Whole System	M(0)	11.04	0.53	<0.001
	k	4.01E-02	4.22E-03	<0.001

Table 7.2.2.4-10: Modeling endpoints for metabolite dissipation evaluated at M-I level

	DT_{50} [days]
BF 500-3 Sediment	78.55
BF 500-3 Whole System	92.54
BF 500-11 Water	25.22
BF 500-11 Whole System	22.62
BF 500-14 Water	15.88
BF 500-14 Whole System	17.29

Degradation

Evaluations of the degradation kinetics of BF 500-3, BF 500-11 and BF 500-14 were carried out for the water-sediment system. In contrast to the dissipation kinetics, in which only the metabolites are considered from the point of maximum occurrence, in the evaluation of the degradation kinetics both parent and metabolite are considered using all data points from DAT = 0 onwards.

The formation and degradation of BF 500-3 in this evaluation could not be adequately reproduced by the SFO model, as the model generally overestimated the concentrations in the early stages of the simulation, but underestimated maximum occurrence concentrations. There was no justification to remove any points as experimental outliers. For the metabolite BF 500-3 the degradation rate could not be adequately determined.

For the remaining two metabolites the formation and degradation in the water-sediment system could be adequately described by the SFO kinetic model, with the t-test passed in all cases. The resulting modeling endpoints are given in Table 7.2.2.4-11.

Table 7.2.2.4-11: Modeling endpoints for metabolite system degradation evaluated at M-I level

	$DegT_{50}$ [days]
BF 500-11	22.90
BF 500-14	8.21

III. CONCLUSION

Kinetic evaluations of an irradiated water/sediment study were carried out according to the FOCUS kinetics recommendations [*FOCUS (2006)*] to determine modeling endpoints for pyraclostrobin. The experimental data were evaluated using single first order (SFO) kinetic models at levels P-I, P-II and M-I. In addition to the parent compound, the metabolites BF 500-3, BF 500-11 and BF 500-14 were considered.

At the M-I level dissipation kinetics were evaluated for the metabolites BF 500-11 and BF 500-14 in the water compartment and for BF 500-3 in the sediment compartment. Degradation kinetics in the complete system were evaluated for all three metabolites. The metabolite BF 500-13 could not be evaluated on the basis of the experimental data.

The endpoints for FOCUS surface water modeling according to the selection scheme given in the FOCUS Kinetics Guidance are given in Table 7.2.2.4-12 to Table 7.2.2.4-16.

Table 7.2.2.4-12: Endpoints for pyraclostrobin for FOCUS surface water modeling

FOCUS surface water Step	Endpoint	Comment
Step 1	7.22 d	System DegT_{50}, Level P-I
Step 2	7.5 d for water 6.48 d for sediment	Water DegT_{50}, Level P-II Sediment DegT_{50}, Level P-II
Step 3	7.5 d for water 6.48 d for sediment	Water DegT_{50}, Level P-II Sediment DegT_{50}, Level P-II

Table 7.2.2.4-13: Endpoints for metabolite BF 500-3 for FOCUS surface water modeling

FOCUS surface water Step	Endpoint	Comment
Step 1	92.54 d	System DisT_{50}, Level M-I
Step 2	92.54 d for water 92.54 d for sediment	System DisT_{50}, Level M-I System DisT_{50}, Level M-I

Table 7.2.2.4-14: Endpoints for metabolite BF 500-11 for FOCUS surface water modeling

FOCUS surface water Step	Endpoint	Comment
Step 1	22.62 d	System DisT_{50}, Level M-I
Step 2	22.62 d for water 22.62 d for sediment	System DisT_{50}, Level M-I System DisT_{50}, Level M-I

Table 7.2.2.4-15: Endpoints for metabolite BF 500-13 for FOCUS surface water modeling

FOCUS surface water Step	Endpoint	Comment
Step 1	1000 d	Conservative default value
Step 2	1000 d for water 1000 d for sediment	Conservative default value

Table 7.2.2.4-16: Endpoints for metabolite BF 500-14 for FOCUS surface water modeling

FOCUS surface water Step	Endpoint	Comment
Step 1	17.29 d	System DisT$_{50}$, Level M-I
Step 2	17.29 d for water 17.29 d for sediment	System DisT$_{50}$, Level M-I System DisT$_{50}$, Level M-I

Report: CA 7.2.2.4/2
Ebert D., 2012a
Degradation of BAS 500 F in water/sediment under irradiated conditions
2011/1101715
Guidelines: OECD 308, EPA 835.4300
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The degradation of ^{14}C-pyraclostrobin in aerobic water/sediment systems was investigated under irradiated conditions. Test vessels were treated separately with chlorophenyl-^{14}C- and tolyl-^{14}C-labeled pyraclostrobin with a concentration corresponding to a field application rate of about 500 g a.s. ha^{-1} when assuming overspray over a 1 m deep water body.

The test vessels were connected to an aeration system and placed in a climatic chamber (phytotron) providing a uniform day/night cycle with 13 hours light (constant light intensity of about 28 kilolux) and 11 hours dark. The temperature in the test vessels was kept in a range of about 21 - 25°C during daylight and 18 - 20°C during night.

Samples were taken at 0, 0.25, 1, 3, 7, 10, 14, 21, 29, 35, and 42 days after treatment (DAT). Water samples and sediment extracts were analyzed by radio-HPLC. The amount of non-extractable residues was determined by combustion and liquid scintillation counting (LSC). Volatiles were trapped in appropriate trapping solutions and also analyzed by LSC. Metabolite identification was done by co-chromatography with reference substances and by HPLC-MS/MS analysis.

Pyraclostrobin dissipated quickly from the water phase reaching ≤ 35% TAR already after 1 day and < 0.5% TAR at the end of incubation.

Numerous medium polar and polar metabolites were formed in water, of which BF 500-11 (max. 5.4% TAR), and BF 500-14 (max. 2.5% TAR) were included in the kinetic analysis. All identified metabolites decreased again during the course of the study or even disappeared completely during the last sampling intervals. Several unknown products were detected in the water during the incubation time; however, none of them ever exceeded 3.8% TAR and all appeared to be of transient nature.

In the sediment, pyraclostrobin quickly reached its maximum amounts after three days (54 - 63% TAR) and declined moderately fast to 15 - 18% TAR at the end of incubation by incorporation into the humic substance matrix.

Only low amounts of metabolites were extractable from the sediment. The des-methoxy- metabolite (BF 500-3) was formed in maximum amounts of 4 - 6% TAR and declined again towards the end of incubation to 1 - 3% TAR. The two dimeric structures BF 500-6 (500M01) and BF 500-7 (500M02) never exceeded 3.7 and 2.3% TAR, respectively.

The non-extractable residues reached amounts of 63 - 66% TAR at the end of incubation, with the majority of radioactivity associated with the humic acids (max. 17 - 18% TAR) and only a small portion associated with the fulvic acids (max. 5 - 7% TAR).

Mineralization was detectable but overall very low (max. 3 - 4% TAR). No other volatiles were observed.

In the dark control vessels, pyraclostrobin also disappeared quickly from the water phase, however, the degradation in the sediment (formation of bound residues) was slower compared to the irradiated incubation.

Kinetic analysis was performed following the recommendations of the FOCUS Kinetics workgroup. Pyraclostrobin degraded rather fast in the total water/sediment system with a DT_{50} of 13.5 days under irradiated conditions. It quickly dissipated from the water phase with a DT_{50} of < 0.5 days. In sediment, further degradation led to incorporation into the organic matrix (humic substances) with a DT_{50} of about 20 days. The metabolites degraded with the following half-lives: 29.1 days (BF 500-3, whole system), 9.0 days (BF 500-11, water) and 6.7 days (BF 500-14, sediment).

Overall, the results of this study showed that pyraclostrobin degraded relatively fast under irradiated as well as under dark conditions. Under sunlight-similar conditions, several photolytical breakdown products appeared in the water phase, which contributed to a faster degradation rate also in the sediment compared to dark conditions.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Pyraclostrobin (BAS 500 F)
Chemical name: methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]-oxymethyl} phenyl)-(N-methoxy)carbamate
Molecular formula: $C_{19}H_{18}ClN_3O_4$
Molecular mass: 387.8212 g mol^{-1} (non-labeled)

Label 1
Label: [chlorophenyl-U-^{14}C]-pyraclostrobin
Batch No.: 579-4006
Specific activity: 7.68 MBq mg^{-1}
Radiochemical purity: 99.6%

Label 2
Label: [tolyl-U-^{14}C]-pyraclostrobin
Batch No.: 566-4040
Specific activity: 7.31 MBq mg^{-1}
Radiochemical purity: 98.7%

2. Test system

A natural water and sediment system was sampled: “Berghäuser Altrhein”, a pond-like side arm of the river Rhine, south of Speyer surrounded by a forest.

The sediment was passed through a 2 mm sieve, and the water was filtered through a 0.2 mm sieve. Sieving of sediment and filling of sediment and water into the test vessels was done at the day of collecting the water/sediment system. The physico-chemical properties of the systems are summarized in Table 7.2.2.4-17.

Table 7.2.2.4-17: Characterization of the water/ sediment system

Designation **Origin**		**Berghäuser Altrhein** **Speyer, Germany**	
Water			
pH		7.35	
Hardness	[mmol $CaCO_3$ L^{-1}]	1.78	
		Beginning	End
Dissolved organic C	[mg L^{-1}]	8.0	9.9
Total N	[mg L^{-1}]	0.68	0.81
Total P	[mg L^{-1}]	0.19	0.033
Bacteria	[cfu mL^{-1}]	6.2×10^3	4.0×10^4
Fungi	[cfu mL^{-1}]	6	2
Actinomycetes	[cfu mL^{-1}]	0	0
Sediment			
Textural class		USDA	DIN
Sand Silt Clay	[%] [%] [%]	13.3 57.3 29.4 Silt clay loam	9.3 61.3 29.4 Loamy silt
pH (H_2O) pH ($CaCl_2$)		8.0 7.4	
CEC	[$cmol^+$ kg^{-1}]	34.4	
		Beginning	End
Organic C	[%]	5.77	6.51
Total N	[%]	0.45	0.48
Total P	[mg kg^{-1}]	470	456
Bacteria	[cfu g^{-1}]	1.4×10^8	4.0×10^7
Fungi	[cfu g^{-1}]	3.7×10^5	3.9×10^5
Actinomycetes	[cfu g^{-1}]	3.1×10^5	2.1×10^5

CEC = cation exchange capacity

B. STUDY DESIGN

1. Experimental conditions

A total of 45 flasks were prepared: 15 flasks for incubation under irradiated conditions and 5 flasks for the dark control for each label. In addition, 3 flasks were prepared for system characterization at the end of the incubation and 2 flasks for continuous temperature recording.

The flasks were filled with about 130 g of wet sediment and 290 mL of water. This corresponded to a sediment layer of about 2 cm and a water layer of about 6 cm. After being filled with sediment and water, the flasks were allowed to equilibrate for 21 days under dark conditions.

Appropriate amounts (20 µL) of the respective application solutions were pipetted to the water surface to achieve a nominal amount of about 15 µg test item per test vessel. This corresponds to a field application rate of about 500 g active substance/ha assuming overspray over a 1 m deep water body. The amount of test item per test vessel is calculated for a 300 mL water volume.

The test vessels were capped air-tight and the upper 1-2 cm water layer was slightly agitated to keep the oxygen saturation on a sufficient high level. Either one or two of the treated test vessels were connected to a volatile trapping system of two gas washing flasks containing different trapping solutions for potential ^{14}C-volatiles (ethylene glycol, 0.5 M NaOH).

Equilibration and subsequent incubation was carried out in a climatic chamber (phytotron) with temperature and light control. The test vessels were connected to a system providing a constant stream of air to be able to establish a full ^{14}C-material balance.

Incubation was conducted simulating a uniform day/night cycle with 13 hours irradiation and 11 hours dark (light intensity: 28 kilolux) in a phytotron chamber with the light being very similar to natural sunlight. The air temperature in the phytotron was kept in the range between 16 and 17.5°C, the temperature in the test vessels was kept in a range of about 21 - 25°C during daylight and 18 - 20°C during night.

The equilibration was monitored by measuring redox potential of water and sediment, temperature, O_2 content and pH of randomly selected flasks at intervals of a few days. After treatment, the same parameters were measured in each sample before workup during the irradiation phase. Dark control samples were also processed.

2. Sampling

Samples for the irradiated experiment were taken at 0, 0.25, 1, 3, 7, 10, 14, 21, 29, 35, and 42 days after treatment (DAT). Since two radio-labels were tested separately, they can be considered as replicates for the degradation results of the test item. Dark control samples were worked up after 14, 29 and 42 days.

For sampling, the parameters temperature, O_2 content, pH and redox potential of the water and the redox potential of the sediment were measured. Then water and sediment were separated by careful decantation. The volatile traps were disconnected from the air stream and stored at room temperature until measurement.

3. Analytical procedures

Water
The radioactivity in the water samples was measured by LSC (3 x 1 or 2 mL aliquots). Aliquots of the water samples were analyzed by HPLC without further workup.

Sediment
The wet sediment (total amount) was extracted once with 80 mL acetonitrile, which is considered overall as an acetonitrile/water extraction due to the water left in the sediment. This extraction was followed by one extraction with 100 mL acetonitrile/water (1:1, v:v) and two extractions with 100 mL pure acetonitrile. Each extraction step was performed on a rotary shaker at 250 rpm for 15 minutes. The phases were separated by centrifugation and analyzed for radioactivity by LSC.

The four corresponding acetonitrile and acetonitrile/water extracts were combined, dried and re-dissolved in acetonitrile. After centrifugation two aliquots of the supernatants were checked for radioactivity to ensure that all radioactivity was recovered after rotary evaporation and centrifugation. The concentrated extracts were finally subjected to HPLC analysis.

The extracted sediment residue was dried at room temperature under a stream of nitrogen and homogenized in a laboratory mill. The amount of non-extractable radioactive residues (NER) in the extracted sediment was determined by combustion of 4 aliquots in an oxidizer. The total amount of radioactivity in the sediment was determined by calculating the sum of extractable and non-extractable residues.

Characterisation of bound residues

The non-extractable radioactivity in sediment was further characterized by separation into fulvic acids, humic acids and humins. For each sampling time of 10, 29, and 42 days, one tolyl-label and one chlorophenyl-label treated sediment sample was selected, respectively. For each sample, the dried sediment (25 g) was extracted three times with 0.5 M NaOH. The radioactivity in the extracts of each extraction step was determined by LSC.

The corresponding extracts of a sample were then combined and acidified with conc. HCl to pH 1-2 to precipitate the acid-insoluble humic acids. For the precipitation phase, the samples were kept in a refrigerator. After centrifugation, the radioactivity in the supernatant (fulvic acids) was determined by LSC.

The precipitate (humic acids) was redissolved in 0.5 M NaOH and the solution measured for radioactivity. The remaining non-extractable radioactivity in the sediment (humins) was determined by combustion.

Since the majority of radioactivity was associated with the high molecular humic acids and humins and the amounts of radioactivity in the fulvic acid fractions were less than 7% TAR, no attempts were made to further characterize the ^{14}C-compounds.

Radioactivity in the volatile trapping solutions was determined by LSC. Although the radioactivity in the volatile traps was very low, two NaOH trapping solutions, one for each radio-label of the 0 - 14 days sampling period, containing 0.7 and 1.7% of the applied radioactivity were treated with conc. HCI, ultrasonicated, additionally flushed with nitrogen gas and then measured again for radioactivity. All radioactivity was purged out of the NaOH by this procedure leading to the conclusion that it consisted originally of $^{14}CO_2$.

4. Kinetic modeling

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] in order to derive persistence and modeling aquatic degradation endpoints. The analysis was done by non-linear regression methods (Marquardt algorithm, ordinary least squares optimization) using the KinGUII (version 2.2011) and ModelMaker (version 3.0.4) software packages.

Kinetic evaluations were performed for pyraclostrobin and three of its metabolites (BF 500-3, BF 500-11, BF 500-14) considering the different levels proposed by the FOCUS kinetics guidance. For the parent substance, the analysis at P-I level (one-compartment approach implemented in KinGUII) was done for degradation in the whole system as well as the respective dissipations from the water and sediment phases of the test system. At the P-II level (two-compartment approach implemented in ModelMaker), the kinetic analysis considered the degradation in water and sediment taking into account the partitioning between the two phases.

For the metabolites (Level M-I), both dissipation and degradation calculations were performed using KinGUII, although the considered radio-labels and system compartments differed for each metabolite (see section on data handling).

The goodness-of-fit of the model was assessed by visual assessment as well as by means of statistical measures as recommended by the FOCUS work group on degradation kinetics. Visual assessment of the model fit, considering the concentration time curve as well as the residual plots, is the principal indicator for the appropriateness of a kinetic model. In addition, the decision on the best kinetic model is supported by χ^2 statistics, and the kinetic model with smaller error levels to pass the χ^2 test is usually preferred. The χ^2 statistics was calculated using mean of replicate measurements when available.

Data handling

For each time point, replicate measurements (chlorophenyl- and tolyl-label) for each time point were used in the parameter estimation for the parent compound.

Degradation in the whole system and dissipation from the water phase of the parent compound (P-I level) were evaluated starting at DAT 0. The dissipation of the parent from the sediment phase (P-I level) was done starting at the day of maximum occurrence. In these cases, the zero time (T_0) was corrected to the time of maximum observed and all later times were adjusted accordingly as days after maximum concentrations (DAMC).

At the P-II level, the initial concentration of the sediment compartment was assumed to be 0. Furthermore, at the P-I and P-II levels of the analysis the measured initial concentrations of the parent substance in the whole system or in the water was set to the material balance at DAT 0, as recommended by the FOCUS Kinetics guidance.

At the M-I level, the dissipation calculations were conducted from the point of maximum occurrence of the considered metabolite, with T_0 corrected accordingly. For the degradation, the recovered amount of the parent substance at DAT 0 was set equal to the material balance of the whole system and the initial concentration of the metabolites was fixed to zero.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The distribution of radioactivity in the different compartments of the water/sediment system treated with ^{14}C-labels of pyraclostrobin is presented in Table 7.2.2.4-18 and Table 7.2.2.4-19.

The material balances in the test vessels ranged between 93.6 and 98.6% of the total applied radioactivity (TAR) for the chlorophenyl-label and between 94.0 and 98.2% TAR for the tolyl-label.

The radioactivity in the water of the irradiated test vessels quickly declined with both labels to 38 - 39% TAR after one day to about 4% at the end of incubation after 42 days. The reduction of radioactivity in the water phases of the dark controls was in principle comparable, reaching 3 - 5% TAR at the end of incubation.

Corresponding to the decline of radioactivity in the water phase, the total radioactivity in the sediment quickly increased within the first 14 days to 82 and 85% TAR in the irradiated vessels, and 91 and 80% TAR in the dark controls for chlorophenyl and tolyl-label, respectively. At the end of the incubation the total amount of radioactivity increased even further to 88 - 89% TAR in the irradiated vessels and 90 - 93% TAR in the dark controls.

At the earlier sampling times, the major part of radioactivity in sediment was always extractable, but under irradiated conditions, the non-extractable part increased with time up to 63 - 66% TAR after 42 days. In the dark controls, the non-extractable radioactivity also increased, but reached only 50 - 51% TAR at the end of the incubation.

Volatiles

Overall, the degradation of pyraclostrobin in water/sediment systems was characterized by a very low mineralization rate. The amount of $^{14}CO_2$ never exceeded 5% TAR within 100 days. No other volatiles were detected.

Table 7.2.2.4-18: Material balance and distribution of radioactivity after application of chlorophenyl-^{14}C-pyraclostrobin to water/sediment system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	Water	Sediment							Volatiles		Material balance
	Total	ACN/ H_2O 1	ACN/ H_2O 2	ACN 1	ACN 2	Total extract-ability	NER	Total	Ethylene glycol	NaOH (CO_2)	
Irradiated											
0	94.9	1.3	0.3	0.1	0.1	1.8	0.0	1.8	n.p.	n.p.	96.8
0.25	58.7	26.7	7.7	2.9	1.0	38.4	1.5	39.9	0.0	0.0	98.6
1	37.9	38.6	11.0	4.2	1.3	55.1	3.4	58.5	0.0	0.0	96.4
3	22.2	45.2	14.2	4.0	1.9	65.2	8.7	73.9	0.1	0.2	96.4
7	14.2	39.2	12.6	5.7	2.1	59.7	20.4	80.0	0.0	0.8	95.0
10	10.2	36.4	13.5	5.2	2.3	57.5	26.9	84.3	0.0	0.4	94.9
14	10.6	31.0	8.8	6.7	3.2	49.7	32.2	82.0	0.1	1.7	94.3
21	6.5	22.7	8.5	6.5	2.9	40.7	46.2	87.0	0.0	1.8	95.3
29	6.3	19.4	7.7	6.0	2.2	35.3	49.1	84.4	0.0	2.8	93.6
35	4.4	15.1	6.1	6.0	2.9	30.1	57.5	87.6	0.1	3.5	95.6
42	4.0	12.2	4.0	5.4	3.0	24.7	62.9	87.6	0.0	4.2	95.7
Dark control											
14	6.7	42.5	9.0	9.1	5.6	66.3	24.6	90.9	0.0	0.1	97.7
29	2.9	29.6	9.8	9.2	3.2	51.7	41.5	93.3	0.0	0.4	96.6
42	2.8	23.7	7.2	8.7	3.8	43.4	49.9	93.3	0.0	0.7	96.8

ACN = acetonitrile
DAT = days after treatment
n.p. = not performed
NER = non-extractable residues
TAR = total applied radioactivity

Table 7.2.2.4-19: Material balance and distribution of radioactivity after application of tolyl-^{14}C-pyraclostrobin to water/sediment system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	Water	Sediment							Volatiles		Material balance
	Total	ACN/ H_2O 1	ACN/ H_2O 2	ACN 1	ACN 2	Total extract-ability	NER	Total	Ethylene glycol	NaOH (CO_2)	
Irradiated											
0	93.3	0.3	0.1	0.1	0.0	0.6	0.1	0.7	n.p.	n.p.	94.0
0.25	63.2	22.8	6.1	2.5	0.8	32.2	1.3	33.5	0.0	0.0	96.7
1	38.8	37.8	11.3	3.9	1.4	54.4	3.5	58.0.	0.0	0.0	96.8
3	25.5	42.6	13.2	3.7	1.7	61.2	10.5	71.7	0.0	0.1	97.3
7	17.2	39.2	12.2	5.5	2.1	59.0	21.8	80.9	0.0	0.2	98.2
10	11.5	34.1	13.6	4.8	2.2	54.7	30.9	85.6	0.0	0.4	97.5
14	10.8	28.7	8.6	5.4	3.1	45.8	39.1	84.9	0.0	0.4	96.0
21	7.2	24.0	8.6	6.2	2.7	41.5	46.8	88.2	0.0	1.0	96.5
29	5.5	14.9	6.1	5.7	2.2	28.8	59.8	88.6	0.0	2.0	96.1
35	5.0	13.2	5.1	5.2	2.5	26.1	62.9	89.0	0.0	2.5	96.4
42	3.8	11.7	4.3	4.8	2.5	23.4	65.9	89.3	0.0	3.4	96.5
Dark control											
14	15.5	37.0	10.2	6.1	3.6	56.8	22.8	79.7	0.0	0.3	95.5
29	8.8	26.7	8.9	7.5	2.5	45.6	39.7	85.4	0.0	1.0	95.2
42	5.3	21.7	6.9	6.9	3.0	38.5	51.4	89.9	0.0	1.7	96.9

TAR = total applied radioactivity
DAT = days after treatment
ACN = acetonitrile
NER = non-extractable residues
n.p. = not performed

B. TRANSFORMATION OF PARENT COMPOUND

Characterization and identification of residues in water and sediment extracts

An overview of active ingredient and metabolites for the water samples and sediment extracts is presented in Table 7.2.2.4-20 to Table 7.2.2.4-22.

A proposed route of degradation of pyraclostrobin in water/sediment systems is given in Figure 7.2.2.4-1.

Pyraclostrobin dissipated quickly from the water phase reaching ≤ 35% TAR already after 1 day and < 0.5% TAR at the end of incubation after 42 days.

Water

Numerous medium polar and polar metabolites were formed of which BF 500-5 (max. 0.9% TAR), BF 500-11 (max. 5.4% TAR), BF 500-12 (max. 1.4% TAR), BF 500-13 (max. 0.9% TAR) and BF 500-14 (max. 2.5% TAR) could be identified by co-chromatography with reference compounds. BF 500-11, BF 500-13 and BF 500-14 were confirmed by HPLC/MS-MS analysis. All of those identified metabolites decreased again or disappeared completely during the last sampling intervals.

The des-methoxy-metabolite of pyraclostrobin (BF 500-3) could also be sporadically detected, which is explainable since the degradation route of pyraclostrobin is supposed to start with the des-methoxylation at the carbamate group. However, the appearance of this compound with 2.5% TAR in the 0 day sample of the tolyl-label treated vessel may also be attributed to the fact that it was already present as impurity in the treatment solution.

Several unknown products were detected in the water during the incubation time, however, none of them ever exceeded 3.8% TAR, and all appeared to be of transient nature.

As expected, the photolysis breakdown products like BF 500-14, BF 500-13, and BF 500-12 were not detected in the dark controls. One peak (approximately 51.4 min) detected in the dark control waters of the chlorophenyl-label test vessels matched the retention time of BF 500-5 (considering the delay between UV and ^{14}C-detector); however, no HPLC/MS-MS confirmation could be performed due to the overall low radioactivity and so the structure has to be considered as not confirmed. It is however very unlikely that a typical photoproduct like BF 500-5 appears under dark conditions.

Sediment
Because of the fast precipitation, pyraclostrobin reached its maximum amounts of 63 and 54% TAR, for chlorophenyl-label and tolyl-label, respectively, at three days after treatment. Afterwards it declined moderately fast by incorporation into the organic sediment matrix, amounting to 18 and 15% TAR at the end of incubation.

Metabolites in the sediment extract were detected only at low amounts among which BF 500-3, BF 500-6 and BF 500-7 could be identified by co-chromatography with reference compounds and HPLC/MS-MS analysis.

The des-methoxy-metabolite (BF 500-3) was formed at maximum amounts of 4 - 6% TAR and declined again towards the end of incubation to 1 - 3%. The two dimeric structures BF 500-6 (500M01) and BF 500-7 (500M02) never exceeded 3.7 and 2.3% TAR, respectively.

The extractable amount of pyraclostrobin in the sediment at the end of incubation was higher in the dark control samples than under irradiated conditions. In the time interval 14 to 42 days, the active substance decreased under dark conditions from 59 to 32% TAR with the chlorophenyl-label and from 49 to 29% TAR with the tolyl-label. The metabolite BF 500-3 was detected only in low amounts of <3%, whereas BF 500-6 and BF 500-7 reached up to 7% and 4% TAR, respectively.

Non-extractable residues
The non-extractable (bound) residues reached high amounts of 63 and 66% TAR during the incubation and were therefore further characterized by NaOH-extraction and separation into fulvic acids, humic acids and non-extractable humins (Table 7.2.2.4-24).

For all investigated samples, less than half of the radioactivity associated with the organic matrix in the sediment could be released by NaOH extraction. After separation of fulvic and humic acids, the majority of radioactivity stayed in the humic acids (max. 17 - 18% TAR) and only a small portion was measured in the fulvic acids (max. 5 - 7% TAR).

Since under irradiated conditions, the formation of non-extractable residues was faster than under dark conditions, it can be concluded that the photolytical breakdown in the water phase also contributed to the overall faster degradation of pyraclostrobin in the sediment, or rather on the sediment surface. The similar distribution of the two radiolabels between the humic substance fractions showed that the two ring moieties were both readily incorporated into the humic substances in the sediment.

Table 7.2.2.4-20: Metabolite overview for water after application of chlorophenyl-^{14}C-pyraclostrobin to system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	^{14}C total	Unknown tR 7.2	Unknown tR 8.1	Unknown tR 8.7	BF 500-5 tR 51.4	BF 500-14 tR 58.8	BF 500-12 tR 69.6	BF 500-3 tR 92.9	Pyraclostrobin tR 94.4	Others*
Water – irradiated										
0	94.9	n.d.	n.d.	n.d.	0.9	n.d.	n.d.	n.d.	94.0	n.d.
0.25	58.7	n.d.	n.d.	n.d.	0.7	1.2	1.4	0.5	53.7	1.2
1	37.9	n.d.	2.5	n.d.	0.4	1.0	n.d.	n.d.	32.9	0.9
3	22.2	3.8	2.2	n.d.	n.d.	2.5	n.d.	n.d.	13.3	0.4
7	14.2	2.2	2.1	2.4	n.d.	1.7	n.d.	n.d.	5.7	n.d.
10	10.2	2.5	2.3	0.7	n.d.	1.0	n.d.	0.2	2.5	1.1
14	10.6	2.0	2.5	1.6	n.d.	1.0	n.d.	n.d.	3.6	n.d.
21	6.5	1.2	2.9	n.d.	n.d.	0.5	n.d.	0.1	1.6	0.2
29	6.3	1.8	2.6	1.1	n.d.	0.3	n.d.	n.d.	0.4	0.1
35	4.4	1.5	2.3	0.4	n.d.	n.d.	n.d.	n.d.	0.2	n.d.
42	4.0	1.1	2.1	0.3	n.d.	n.d.	n.d.	n.d.	0.4	n.d.
Water - dark control										
14	6.7	n.d.	n.d.	n.d.	1.0	n.d.	n.d.	n.d.	5.2	0.5
29	2.9	0.2	0.3	0.2	0.3	n.d.	n.d.	n.d.	1.4	0.5
42	2.8	0.4	0.6	0.1	0.3	n.d.	n.d.	n.d.	1.4	n.d.

* sum of several peaks, each single peak < 2% TAR
tR = retention time [min], approx. value
n.d. = not detected

Table 7.2.2.4-21: Metabolite overview for sediment after application of chlorophenyl-^{14}C-pyraclostrobin to system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	^{14}C total	BF 500-3 tR 81.5	Pyraclostrobin tR 83.2	BF 500- 6 tR 105.7	BF 500- 7 tR 110.3	Others*
Sediment extract - irradiated						
0	1.8	n.d.	n.d.	n.d.	n.d.	n.d.
0.25	38.4	0.5	37.9	n.d.	n.d.	n.d.
1	55.1	0.8	54.2	0.1	n.d.	n.d.
3	65.2	1.4	63.0	0.5	0.2	n.d.
7	59.7	4.2	53.3	0.9	0.6	0.5
10	57.5	2.4	52.3	1.7	1.1	n.d.
14	49.7	2.2	44.0	1.9	1.0	0.7
21	40 7	2.3	33.3	2.5	1.8	0.8
29	35 3	2.0	28.1	3.1	1.8	0.3
35	30.1	1.8	21.5	3.7	1.8	1.3
42	24.7	1.0	17.5	3.6	2.2	0.4
Sediment extract- dark control						
14	66.3	0.9	58.5	3.6	2.4	0.9
29	51.7	0.9	41.1	5.9	3.1	0.7
42	43.4	0.7	31.8	7.0	3.6	0.4

* sum of several peaks, each single peak < 2% TAR
tR = retention time [min], approx. value
n.d. = not detected
0 d sediment not analyzed

Table 7.2.2.4-22: Metabolite overview for water after application of tolyl-^{14}C-pyraclostrobin to system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	^{14}C total	UK tR 7.2	UK tR 8.0	UK tR 29.2	UK tR 35.2	BF 500-13 tR 41.3	BF 500-11 tR 43.9	BF 500-14 tR 58.8	BF 500-12 tR 69.6	BF 500-3 tR 93.0	Pyraclostrobin tR 94.4	Others*
Water - irradiated												
0	93.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.5	86.5	4.4
0.25	63.2	n.d.	n.d.	n.d.	3.2	n.d.	3.0	1.9	1.0	0.5	46.7	6.9
1	38.8	n.d.	n.d.	0.7	2.7	n.d.	5.4	1.7	n.d.	1.3	22.7	4.2
3	25.5	1.5	n.d.	2.2	1.4	n.d.	4.8	2.2	n.d.	n.d.	9.5	4.0
7	17.2	1.3	2.4	2.6	n.d.	0.6	3.5	1.6	n.d.	n.d.	3.7	1.4
10	11.5	1.4	1.4	1.4	0.5	n.d.	2.4	1.1	n.d.	n.d.	1.5	1.7
14	10.8	n.d.	1.7	1.1	n.d.	0.6	2.2	0.5	n.d.	n.d.	2.5	2.1
21	7.2	n.d.	1.0	0.6	n.d.	0.9	1.4	0.4	n.d.	0.2	1.1	1.7
29	5.5	n.d.	2.0	n.d.	n.d.	0.5	0.7	n.d.	n.d.	n.d.	0.2	2.1
35	5.0	1.6	1.8	n.d.	n.d.	0.4	0.1	n.d.	n.d.	0.3	0.4	0.3
42	3.8	1.4	1.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.4	0.8
Water - dark control												
14	15.5	2.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13.4	n.d.
29	8.8	0.9	1.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	6.1	0.4
42	5.3	0.9	1.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.8	0.3

* sum of several peaks, each single peak < 2% TAR
n.d. = not detected
tR = retention time [min], approx. value
UK = unknown

Table 7.2.2.4-23: Metabolite overview for sediment after application of tolyl-^{14}C-pyraclostrobin to system Berghäuser Altrhein and incubation under irradiated conditions [%TAR]

DAT	^{14}C total	BF 500-3 tR 81.5	Pyraclostrobin tR 83.2	BF 500- 6 tR 105.7	BF 500- 7 tR 110.3	Others*
Sediment extract - irradiated						
0	0.6	n.d.	n.d.	n.d.	n.d.	n.d.
0.25	32.2	1.6	30.2	n.d.	n.d.	0.4
1	54.4	3.8	49.6	n.d.	n.d.	1.0
3	61.2	4.6	54.2	0.3	0.3	1.8
7	59.0	2.5	53.8	1.1	0.7	0.9
10	54.7	0.9	53.7	n.d.	n.d.	n.d.
14	45.8	4.4	38.0	1.6	0.9	0.9
21	41.5	5.9	30.8	1.4	1.0	2.4
29	28.8	2.8	20.7	3.1	2.3	n.d.
35	26.1	2.9	17.5	2.9	1.4	1.3
42	23.4	2.8	14.5	3.2	1.9	1.1
Sediment extract – dark control						
14	56.8	2.5	49.3	2.3	1.4	1.3
29	45.6	1.8	36.4	4.5	2.5	0.5
42	38.5	1.6	28.8	4.2	2.5	1.4

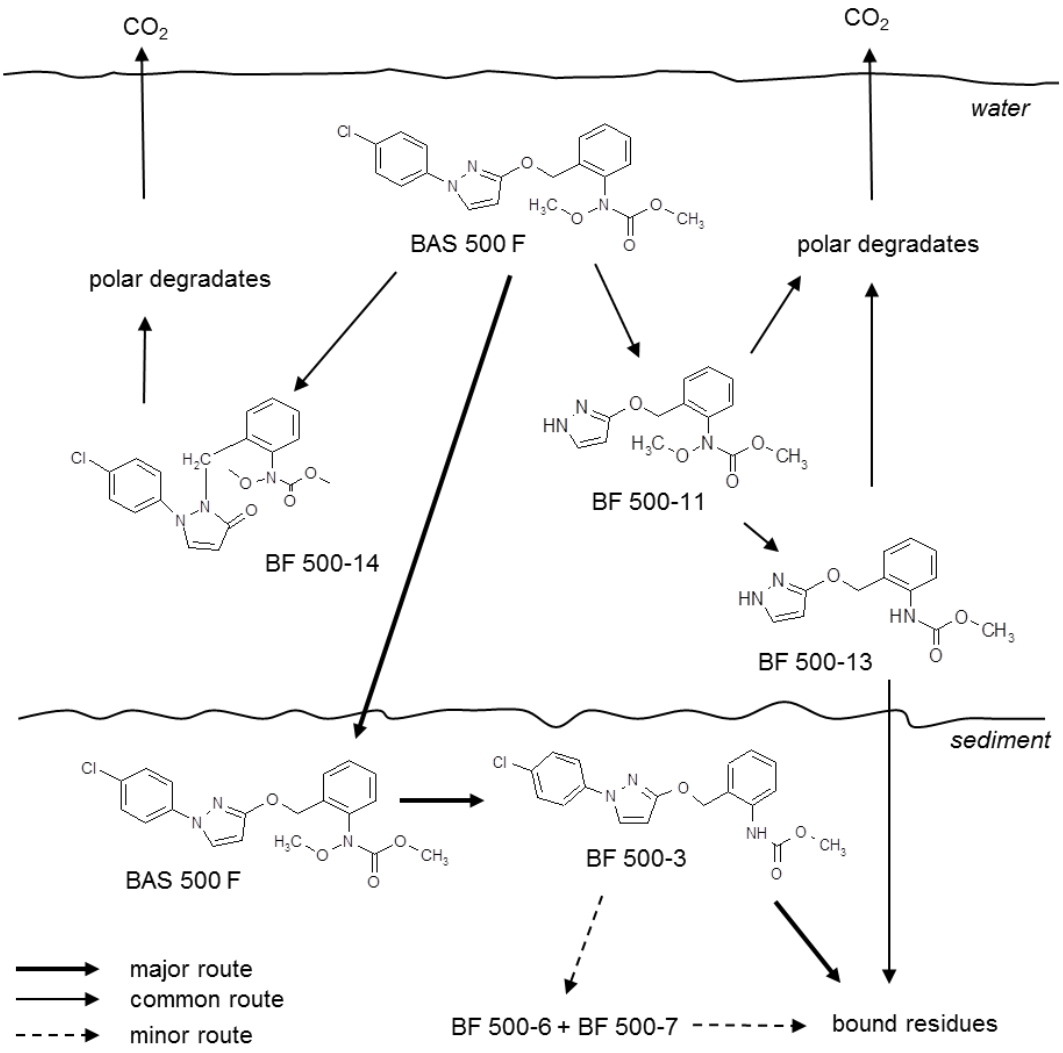
* sum of several peaks, each single peak < 2% TAR
tR = retention time [min], approx. value
n.d. = not detected
0 d sediment not analyzed

Table 7.2.2.4-24: Distribution of radioactivity between fulvic acids, humic acids, and humins after application of ^{14}C-pyraclostrobin to water/sediment systems [%TAR]

DAT	NER	Fulvic acids	Humic acids	Humins	Sum	Recovery [%]
Chlorophenyl label						
10	26.9	3.0	7.8	20.1	31.0	115.3
29	49.1	5.6	14.2	26.1	45.9	93.4
42	62.9	6.8	17.1	34.7	58.6	93.3
Tolyl label						
10	30.9	2.2	9.0	17.6	28.8	93.2
29	59.8	5.0	17.3	34.4	56.7	94.9
42	65.9	5.3	17.7	36.7	59.7	90.6

DAT = days after treatment
NER = non-extractable radioactivity
TAR = total applied radioactivity

Figure 7.2.2.4-1: Proposed route of degradation of pyraclostrobin in water/sediment systems



Dissipation and degradation rates

The dissipation and degradation rates in water, sediment and the whole system could be calculated for the active substance pyraclostrobin and its metabolites BF 500-3 (sediment), BF 500-11 and BF 500-14 (both water).

The kinetics of the dissipation and degradation of BF 500-3 were established for the whole system, but using only the results of the chlorophenyl-label. The measurements for the tolyl-label in water showed a peculiarly high initial concentration of the metabolite at time zero, which was quite unusual for a metabolite forming in sediment and was not observed for the chlorophenyl-label. This fact skewed the description of the formation kinetic, and the apparition of the maximum occurrence concentration only very late toward the end of the experiment left too few measurement points to conduct the dissipation analysis. For these reasons, the measurements from the tolyl-label were discarded in the calculations of the dissipation and the degradation of BF 500-3. Furthermore, the dissipation of BF 500-3 was calculated for the whole system. While it was mainly found in sediment, the small quantities in the water phase were also considered as a worst-case perspective.

The metabolite BF 500-11 was only studied in water for both dissipation and degradation as it only appeared in this compartment. Additionally, no replicate was available as this metabolite formation implies the elimination of the molecular segment carrying the chlorophenyl-label, thus only the tolyl-label could be used to quantify it. Likewise, the metabolite BF 500-14 was studied from the tolyl-label in the water compartment as it was not observed in sediment.

The best-fit (persistence) and modelling values for pyraclostrobin after a P-I analysis are listed in Table 7.2.2.4-25. No reliable degradation rates with a P-II analysis could be determined.
Persistence and modelling endpoints for the metabolites of pyraclostrobin are provided in Table 7.2.2.4-26 (dissipation) and Table 7.2.2.4-27 (degradation). No degradation rate for metabolite BF 500-3 could reliably be determined.

Table 7.2.2.4-25: Persistence and modeling endpoints for pyraclostrobin (P-I)

	Persistence endpoints			Modeling endpoints	
	Model	**DT_{50} [d]**	**DT_{90} [d]**	**Model**	**DT_{50} [d]**
Whole system[1]	HS	13.5	54.4	SFO	15.4
Water[2]	DFOP	0.3	3.9	DFOP	1.2*
Sediment[2]	SFO	20.1	66.7	SFO	20.1

[1] degradation rate
[2] dissipation rate
* back-calculated from DT_{90} ($DT_{50} = DT_{90} / 3.32$) as required by FOCUS kinetics

Table 7.2.2.4-26: Persistence and modeling endpoints for the dissipation of the metabolites of pyraclostrobin (M-I)

	Persistence endpoints			Modeling endpoints	
	Model	**DT_{50} [d]**	**DT_{90} [d]**	**Model**	**DT_{50} [d]**
BF 500-3 - Wholesystem	not calculated			HS	29.1*
BF 500-11 – Water	SFO	9.0	29.8	SFO	9.0
BF 500-14 – Water	SFO	6.7	22.3	SFO	6.7

* back-calculated from DT_{90} (DT_{50} = DT_{90} / 3.32) as required by FOCUS kinetics

Table 7.2.2.4-27: Persistence and modeling endpoints for the metabolites degradation (M-I)

	Persistence endpoints			Modeling endpoints	
	Model	**$DegT_{50}$**	**$DegT_{90}$**	**Model**	**DT_{50}**
BF 500-11 – Water	SFO	6.6	22	SFO	6.6
BF 500-14 – Water	SFO	6.9	23	SFO	6.9

III. CONCLUSION

Overall, it can be concluded that pyraclostrobin degrades rather fast in the total water/sediment system with a best-fit $DegT_{50}$ of 13.5 days under irradiated conditions. It quickly dissipates from the water phase with a DT_{50} of < 0.5 days. In sediment, further degradation leads to incorporation into the organic matrix (humic substances) with a DT_{50} of about 20 days.

Summary of degradation and dissipation endpoints for pyraclostrobin in different water/sediment systems

Table 7.2.2.4-28: Summary table on best-fit endpoints of pyraclostrobin obtained in water/sediment studies

Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	Best-fit $DegT_{50}/DegT_{90}$ whole system [d]	Kinetic model	Best-fit DT_{50}/DT_{90} water [d]	Kinetic model	Best-fit DT_{50}/DT_{90} sediment [d]	Kinetic model
BASF DocID 1999/11241 and 2012/1165029 – dark aerobic conditions									
System A (c)	8.42	7.1	20	23.3 / 77.4	SFO	2.13 / 28.8	FOMC	25.7 / 85.5	SFO
System A (t)	8.42	7.1	20	26.8 / 89.1	SFO	2.30 / 17.5	DFOP	29.8 / 98.9	SFO
System B (c)	8.09	7.3	20	n.c. [1]	-	0.636 / 6.19	DFOP	n.c. [1]	-
System B (t)	8.09	7.3	20	n.c. [1]	-	0.443 / 6.28	DFOP	n.c. [1]	-
BASF DocID 1999/11791 and 2012/1021122 – irradiated aerobic conditions									
Level P-I [2]									
Kellmetsch-weiher (c+t)	8.58	7.5	various [3]	7.22 / 23.98 [4]	SFO	4.47 / 14.84 [4]	SFO	5.93 / 19.69 [4]	SFO
Level P-II									
Kellmetsch-weiher (c+t)	8.58	7.5	various [3]	n.a.	-	7.50 / 24.9 [4,5]	SFO	6.48 / 21.51 [4,5]	SFO
BASF DocID 2011/1101715 – irradiated aerobic conditions									
Berghäuser Altrhein (c+t)	7.35	7.4	18-25 [3]	13.5 / 54.4	HS	0.3 /3.9	DFOP	20.1 /66.7	SFO

(t), (c) - tolyl or chlorophenyl-labeled test item used
n.a. not applicable
n.c. not calculated
[1] No reliable endpoints derived in kinetic evaluation.
[2] Endpoints at P-I level are reported as modeling endpoints and no biphasic kinetic models were tested in the study. However, as the SFO model provides excellent fits (excellent visual fit, very low χ^2 error and type I error rate), the endpoints are appropriate for use as trigger endpoints.
[3] Outdoor temperatures and light conditions were simulated in a climatic chamber.
[4] DT_{90} not reported; calculated from SFO DT_{50} as $DT_{90} = DT_{50} \times 3.32$
[5] The P-II level provides degradation endpoints for water and sediment compartment, in contrast to P-I where dissipation endpoints are estimated for water and sediment.

Table 7.2.2.4-29: Summary table on modeling endpoints of pyraclostrobin obtained in water/sediment studies

Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	Modeling $DegT_{50}$ whole system [d]	Kinetic model	Modeling DT_{50} water [d]	Kinetic model	Modeling DT_{50} sediment [d]	Kinetic model
BASF DocID 1999/11241 and 2012/1165029 – dark aerobic conditions									
Level P-I									
System A (c)	8.42	7.1	20	23.3	SFO	8.67 [1]	FOMC	25.7	SFO
System A (t)	8.42	7.1	20	26.8	SFO	5.27 [1]	DFOP	29.8	SFO
System B (c)	8.09	7.3	20	n.c. [2]	-	1.86 [1]	DFOP	n.c. [2]	-
System B (t)	8.09	7.3	20	n.c. [2]	-	1.89 [1]	DFOP	n.c. [2]	-
BASF DocID 1999/11791 and 2012/1021122 – irradiated aerobic conditions									
Level P-I									
Kellmetsch-weiher (c+t)	8.58	7.5	various [3]	7.22	SFO	4.47	SFO	5.93	SFO
Level P-II									
Kellmetsch-weiher (c+t)	8.58	7.5	various [3]	n.a.	-	7.50 [4]	SFO	6.48 [4]	SFO
BASF DocID 2011/1101715 – irradiated aerobic conditions									
Level P-I									
Berghäuser Altrhein (c+t)	7.35	7.4	18-25 [3]	15.4	SFO	1.2 [1]	DFOP	20.1	SFO

(t), (c) - tolyl or chlorophenyl-labeled test item used
n.a. not applicable
n.c. not calculated
[1] back-calculated as $DT_{50} = DT_{90} / 3.32$ according to FOCUS kinetics
[2] No reliable endpoints derived in kinetic evaluation.
[3] Outdoor temperatures and light conditions were simulated in a climatic chamber.
[4] The P-II level provides $DegT_{50}$ for water and sediment compartment, in contrast to P-I where dissipation rates are estimated for water and sediment.

Table 7.2.2.4-30: Maximum occurrence of pyraclostrobin metabolites in water/ sediment studies [1]

Metabolite	Matrix	BASF DocID	System	Incubations	Parent label	Maximum % AR
BF 500-3	water	1999/11241 [2]	Kastenbergheide	dark	tolyl chlorophenyl	0.4 [3] 0.2 [3]
			Bergh. Altrhein	dark	tolyl chlorophenyl	2.4 [3] 1.4 [3]
		1999/11791 [2]	Kellmetschweiher	irradiated	tolyl chlorophenyl	5.0 3.5
		2011/1001715	Kellmetschweiher	irradiated	tolyl chlorophenyl	2.5 0.5
		2011/1001715	Kellmetschweiher	dark control	tolyl chlorophenyl	0.1 n.d.
	sediment	1999/11241 [2]	Kastenbergheide	dark	tolyl chlorophenyl	9.7 [3] 20.1 [3]
			Bergh. Altrhein	dark	tolyl chlorophenyl	65.3 [3] 66.1 [3]
		1999/11791 [2]	Kellmetschweiher	irradiated	tolyl chlorophenyl	16.9 15.9
		2011/1001715	Kellmetschweiher	irradiated	tolyl chlorophenyl	5.9 4.2
		2011/1001715	Kellmetschweiher	dark control	tolyl chlorophenyl	2.5 0.9
BF 500-5	water	2011/1001715	Kellmetschweiher	irradiated	chlorophenyl	0.9
		2011/1001715	Kellmetschweiher	dark control	chlorophenyl	1.0
BF 500-6	sediment	1999/11241 [2]	Kastenbergheide	dark	tolyl chlorophenyl	7.3 [3] 6.3 [3]
			Bergh. Altrhein	dark	tolyl chlorophenyl	0.9 [3] n.d. [3]
		2011/1001715	Kellmetschweiher	irradiated	tolyl chlorophenyl	3.1 3.7
		2011/1001715	Kellmetschweiher	dark control	tolyl chlorophenyl	4.5 7.0
BF 500-7	sediment	1999/11241 [2]	Kastenbergheide	dark	tolyl chlorophenyl	5.7 [3] 7.0 [3]
			Bergh. Altrhein	dark	tolyl chlorophenyl	n.d. n.d.
		2011/1001715	Kellmetschweiher	irradiated	tolyl chlorophenyl	2.3 2.2
		2011/1001715	Kellmetschweiher	dark control	tolyl chlorophenyl	2.5 3.6
BF 500-11	water	1999/11791 [2]	Kastenbergheide	irradiated	tolyl	11.4
		2011/1001715	Bergh. Altrhein	irradiated	tolyl	5.4
	sediment	1999/11791 [2]	Kastenbergheide	irradiated	tolyl	0.6
BF 500-13	water	1999/11791 [2]	Kastenbergheide	irradiated	tolyl	15.4
		2011/1001715	Bergh. Altrhein	irradiated	tolyl	0.9
	sediment	1999/11791 [2]	Kastenbergheide	irradiated	tolyl	2.1

Metabolite	Matrix	BASF DocID	System	Incubations	Parent label	Maximum % AR
BF 500-14	water	1999/11791 [2]	Kastenbergheide	irradiated	tolyl chlorophenyl	10.8 11.4
		2011/1001715	Bergh. Altrhein	irradiated	tolyl chlorophenyl	2.2 2.5
	sediment	1999/11791 [2]	Kastenbergheide	irradiated	tolyl chlorophenyl	0.6 0.7

[1] Only metabolites listed which occur in a compartment at > 5% AR.
[2] already peer-reviewed during previous EU evaluation
[3] Values taken from appendix of original report; for modeling, mean values as listed in the table section of the original report and former dossier tables are used.

Table 7.2.2.4-31: Summary table on kinetic endpoints of pyraclostrobin metabolites obtained in water/sediment studies

Study BASF DocID	Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	DT_{50} whole system [d]	Kinetic model	DT_{50} water [d]	Kinetic model	DT_{50} sediment [d]	Kinetic model
BF 500-3										
1999/11241 dark	System A	8.42	7.1	20	n.c.	-	n.c.	-	n.r.	-
1999/11241 dark	System B	8.09	7.3	20	n.c.	-	n.c.	-	54.8 [1]	SFO
1999/11791 & 2012/1021122 irradiated	Kellmetsch-weiher	8.58	7.5	various [2]	92.5 [3]	SFO	n.c.	-	78.6 [3]	SFO
2011/1101715 irradiated	Berghäuser Altrhein	7.35	7.4	18-25 [2]	29.1 [3,5]	HS	n.c.	-	n.c.	-
BF 500-6										
1999/11241 dark	System A	8.42	7.1	20	n.c.	-	n.c.	-	116.3 [1]	SFO
1999/11241 dark	System B	8.09	7.3	20	n.c.	-	n.c.	-	n.c.	
BF 500-7										
1999/11241 dark	System A	8.42	7.1	20	n.c.	-	n.c.	-	80.0 [1]	SFO
1999/11241 dark	System B	8.09	7.3	20	n.c.	-	n.c.	-	n.c.	-
BF 500-11										
1999/11791 & 2012/1021122 irradiated	Kellmetsch-weiher	8.58	7.5	various [2]	22.6 [3]	SFO	25.2 [3]	SFO	n.c.	-
					22.9 [4]	SFO	n.a.	-	n.a.	-
2011/1101715 irradiated	Berghäuser Altrhein	7.35	7.4	18-25 [2]	n.c.	-	9.0 [3]	SFO	n.c.	-
					n.a.	-	6.6 [4]	SFO	n.a.	-

Study BASF DocID	Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	DT_{50} whole system [d]	Kinetic model	DT_{50} water [d]	Kinetic model	DT_{50} sediment [d]	Kinetic model
BF 500-14										
1999/11791 & 2012/1021122 irradiated	Kellmetsch-weiher	8.58	7.5	various [2]	17.3 [3]	SFO	15.9 [3]	SFO	n.c.	-
					8.2 [4]	SFO	n.a.	-	n.a.	-
2011/1101715 irradiated	Berghäuser Altrhein	7.35	7.4	18-25 [2]	n.c.	-	6.7 [3]	SFO	n.c.	-
					n.a.	-	6.9 [4]	SFO	n.a.	-

n.a. not applicable
n.c. not calculated
n r. not reported in study; calculated value extrapolated too far beyond the period of investigation
[1] calculated with multi-compartment model with ModelMaker
[2] Outdoor temperatures and light conditions were simulated in a climatic chamber.
[3] DT_{50} considering metabolite decline only
[4] $DegT_{50}$ considering simultaneous formation and degradation of metabolite
[5] back-calculated as $DT_{50} = DT_{90} / 3.32$ according to FOCUS kinetics

CA 7.2.3 Degradation in the saturated zone

Due to its low leaching potential, pyraclostrobin is not expected to reach deeper soil layers or saturated zones. Therefore, investigations on the degradation in the saturated zone are considered to be not necessary.

CA 7.3 Fate and behaviour in air

Pyraclostrobin is characterized by a low vapor pressure (2.6 x 10^{-8} Pa at 20°C) and a low volatilization from soil and plant surfaces (< 3% in 24h). Furthermore, in the air it is rapidly degraded by photochemical processes.

CA 7.3.1 Route and rate of degradation in air

No new experimental data are available, but a new calculation of the photochemical oxidative degradation in air (Atkinson) according to the newest model is provided below.

Report: CA 7.3.1/1
Hassink J., 2013a
Photochemical oxidative degradation of BAS 500 F (QSAR estimates)
2013/1350648
Guidelines: EC 1107/2009 of the European Parliament
GLP: no (not applicable)

Executive Summary

The degradation rates for reactions of pyraclostrobin with OH-radicals and ozone in the atmosphere were calculated using the AOPWIN program based on ATKINSON's increment method. Based on the resulting degradation rate of k_{OH} = 206.3747 x 10^{-12} cm^3 $molecule^{-1}$ s^{-1}, the atmospheric degradation half-life of the substance via this reaction route was estimated to be $t_{1/2}$ = 0.05 d (12 h day). Although O_3 is likely to react with pyraclostrobin, the degradation rate resulting from ozone attack could not be estimated.

Due to the rapid degradation in air, it can be concluded that there is no risk of long-range transport of pyraclostrobin.

I. MATERIAL AND METHODS

The degradation rate resulting from attack of OH-radicals was calculated with the AOPWIN Program (Atmospheric Oxidation Program for Microsoft Windows 3.1, Version 1.88, Syracuse Research Corp. 1997) based on ATKINSON's increment method [*Atkinson, R. (1987) A Structure-Activity Relationship for the Estimation of Rate Constants for the Gas-Phase Reactions of OH Radicals with Organic Compounds, Int.J.Chem.Kin. 19, 799*]. The degradation rate resulting from attack of ozone was calculated according to an OECD method [*Anonymous (1992): The rate of photochemical transformation of gaseous organic compounds in air under tropospheric conditions. OECD Environment Monographs No. 61, OECD, Paris*].

The degradation rate of pyraclostrobin with OH-radicals was estimated based on the structural formula. The SMILE notation used for pyraclostrobin in AOPWIN was:

COC(=O)N(c1ccccc1COc2nn(cc2)c3ccc(cc3)CL)OC

II. RESULTS AND DISCUSSION

Assuming a pseudo-first order reaction, the degradation half-life was calculated by taking into account the diurnally and seasonally averaged concentration of hydroxyl-radicals in the troposphere. The total rate constant was estimated to be k_{OH} = 206.3747 x 10^{-12} cm^3 $molecule^{-1}$ s^{-1}.

Considering a weighted global average tropospheric hydroxyl-radical concentration of 1.5 x 10^6 mol cm^{-3}, the half-life for the degradation of pyraclostrobin by OH-radicals was calculated according to Equation 7.3.1-1.

Equation 7.3.1-1 Estimation of the atmospheric degradation half-life ($t_{1/2}$) of pyraclostrobin

$$t_{1/2} = \ln 2 / (206.3747\ 10^{-12} \times 1.5\ 10^{6})\ s$$
$$= 0.622\ h$$
$$= \underline{0.05\ d\ (12\ h\ day)}$$

Although pyraclostrobin contains reactive sites for an ozone attack, a reasonable approximation by AOPWIN was not possible. Therefore, although O_3 is likely to react with pyraclostrobin, no degradation estimation could be given.

III. CONCLUSION

Based on the results of the atmospheric degradation half-life of pyraclostrobin ($t_{1/2}$ = 0.05 d), it can be concluded that the substance will be rapidly degraded by photochemical processes in the troposphere. Hence, due to the rapid degradation in air, it can be concluded that there is no risk of long-range transport of pyraclostrobin.

CA 7.3.2 Transport via air

Pyraclostrobin has a very low volatilization potential and is degraded very fast by photochemical processes. Consequently, there is no risk of long-range transport of pyraclostrobin.

CA 7.3.3 Local and global effects

No effects are expected since transport via air is very unlikely (for details see above).

CA 7.4 Definition of the residue

CA 7.4.1 Definition of the residue for risk assessment

According to the results presented in M-CA 7.1 – 7.3 the following compounds have to be considered for the environmental risk assessment:

Soil:
Pyraclostrobin and its aerobic soil metabolites BF 500-6, BF 500-7

Both metabolites were tested in chronic earthworm and collembola studies due to their potential persistence in soil. Based on the results it can be concluded that the risk of metabolites for soil organisms is negligible.

Groundwater:
Pyraclostrobin, its aerobic soil metabolites BF 500-6, BF 500-7, and its anaerobic soil metabolites BF 500-3, BF 500-4 and BF 500-5

Based on the high adsorption and/or the fast degradation rates in soil, neither the parent molecule nor one of its metabolites poses any risk of leaching to groundwater. The predicted annual leachate concentrations of pyraclostrobin and all soil metabolites were below 0.001 µg L^{-1}.

Surface Water:
Pyraclostrobin and its metabolites BF 500-5, BF 500-11, BF 500-13, BF 500-14

The aquatic toxicity of all metabolites was tested and was found to be three orders of magnitude lower for the most sensitive species than parent. Sufficient margins of safety were reached after FOCUS surface water step 1-2 calculations and it is concluded that the risk of metabolites for aquatic organisms is negligible.

Sediment:
Pyraclostrobin and its metabolites BF 500-3, BF 500-6 and BF 500-7

The sediment toxicity of the metabolites was tested in chronic *Chironomus* studies. Sufficient margins of safety were reached already after FOCUS surface water step 1-2 calculations (worst case) and it is concluded that the risk of metabolites for sediment organisms is very low.

Air:
Pyraclostrobin

No volatile metabolite was detected.

CA 7.4.2 Definition of the residue for monitoring

According to the results of the risk assessment the following compounds should be considered for environmental monitoring:

Soil: Pyraclostrobin (parent only)

Ground Water: Pyraclostrobin (parent only)

Surface Water: Pyraclostrobin (parent only)

Sediment: Pyraclostrobin (parent only)

Air: Pyraclostrobin (parent only)

CA 7.5 Monitoring data

According to the knowledge of the applicant, there are currently no published environmental monitoring data available for pyraclostrobin or its metabolites, which would provide knowledge on the environmental behaviour not covered by this dossier.

During literature search a few publications were found dealing with environmental monitoring of various pesticides in surface water, groundwater and/or sediment outside EU (e.g. US, Australia, Costa Rica) mentioning also pyraclostrobin. The results showed that pyraclostrobin could be detected in local areas characterized by intensive fungicide use during the spraying season, however, frequency of detection as well as measured concentrations were usually very low.

One publication described an air monitoring in France (near Straßbourg) in April/May 2007. Pyraclostrobin was included as an analyte, however, it was not detected.

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1162249 (version 1)
27-Feb-2017	A new field effect study for voles was added in 8.1.2.2 and table 8.1-1 was revised accordingly. 8.1.4 was amended reflecting 2 new amphibian studies that have been included in M-CP 10 of the BAS 500 06 F dossier. Additional MDD calculations were summarised in 8.2.8. A new bumblebee study was added in 8.3.1 and table 8.3.1-1 was revised accordingly. New or changed text is marked in yellow.	BASF DocID 2017/1032926 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Pyraclostrobin (BAS 500 F), a fungicide for use in cereals, maize, potatoes, legumes, fruits, vegetables and various other crops, is registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive No 2004/30/EC and 2009/25/EC. Inclusion entered into force on 10 March 2004. The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011. Approval extension was granted until 31 January 2017 by Regulation No 823/2012/EU.

All relevant information on the first Annex I review and the endpoints used in ecotoxicological risk assessments can be found in the monograph of pyraclostrobin and in the SANCO/1420/2001-Final document (EU Review Report).

For the current registration renewal under Regulation 1107/2009, a data gap analysis according to new guidelines and new guidance documents was performed and new studies or evaluations were initiated where considered necessary. All new data are provided in this section or in the respective sections of the dossiers for the two new representative formulations.

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. Summaries of relevant and reliable public literature data on pyraclostrobin are provided in this section (or in the respective sections of the dossiers for the two new representative formulations) as appropriate. Further information on the literature assessment and respective justifications can be found in M-CA 9.

An overview of metabolites relevant for the environment is given in M-CA 7. The table is including the different code numbers that are available for each metabolite. In the following chapters and study summaries synonym metabolite codes are given in brackets where deemed to be helpful.

CA 8.1 Effects on birds and other terrestrial vertebrates

Data from new studies conducted with the active substance pyraclostrobin or the representative formulation BAS 500 06 F and relevant for the risk assessment of birds and mammals are summarized below. An overview on the reports and documents is given in Table 8.1-1.

Besides the here summarized substance-specific study reports and documents, further information (either generic or formulation-specific studies and information) used in the risk assessment for birds and mammals will be given in M-CP 10.1.1 and M-CP 10.1.2.

Table 8.1-1: Overview of study reports and documents whose summaries are given in M-CA 8.1.1 and 8.1.2

Data point (all M-CA)	References (BASF DocID)	Year	Title
8.1.1.1/1	2013/1400375	2014	BAS 500 F (Pyraclostrobin) - Acute Toxicity in the canary (Serinus canaria) after single oral administration (LD_{50}).
8.1.2.1/1	2009/1108893	2009	BAS 500 06 F: Acute toxicity testing study - Repeated dose oral toxicity study in Wistar rats.
8.1.2.1/2	2011/1146588	2011	BAS 500 06 F - Repeated dose oral toxicity study in Wistar rats - Administration via the diet over 5 days.
8.1.2.1/3	2012/1129348	2014	BAS 500 06 F: Study to assess avoidance and effects in wood mice *(Apodemus sylvaticus)* upon dietary exposure.
8.1.2.1/4	2014/1001603	2014	A body burden model to assess the acute dietary risk posed by formulated Pyraclostrobin (BAS 500 06 F) to the common vole, *Microtus arvalis*.
8.1.2.2/1	2014/1000041	2014	Field study on the acute and long-term effects of a pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray in spring to cereals on populations of small mammals (wood mice and common voles) in Central Europe (Germany).
8.1.2.2/2	2015/1126803	2016	Field study on the acute and long-term effects of a pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray on meadows to populations of common voles in Central Europe (Germany)
8.1.2.2/3	2013/1045207	2013	Study on the residue behaviour of Pyraclostrobin (BAS 500 F) on wheat (young plants) after treatment with BAS 500 06 F under field conditions in North and South Europe.
8.1.2.2/4	2013/1044539	2013	Study on the residue behavior of Pyraclostrobin (BAS 500 F) on pea (young plants) after the application of BAS 500 06 F under field conditions in France (North), Germany, The United Kingdom, Italy and Spain, 2012.
8.1.2.2/5	2013/1078114	2013	Dissipation of BAS 500 F - pyraclostrobin on young plants (wheat and peas). Trials conducted in the Northern Zone of Europe. Calculation of DT50 / DT90 dissipation times.
8.1.2.2/6	2013/1291161	2013	Dissipation of BAS 500 F - pyraclostrobin on young plants (wheat and peas). Trials conducted in the Southern Zone of Europe. Calculation of DT50 / DT90 dissipation times.

Information on M-CA 8.1.1 and 8.1.2

Effects on birds and effects on mammals are discussed in M-CA 8.1.1 and 8.1.2, respectively. Table 8.1-2 provides information on the EU- agreed toxicity endpoints and additional toxicity studies relevant for assessing the risk to birds and mammals.

Table 8.1-2: Summary of EU-agreed and new toxicity studies relevant for assessing the risk to birds and mammals[1)]

Test system	Test species	Reference (BASF DocID)	EU-agreed
Birds			
Acute oral toxicity	*Colinus virginianus*	1997/11136	yes (still valid for AIR 3 according to EFSA/2009/1438)
	Serinus canaria	2013/1400375	no (new study, please refer to CA 8.1.1.1/1 for detailed study summary)
Short-term dietary toxicity	*Colinus virginianus*	1998/10932	yes (but no longer part of core data package according to EFSA/2009/1438)
	Anas platyrhynchos	1998/10933	
Sub-chronic toxicity and reproduction	*Colinus virginianus*	1999/11207	yes (still valid for AIR 3 according to EFSA/2009/1438)
	Anas platyrhynchos	1999/11206	
Mammals			
Acute oral toxicity	Rat	1998/10965	yes (still valid for AIR 3 according to EFSA/2009/1438)
2-generation reproductive toxicity	Rat	1999/11869	
Development toxicity	Rat	1999/11511	
	Rat	1999/11512	
	Rabbit	2001/1003803	

1) EU agreed means assessed during the previous EU evaluation process, but not necessarily included in the list of endpoints as given in the Review Report for the active substance pyraclostrobin (SANCO/1420/2001-Final. 8. September 2004).

CA 8.1.1 Effect on birds

CA 8.1.1.1 Acute oral toxicity to birds

Report: CA 8.1.1.1/1
[redacted] 2014a
BAS 500 F (Pyraclostrobin) - Acute Toxicity in the canary (Serinus canaria) after single oral administration (LD50)
2013/1400375
Guidelines: EPA 850.2100, EPA 850.2000, EPA 712-C-025
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

An avian acute oral toxicity test with the active substance pyraclostrobin (BAS 500 F) was conducted. The objective of the study was to evaluate the acute toxicity of the substance in Canaries (*Serinus canaria*) and to determine the oral LD_{50} in the Canary after a single oral administration of the test substance and a 14-day post observation period.

The test substance was administered via a single oral dose of 0 (control), 210, 340, 551, 893 and 1446 mg a.s./kg body weight to groups of 28 weeks old Canaries. Ten birds (5 males and 5 females) were used in each test substance group. The doses were administered undiluted in two gelatin capsules directly into the crop. Birds that have been fasted for about 3 to 5 hours were administered the test substance. Birds of all groups received food and water *ad libitum* throughout the test. The test was terminated after 14 days.

All groups were observed for mortality, signs of clinical toxicity, impact on food consumption and body weight for 14 consecutive days post dosing. Gross-pathological examinations were conducted on all birds sacrificed at the termination of the test.

No mortality occurred throughout the duration of the study in the control and in the 210, 893 and 1446 mg a.s./kg b.w. dose groups. There was one mortality each in males of the dose groups of 340 and 551 mg a.s./kg b.w who were considered not to be treatment related as there was no mortality in the higher dose groups. No regurgitation was observed during the first hour after dosing in any of the dose groups. Thus all birds received the full dose. No substance-related impairment of food uptake in comparison to the control was observed in any of the dose groups. No statistically significant (Dunnett test) substance-related reduction of the body weights in the male and female birds was observed on day 7 and at day 14 (sacrifice) in any of the dose groups compared to the control. The body weight development of the females was not statistically different from the control in any of the dose groups. In males the body weight development on some occasions was higher than in the control group, however, without any dose-response pattern. It was not considered to be treatment-related. All birds were examined macroscopically (post-mortem) after study termination. No abnormalities caused by the test substance were observed in birds that died and sacrificed birds.

In an acute oral toxicity test with the canary (*Serinus canaria*), the LD_{50} of pyraclostrobin was found to be >1446 mg a.s./kg b.w. The NOEL was ≥ 1446 mg a.s./kg b.w.

I. MATERIAL AND METHODS

Test item:	BAS 500 F (Reg. No. 304428), batch no. COD 001236, purity: 99.02% (tolerance ± 1.0%).
Test species:	Canary (*Serinus canaria*), visually indistinguishable from wild birds; adults, age: approx. 28 weeks old (before beginning of first egg-laying period); supplier: Zoowelt, Bechtheim, Germany.
Test design:	Birds were administered single doses of 210, 340, 551, 893 and 1446 mg a.s./kg body weight of the test substance pyraclostrobin (BAS 500 F) undiluted in two gelatin capsules directly into the crop; 5 males and 5 females per dose group were used. The birds were observed for regurgitation for 1 - 3 hours after dosing. An observation period of 14 days followed, during which mortalities and signs of toxicity were recorded, four times on day of dosing and daily thereafter; assessment of body weight was carried out on the day before dosing and on days 7 and 14 after dosing; mean food consumption (g/bird/day) was calculated from the daily food consumption per cage separately for male and female birds after dosing. Gross-pathological post-mortem examinations of all birds at study termination on day 14 after dosing.
Endpoints:	Mortality, clinical signs, feed consumption, body weight (b.w.), and gross-pathological examinations were conducted on all birds sacrificed at the termination of the test. Calculation of LD_{50} and NOEL.
Test concentrations:	0 (Control), 210, 340, 551 and 893 mg a.s./kg body weight (nominal).
Test conditions:	Birds fasted for about 3 h to 5 h before administration of the test substance; temperature: 19.1°C (minimum) and 21.1°C (maximum); values within the limits of 21°C ± 2°C; relative humidity: 36.8% and 76.8%; values not within limits of 45% - 70%, time below limit 11 days and 7 hours. The deviations had no relevant influence on the study results, since the limits were exceeded only to a small extent and no adverse effects were observed in the control group; photoperiod: 8 hours light : 16 hours dark, light intensity: lux 20 - 38 lux.
Analytics:	The test substance concentrations were analyzed using HPLC.
Statistics:	Descriptive statistics. No statistical calculation of the LD_{50} was performed since no substance related mortality was observed in the tested dose. Dunnett test was used for body weight data and Fisher's exact test for pair-wise comparison of mortality data.

II. RESULTS AND DISCUSSION

Analytical measurements: No analytical determinations of the test substance in the carrier were necessary since the test substance was applied without carrier.

Biological results: No mortality occurred throughout the duration of the study in the control and in the 210, 893 and 1446 mg a.s./kg b.w. dose groups. There was one mortality each in males of the dose groups of 340 and 551 mg a.s./kg b.w who were considered not to be treatment related as there was no mortality in the higher dose groups. No regurgitation was observed during the first hour after dosing in any of the dose groups. Thus all birds received the full dose. No substance-related impairment of food uptake in comparison to the control was observed in any of the dose groups. No statistically significant (Dunnett test) substance-related reduction of the body weights in the male and female birds was observed on day 7 and at day 14 (sacrifice) in any of the dose groups compared to the control. The body weight development of the females was not statistically different from the control in any of the dose groups. In males the body weight development on some occasions was higher than in the control group, however, without any dose-response pattern. It was not considered to be treatment-related. All birds were examined macroscopically (post-mortem) after study termination. No abnormalities caused by the test substance were observed in birds that died and sacrificed birds. The relevant data and endpoints are summarized in the table below.

Table 8.1.1.1-1: Acute toxicity of pyraclostrobin (BAS 500 F) to the canary (*Serinus canaria*)

	Dose rate [mg a.s./kg b.w.]					
	0 (control)	**210**	**340**	**551**	**893**	**1446**
Number of birds per dose group	10	10	10	10	10	10
Number of dead birds	0	0	1[1)]	1[1)]	0	0
Dead birds percentage [%]	0	0	10	10	0	0

Endpoints	**Dose [mg a.s./kg b.w.]**
Highest dose causing no substance-related mortality	1446
LD_{50} (14 d)	>1446
NOEL	≥ 1446

b.w. = body weight

1) One male bird of group 2 died five days after dosing and one male bird of group 3 died one day after dosing. According to the test guideline 10% mortality is acceptable for the control group. Because the mortality in these both groups did not exceed 10% and in the higher dose groups (893 and 1446 mg/kg body weight) no mortality was observed, it was concluded that the mortality can be clearly not attributed to the test substance.

III. CONCLUSION

In an acute oral toxicity test with the canary (*Serinus canaria*), the LD_{50} of pyraclostrobin was found to be >1446 mg a.s./kg b.w. The NOEL was ≥ 1446 mg a.s./kg b.w.

CA 8.1.1.2 Short-term dietary toxicity to birds

The two short-term toxicity studies in bobwhite quail and mallard duck evaluated and accepted during the previous Annex I inclusion process are no longer part of the required data as outlined in EFSA/2009/1438 and the new EU Regulation 283/2013. For completeness the study details are summarised in the table below.

Table 8.1.1.2-1: Available short-term toxicity studies with pyraclostrobin

Test system	Test species	Results	Guideline	Reference (BASF DocID)
Short-term dietary toxicity	*Colinus virginianus*	LC_{50} > 5000 mg a.s./kg diet; LC_{50} > 1176 mg a.s./kg bw/d[1)]	US EPA FIFRA Guideline § 71-2 (Oct. 1984); OECD 205 (Apr. 1984)	1998/10932[2)]
	Anas platyrhynchos	LC_{50} > 5000 mg a.s./kg diet; LC_{50} > 1320 mg a.s./kg bw/d[1)]	US EPA FIFRA Guideline § 71-2 (Oct. 1984); OECD 205 (Apr. 1984)	1998/10933[3)]

1) Daily Dose [mg/kg bw/d] calculated based on study data for food consumption and body weight
2) Study title: "BAS 500 F - Avian Dietary LC_{50} test in chicks of the Bobwhite Quail (Colinus virginianus)"
3) Study title: "BAS 500 F - Avian Dietary LC_{50} test in chicks of the Mallard Duck (Anas platyrhynchos L.)"

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

Two reproductive bird toxicity studies are available for pyraclostrobin, one in bobwhite quail and one in mallard duck. Both studies were already evaluated during the previous Annex I inclusion process and are still relevant for deriving the reproductive toxicity endpoint. Details on the studies are shown in M-CP 10.1 (e.g. Table 10.1.1.-2 in M-CP of BAS 500 06 F).

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

Acute oral toxicity to mammals is discussed in M-CA 5.2.1. The studies previously evaluated during the Annex I inclusion process are considered to be still valid and sufficient. Therefore, no new studies have been conducted with the active substance. In the following two new 5 day dietary toxicity studies conducted with the representative formulation BAS 500 06 F are summarized.

Please note that the following studies CA 8.1.2.1/1 and CA 8.1.2.1/2 were erroneously listed as CP 10.1.2.1/1 and CP 10.1.2.1/2 in the Application submitted in January 2014 for the renewal of approval of pyraclostrobin.

Report:	CA 8.1.2.1/1 [redacted] et al., 2009a BAS 500 06 F: Acute toxicity testing study - Repeated dose oral toxicity study in Wistar rats 2009/1108893
Guidelines:	EPA 540/9-82-024, EPA 540/9-85-008, EPA 850.2200, EPA 712-C-96-140, OECD 205, OECD 423
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

A 5-day mammal dietary toxicity test with the formulation BAS 500 06 F was conducted to determine mortality and sublethal effects in small mammals after oral dietary administration of BAS 500 06 F. In addition, the food avoidance behavior of small mammals was investigated. Behavior such as food avoidance, can reduce exposure by preventing animals from ingesting body burdens exceed harmful thresholds. Furthermore, the no observed effect concentration (NOEC) should be determined.

The test substance was administered at dietary concentrations of 5000 and 12000 ppm BAS 500 06 F. Five young female rats were used in each concentration group and in the control group. All groups were observed for mortality, water consumption, signs of clinical toxicity, impact on food consumption and body weight during the exposure period of 5 days and a treatment-free post-exposure period of 9 days. The animals received feed ad libitum.
No test substance related mortality was observed in any test group throughout the entire study period. No clinical signs of toxicity related to the test substance were observed in the treatment group of 5000 ppm test substance in the diet. In the highest treatment group of 12000 ppm test substance in the diet piloerection in all animals was observed from day 3 to 5. Significantly reduced food consumption was observed in all treated test groups during administration period. This effect was assessed as a problem of palatability resulting in avoidance of food contaminated with BAS 500 06 F containing the active ingredient pyraclostrobin. Impaired body weight data were obtained during administration period in all treated test groups. This finding was assessed as a consequence of the reduced food consumption rather than an incipient sign of general systemic toxicity.

All animals were examined macroscopically. No macroscopical findings were observed during the gross pathological examinations.

No test substance-related adverse effects of toxicological concern could be revealed. Reduced food consumption and body weight loss were clearly related to food avoidance. A NOEC could not be determined.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 06 F, batch no. 8265, purity: content of a.s. in formulation: 202.7 g pyraclostrobin /L

Test species: rat, strain crl:WI (Han), female rats, age: 70-72 days at start of administration period; source: Charles River Laboratories, Research Models and Services GmbH, Germany.

B. STUDY DESIGN

Test design: Young female rats received different concentrations of the test substance offered in the diet on 5 consecutive days with a treatment-free post-exposure period of 9 days; 5 rats per test substance concentration and for the control group were used. All groups received ground diet and drinking water ad libitum. Assessment for mortality and clinical signs was carried out two times a day on working days (once a day on Saturdays, Sundays and public holidays) of the entire study period. Assessment of body weight was carried out once a day from study days 0 to 8 and on day 10, 12 and 14; food consumption was determined daily from study days 0 to 8 and on day 10, 12 and 14 and calculated as daily mean food consumption (g) per animal and day. Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume. Gross-pathological post-mortem examinations of all surviving specimens were carried out at the end of the study.

Endpoints: Mortality, clinical signs, feed consumption, water consumption, body weight (b.w.). Gross-pathological examinations were conducted on rats sacrificed at the definitive termination of the test. Calculation of NOEC.

Test concentrations: Dietary concentrations of 0 (Control), 5000 and 12000 ppm BAS 500 06 F in the diet. The corresponding actual mean daily intake rates were 0 (Control), 264 and 240 mg BAS 500 06 F/kg b.w./day.

Test conditions: Temperature: 20°C - 24°C, relative humidity: 30% - 70%; with only minimal deviations from these limits; the day/night cycle was a 12 hour light cycle photoperiod: 12 hours light: 12 hours dark.

Statistics: Descriptive statistic, Dunnett test for body weight data.

II. RESULTS AND DISCUSSION

Analytical measurements: The results of the analytical verification of the test substance concentration in the diet were within a range of 89.3% to 96.4% of the nominal concentrations during the test. The biological results are therefore based on the nominal values.

Biological results: The test substance was administered at dietary concentrations of 0 (Control), 5000 and 12000 ppm BAS 500 06 F. The corresponding actual mean daily intake rates are 0 (Control), 264 and 240 mg BAS 500 06 F/kg b.w./day.
No test substance related mortality was observed in any test group throughout the entire study period. No clinical signs of toxicity related to the test substance were observed in the treatment group of 5000 ppm test substance in the diet. In the highest treatment group of 12000 ppm test substance in the diet piloerection in all animals were observed from day 3 to 5. Statistical significant reduced food consumption was observed in all treated test groups during administration period. This effect was assessed as a problem of palatability resulting in avoidance of food contaminated with BAS 500 06 F containing the active ingredient pyraclostrobin. Impaired body weight data were obtained during administration period in all treated test groups. This finding was assessed as a consequence of the reduced food consumption rather than an incipient sign of general systemic toxicity.

All animals were examined macroscopically. No macroscopical findings were observed during the gross pathological examinations. Results are presented in the table below.

Table 8.1.2.1-1: Mammalian dietary toxicity of BAS 500 06 F to rats

Dose Group [ppm BAS 500 06 F in diet]	Control	5000	12000
Mean test substance intake (BAS 500 06 F mg/kg b.w./day)	--	264	240
Mortality [%]	0	0	0
Clinical signs	None	None	Yes[1)]

b.w. = body weight
1) Piloerection was observed in all animals on study day 3 to 5.

III. CONCLUSION

In conclusion, this study did not reveal test substance-related adverse effects of toxicological concern. Reduced food consumption and body weight loss were clearly related to food avoidance. A NOEC could not be determined.

Report: CA 8.1.2.1/2
[redacted]., 2011a
BAS 500 06 F - Repeated dose oral toxicity study in Wistar rats - Administration via the diet over 5 days
2011/1146588
Guidelines: <none>
GLP: no

Executive Summary

With regard to the risk assessment for wild mammals, the standard acute tests using oral gavage as dosing method do not reflect the real pattern of exposure of wild mammals in the field following the application of several types of Plant Protection Products (for instance, those applied as foliar sprays). Laboratory testing using more realistic exposure routes (i.e., dietary) and dosing regimes (food available for brief daily intervals, not continuously, ensuring high feeding rates) would result in toxicity data better suitable for a more realistic assessment of the risk to wild mammals.

Hence, 5-day mammal dietary toxicity test with the formulation BAS 500 06 F was conducted to determine mortality and sublethal effects in small mammals after oral dietary administration of BAS 500 06 F under more realistic exposure conditions and dosing regimes than the standard acute toxicity tests.

Mortality and sublethal effects were determined in small mammals after oral dietary administration of BAS 500 06 F for 5 days. In addition, the food avoidance behavior of small mammals was investigated. Behavior such as food avoidance can reduce exposure by preventing animals from ingesting body burdens that exceed harmful thresholds.

BAS 500 06 F was administered over 5 days via the diet to groups of 5 young female Wistar rats as a homogeneous addition to the food. The test concentrations were chosen to cover field-relevant dietary concentrations of pyraclostrobin (BAS 500 F). Due to a transmission and calculation error the dietary concentrations applied in a first study part were too high (20859 ppm, 8773 ppm and 1883 ppm of BAS 500 06 F). Hence a second study part was conducted using the correct dietary concentrations in the diet (1495 ppm, 648 ppm and 149 ppm of BAS 500 06 F). Control rats were offered untreated diet. The administration period was followed by a treatment-free post-exposure period of 9 days.
In order to reflect the feeding behavior of wild animals and to ensure high feeding rates of the animals the feeding was restricted to 2 feeding periods of 2 hours. Two different control groups of 5 young female rats per group were included in the testing. One control group received untreated diet during the restricted feeding windows. Additionally, a second control group was offered untreated diet ad libitum to determine potential variations in food consumption due to restricted feeding.
All groups were observed for mortality, water consumption, signs of clinical toxicity, impact on food consumption and body weight during the exposure period of 5 days and a treatment-free post-exposure period of 9 days. Individual food consumption of animals was determined daily. On study day 0, the food consumption was in addition determined separately for each feeding interval after 20, 45, 70, 95, and 120 minutes for the dose groups as well as the control to estimate in reference to the controls the avoidance response time.

The experimental design was overall successful at training the rats to ingest the full daily intake during the only four hours (two periods of 2 hours each) where food was available. Detailed investigations of the feeding pattern at study day 0 revealed that the food was not ingested evenly throughout each of the 2-hour feeding intervals, but was largely ingested during the first feeding bout of 20 minutes. Hence the experimental designs is suitable to ensure high feeding rates of the test animals concentrated during two brief (<20 minutes) daily periods.
No test substance related mortality was observed in any test group throughout the entire study period. No clinical signs of toxicity related to the test substance were observed in any of the treatment groups. Reduced food consumption was observed in all treated test groups during administration period. This effect was caused by the avoidance of food treated with BAS 500 06 F containing the active ingredient pyraclostrobin. Impaired body weight data were obtained during administration period in all treated test groups. This finding was assessed as a consequence of the reduced food consumption rather than an incipient sign of general systemic toxicity. During the post-administration period a full recovery of food consumption and body weights was observed.
All rats were examined macroscopically. No substance-related abnormalities were detected in rats after sacrifice.

No test substance-related mortality or severe sublethal effects were observed. Reductions on food consumption and body weight loss were clearly related to avoidance of treated food.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 500 06 F, batch no. 3223026, purity: content of a.s. in formulation: 200.7 g pyraclostrobin /L
Test species:	rat, strain crl:WI (Han), female rats, age: 69-71 days at start of administration period; source: Charles River Laboratories, Research Models and Services GmbH, Germany.

B. STUDY DESIGN

Test design:	Due to a transmission and calculation error the dietary concentrations applied in a first study part were too high (20859 ppm, 8773 ppm and 1883 ppm of BAS 500 06 F). Hence a second study part was conducted using the correct dietary concentrations in the diet (1495 ppm, 648 ppm and 149 ppm of BAS 500 06 F). Young female rats received different concentrations of the test substance offered in the diet on 5 consecutive days with a treatment-free post-exposure period of 9 days; 5 rats per test substance concentration and for the two control groups were used. Feeding time was restricted to 2 feeding periods of 2 hours in order to reflect the feeding behavior of wild animals and to ensure high feeding rates. Control group 5 with 5 animals was fed ad libitum, control group 6 was fed during restricted periods. Assessment for mortality and clinical signs was carried out two times a day on working days (once a day on Saturdays, Sundays and public holidays) of the entire study period. Assessment of body weight was carried out once a day on days -8, -2, 0 to 5, 7, 9, 11,13, and 14; food consumption was determined daily from study days -7 to 4 and 6 to 13. Individual food consumption was determined daily and calculated as daily mean food consumption (g) per animal and day. On study day 0, the food consumption was in addition determined separately for each feeding interval after 20, 45, 70, 95, and 120 minutes for the dose groups as well as the control to estimate in reference to the controls the avoidance response time. Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume. Gross-pathological post-mortem examinations of all surviving specimens were carried out at the end of the study.
Endpoints:	Mortality, clinical signs, feed consumption, water consumption, body weight (b.w.). Gross-pathological examinations were conducted on rats sacrificed at the definitive termination of the test.
Test concentrations:	Study part 1: Dietary concentrations of 0 (Control ad libitum and restricted), 1883, 8773, and 20859 ppm BAS 500 06 F. Study part 2: Dietary concentrations of 0 (Control ad libitum and restricted), 149, 648, and 1495 ppm BAS 500 06 F. The corresponding actual mean daily doses are 0 (Controls), 10.4, 44.4 and 96.8 mg BAS 500 06 F/kg b.w./day.
Test conditions:	Temperature: 20°C - 24°C, relative humidity: 30% - 70%; with only minimal deviations from these limits; the day/night cycle was an inverted 12 hour light cycle photoperiod: 12 hours light: 12 hours dark.
Statistics:	Descriptive statistics

II. RESULTS AND DISCUSSION

Analytical measurements: The results of the analytical verification of the test substance concentration in the diet were within a range of 87.66% to 98.1% of the nominal concentrations during the test. The biological results are therefore based on the nominal values.

Biological results: The test substance was administered in study part 1 at dietary concentrations of 0 (Control ad libitum and restricted), 1883, 8773, and 20859 ppm BAS 500 06 F and in study part 2 at dietary concentrations of 0 (Control ad libitum and restricted), 149, 648 and 1495 ppm BAS 500 06 F.
The experimental design was overall successful at training the rats to ingest the full daily intake during the only four hours (two periods of 2 hours each) where food was available. Detailed investigations of the feeding pattern at study day 0 revealed that the food was not ingested evenly throughout each of the 2-hour feeding intervals, but was largely ingested during the first about 20 minutes. Hence the experimental design is suitable to ensure high feeding rates of the test animals concentrated during two brief (<20 minutes) daily periods.
Dose-dependent reductions in food consumption were observed in all treated test groups during the administration period. This effect was caused by the clear avoidance of food treated with BAS 500 06 F containing the active ingredient pyraclostrobin by the laboratory rats. Impaired body weight data were obtained during administration period in all treated test groups. This finding was assessed as a consequence of the reduced food consumption rather than an incipient sign of general systemic toxicity. Marked reductions in food consumption [up to 96-99%] were observed in the highest treatment group (test group 4). This test group was terminated on treatment day 2 because of reduced nutritial condition of animals due to the very strong food avoidance. No test substance related mortality was observed in any test group throughout the entire study period. No severe clinical signs of toxicity related to the test substance were observed in any of the treatment groups. During the post-administration period a full recovery of food consumption and body weights was observed. All rats were examined macroscopically. No substance-related abnormalities were detected in rats after sacrifice. The results are presented in the tables below.

Table 8.1.2.1-2: Mammalian dietary toxicity of BAS 500 06 F to rats – Study part 1

Dose Group [ppm BAS 500 06 F in diet]	**Control 0** (feeding ad libitum)	**Control 1** (restricted feeding)	**1883**	**8773**	**20859**
Mean test substance intake rate (BAS 500 06 F mg/kg b.w./day)	--	--	119.6	241.9	67.5
Corresponding daily dose of active substance [mg /kg b.w./day]	--	--	24.0	48.6	13.5
Mortality [%]	0	0	0	0	n.a.[3)]
Food consumption	--	--	Reduced[1)]	Reduced[2)]	Reduced[4)]
Clinical signs	None	None	None	None	Yes[5)]

a.s. = active substance
b.w. = body weight
n.a. = not applicable
1) Reduced food consumption on study day 0 to 4.
2) Reduced food consumption on study day 0 to 4
3) Test group terminated at treatment day 2 because of reduced nutritial condition of animals due to very strong food avoidance.
4) Markedly (96-99%) reduced food consumption on study day 0 to 2.
5) Reduced nutritional condition and piloerection on study day 2.

Table 8.1.2.1-3: Mammalian dietary toxicity of BAS 500 06 F to rats – Study part 2

Dose Group [ppm BAS 500 06 F in diet]	**Control 5** (feeding ad libitum)	**Control 6** (restricted feeding)	**149**	**648**	**1495**
Mean test substance intake rate (BAS 500 06 F mg/kg b.w./day)	--	--	10.4	44.4	96.8
Corresponding daily dose of active substance [mg /kg b.w./day]	--	--	2.1	8.9	19.4
Mortality [%]	0	0	0	0	0
Food consumption	--	--	--	Slightly reduced[1)]	Reduced[2)]
Clinical signs	None	None	None	None	None

a.s. = active substance
b.w. = body weight
1) Reduced food consumption on study day 0
2) Reduced food consumption on study day 0 to 1

III. CONCLUSION

Dose-dependent reductions in food consumption were observed in all treated test groups during the administration period. Reductions in food consumption and body weight loss were clearly related to avoidance of treated food. No test substance-related mortality or severe sublethal effects were observed.

Please note that the following studies CA 8.1.2.1/3 and CA 8.1.2.1/4 were erroneously listed as CP 10.1.2.2/2 and CP 10.1.2.2/5 in the Application submitted in January 2014 for the renewal of approval of pyraclostrobin.

Report:	CA 8.1.2.1/3 [redacted] 2014a BAS 500 06 F: Study to assess avoidance and effects in wood mice (Apodemus sylvaticus) upon dietary exposure 2012/1129348
Guidelines:	<none>
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive summary

Study objective
A 3-day dietary toxicity test was conducted with the wood mouse (*Apodemus sylvaticus*) to determine toxicity in a risk assessment-relevant wild mammal species after oral dietary administration of the formulation BAS 500 06 F under field relevant conditions (i.e. rapid consumption of a limited resource via the dietary route, food available for brief daily intervals, not continuously).

Standard acute tests using oral gavage as dosing method do not reflect the real dietary pattern of exposure of wild mammals in the field following foliar spray applications. Hence the purpose of the study was to determine potential effects of BAS 500 06 F on wild mammals using exposure routes and dosing regimes better suitable for a more realistic assessment of the risk to wild mammals.

The study was deemed necessary as uncertainties were identified regarding a save use of the formulation BAS 500 06 F in the risk assessment for small mammals. The wood mouse was chosen as test organism, being a species representative of the small mammals likely exposed to the test item under normal use. The employed study design (i.e. length of the deprivation period, recovery times and lightning regime) was chosen to ensure the most stringent design while simultaneously considering animal welfare by alternating between exposure to treated food and exposure to non-treated food.

Besides the evaluation of potential effects of dietary exposure to BAS 500 06 F with respect to mortality, sublethal effects, feed consumption, and body weight, the objective of this study was to determine 1) the highest daily dietary ingestion rate of the test item that could be tolerated by a relevant wild mammal species), and 2) if animals show avoidance behavior in relation to the concentration of the test item. In this context an attempt was made to estimate the time of onset of avoidance using video recording.

Study design
The experiment was split into a pre-exposure period of 11 days, followed by an exposure period of 5 days (i.e. 3 exposure days alternating with 2 non-exposure days), and a treatment-free post-exposure period of 9 days.

During the exposure period (day 0 to day 4) BAS 500 06 F was administered over 3 exposure days (day 0, 2 and 4) via diet blocks to 3 treatment groups with 6 animals (3 males, 3 females) each. Within the exposure period animals received non-treated food on day 1 and 3. The test concentrations (i.e. 150, 1500 and 3000 mg BAS 500 06 F/kg diet; corresponding to 28.93, 289.30 and 578.60 mg a.s. pyraclostrobin/kg diet) were chosen to cover field-relevant dietary concentrations of pyraclostrobin (BAS 500 F) and a factor of 10 beyond to cover for interspecies variation. Treated diet blocks were prepared by first dissolving the test item in water and then adding the ground diet to ensure homogenous mixing prior to combining with untreated binding agent. A control group (3 males, 3 females) was offered untreated diet blocks. For ethical reasons, each of the three exposure days was followed by a non-exposure day with *ad libitum* access to untreated diet for recovery. In order to reflect the feeding behavior of animals in the wild and to ensure high feeding rates, the feeding on exposure days was restricted to 2 feeding periods of 4 hours separated by 2 h of non-exposure.

Mice were observed at least daily for mortality, signs of clinical toxicity, impact on food consumption and body weight during the exposure period and a treatment-free post-exposure period. Individual food consumption of animals was determined daily.

Results and conclusion
No mortalities, symptoms of poisoning (e.g. during pathological observations), and abnormal behavior were observed in any test group.

Dose-dependent reductions in food consumption were observed in all treated test groups during the administration period. The reduction in intake of treated diet blocks was significant at the 1500 and 3000 mg/Kg by wood mice compared to the control. The maximum dose of BAS 500 06 F consumed by mice during 8 hours of daily exposure was approximately 243 ± 25 mg formulation/ kg b.w./day, which is a factor of 2.2 less than the predicted total daily intake of 525.5 ± 25 mg formulation/ kg b.w./d recorded during the pre-exposure period on day -2.

Incorporation of BAS 500 06 F into diet blocks reduced food consumption of mice in the 150, 1500 and 3000 mg BAS 500 06 F/kg treatment groups by up to 40%, 59%, and 75% respectively, when compared to the control. This effect was consistent across the 3-days of exposure. This reduction in feeding on BAS 500 06 F treated blocks leads to clearly lower actual exposure levels as compared to predicted exposure levels based on pre-exposure consumption data.

Bodyweight loss on day 0 (i.e. first exposure day) and day 4 (i.e. last exposure day) was observed as a result of the experimental procedure similarly across the three treatment groups and the control.

During the treatment-free post-exposure period a full recovery of food consumption and body weight was observed, i.e. body weight was seen to exceed baseline weights (weights from day -2) on day 7 and day 9 during post-exposure.

In conclusion, there were no apparent significant adverse clinical or behavioral effects in wood mice exposed to dietary concentrations up to and including the highest test dose of 3000 mg BAS 500 06 F/kg diet.

Please note: the following detailed study summary presents data as given in the FERA report (BASF DocID 2012/1129348), as well as additional calculations. These additional calculations are fully based on the data presented in the FERA report, and are marked in the tables with a foot note. The calculations are included in the study summary in order to facilitate the comparison of the results from the present study with results from other acute toxicity studies with pyraclostrobin and BAS 500 06 F that are presented elsewhere.

I. MATERIAL AND METHODS

Test species: Wood mouse (*Apodemus sylvaticus*), age: over 6 weeks old; source: supplied from a FERA (Sand Hutton, York, YO41 1LZ, UK) breeding colony. Wood mice were housed individually throughout the study period

Test item: BAS 500 06 F, batch no. 0004863761, purity: content of a.s. in formulation: 201.0 g pyraclostrobin /L.

Test concentrations: Dietary concentrations of nominal 0 (control), 150, 1500 and 3000 mg BAS 500 06 F/kg diet (corresponding to analysed 0 (control), 33.04, 288.16 and 555.14 mg pyraclostrobin/kg diet).

Preparation of diet: Diet blocks of approx 10-15 g each were prepared from ground diet (Labdiet Certified Rodent Diet 5002) and egg white. The blocks were prepared to provide the test item incorporated at the required range of concentrations: 150, 1500 and 3000 mg BAS 500 06 F/kg diet.

Test design: 3 treatment groups and 1 control group with 6 animals (3 males and 3 females) per group were tested. Two replicates (one replicate = one mouse) of each treatment group and the control group were assessed on each test day. Note that, as the study did not need to be continuous, all mice began the acclimatization phase at the same time and continued up to day -2. Only two replicates (two mice) from each dose level were tested at a time and those mice not undergoing treatment remained on acclimatization conditions.

Pre-exposure period (-11 to -1): Wood mice were acclimatized to feeding on untreated diet blocks with two restricted 4 h feeding periods per day on 3 days (day -10 to day -6). To maximize their feeding rate wood mice were deprived of food for 12 h before the 1st 4 h feeding period, and between the 1st and 2nd feeding period. Wood mice that readily consumed untreated diet blocks were selected for the experiment. Normal intake rate was determined based on food consumption data for the untreated diet blocks. Based on the data gained over the pre-treatment period mice were randomly assigned to treatments on day -2.

Exposure period (day 0 to 4): Following a 12h period of food deprivation, animals were exposed on day 0 to BAS 500 06 F treated diet blocks for two 4 h periods separated by 2 hours food deprivation, and then access to untreated food for 6 hours for the remainder of the test day. To avoid disturbance, the intake of blocks was monitored remotely using video-recording of a balance reading and animal behaviour, where possible, simultaneously to determine behaviour during the 4 h exposure periods. The exposure day was then followed by a day of recovery (day 1) with ad libitum access to untreated food. This design (one day exposure, one day recovery) was repeated twice, but without video recording, so that each mouse had a total of three days of exposure, to reproduce the possible duration of exposure in the field and estimate the rate of food consumption.

Post-exposure period (day 5 to 9): The last exposure period on day 4 was followed by a recovery period where normal diet was available *ad libitum* for five days.

	Observations: Mice were observed at least daily during the test (when food was changed) until day 9. Assessment of weight of the untreated blocks at the beginning and end of exposure periods on days –10, -8, -6 and –2 in the pre-treatment period before the start of the test. The weight of test diet blocks (i.e. treated and untreated) at the beginning and end of treatment periods on the test day and on each of the 2 follow-on exposure days were assessed as well as the weight of normal ground diet consumed on Day 7 and 9 of the recovery period. The differences in weight of the food blocks at the beginning and end of relevant exposure and treatment periods, respectively, served as basis for the estimates of food consumption. Body weight of the mice was assessed at the beginning of the study (day –14), during the pre-trial period (day -7), before the test day (day -2), end of the test day (day 0), at the end of the follow-on period (day 4) and at the end of days 7 and day 9 during the recovery period.
Endpoints:	Mortality, feed consumption, body weight (b.w.).
Test conditions:	Room temperature was maintained at 16-20°C. Reversed daylight was used such that night corresponded to red light on from 09:00 PM to 01:00 AM (deprivation period starting on days before exposure, i,e. day -1, day 1 and day 3, respectively), dusk periods corresponded to low light on from 08:30 AM to 09:00 AM and 01:00 AM to 01:30 AM and the day period corresponded to main lights on from 01:30 AM to 08:30 AM (on exposure day 0, 2 and 4, respectively, with no food available). The change in light levels was monitored for each designated period at the beginning and end of the experimental phase.
Analytics:	The test substance concentrations in the diet blocks were analyzed using HPLC.
Statistics:	Descriptive statistic, in addition for each endpoint, food consumption (g), bodyweight (g) and time taken to start feeding on Day 0 (minutes) a repeated measures analysis was performed using a mixed-models approach to the analysis of covariance (ANCOVA).

II. RESULTS AND DISCUSSION

Analytical measurements

The results of the analytical verification of the concentration of the active substance pyraclostrobin in the diet were within a range of 89.2 to 106.2% of the nominal concentrations during the test (see Table 8.1.2.1-4). The biological results are based on the analytical values for the active substance.

Table 8.1.2.1-4: Summary of nominal and analysed concentrations of BAS 500 06 F and pyraclostrobin in test items

Nominal concentration of the formulation	Mean concentration of the formulation (n=3)	Nominal concentration of pyraclostrobin	Measured mean concentration of pyraclostrobin (n=3)	Verification of nominal concentration values
[mg BAS 500 06 F/kg]	[mg BAS 500 06 F/kg]	[mg a.s./kg]	[mg a.s./kg]	[%]
150[1)]	170[2)]	31.11[3)]	33.04[4)]	106.2[5)]
1500[1)]	1487[2)]	311.10[3)]	288.16[4)]	92.6[5)]
3000[1)]	2864[2)]	622.20[3)]	555.14[4)]	89.2[5)]

1) Values from Table S3 of the FERA report.
2) Values from Table S3 of the FERA report. Mean concentration of mg BAS 500 06 F/kg calculated based on 201.0 g/L and 1.037g/cm3 as stated in the foot note of table S3 of the FERA report.
3) Values specifically calculated for this study summary based on a density of 1.037g/cm^3 for the formulation and nominal 200 g a.s./L.
4) Values specifically calculated for this study summary from results as presented in Table S3 of the FERA report as mean value from three samples A, B and C for the respective test group concentration.
5) Values specifically calculated for this study summary by comparing nominal concentration values of the a.s. with the analytical values for the a.s.

Biological results

Mice were observed at least daily during the test (when food was changed) until day 9. There were no abnormal behaviour, mortalities and symptoms of poisoning (e.g. pathological observations in mortalities during the treatment period).

1. Food consumption

Incorporation of BAS 500 06 F in the diet lead to reduced mean food consumption by the wood mice. This effect did not change over the 3 days of exposure (day 0, day 2 and day 4). The mean food consumption was reduced up to 40%, 59%, and 75% compared with controls in the 150, 1500 and 3000 mg BAS 500 06 F/kg diet dose group, respectively. The mean food consumption on each of the exposure days (day 0, day 2 and day 4) and food consumption compared with controls (in percent) is shown in Table 8.1.2.1-5. Baseline consumption was defined as the amount eaten on day -2. The control group did show a slight increase in baseline consumption. The difference between control and treated groups was statistically significant for the 1500 mg BAS 500 06 F/kg ($p=0.009$) and 3000 mg BAS 500 06 F/kg ($p\leq0.0001$) treatment groups (Quantics Report_20140101, p. 10) however there was no significant difference between control and 150 mg BAS 500 06 F/kg treatment group.

Table 8.1.2.1-5: Mean food consumption across treatment groups (standard errors are in brackets)

Test group		Control	150 mg BAS 500 06 F/kg	1500 mg BAS 500 06 F/kg	3000 mg BAS 500 06 F/kg
Day 0	Consumption [g][3)]	2.94 (±0.2)	2.30 (±0.1)	1.74 (±0.2)	1.29 (±0.1)
	Compared with control [%][3)]	100	78.2	59.0	43.9
Day 2	Consumption [g][3)]	3.19 (±0.3)	2.40 (±0.2)	2.01 (±0.2)	1.54 (±0.2)
	Compared with control [%][3)]	100	75.0	63.3	48.3
Day 4	Consumption [g][3)]	3.16 (±0.3)	2.92 (±0.4)	1.98 (±0.3)	1.27 (±0.1)
	Compared with control [%][3)]	100	92.4	62.6	40.1

1) Based on a density of 1.037g/cm^3 for the formulation and nominal 200 g a.s./L.
2) Calculated from results as presented in Table S3 (FERA Report) as mean value from three samples A, B and C for the respective test group concentration.
3) Data from Table 4 of the FERA Report

In conclusion, this reduction in feeding on BAS 500 06 F treated blocks leads to clearly lower actual exposure levels as compared to the exposure levels predicted on pre-exposure consumption data, and could in turn be seen as a mechanism to reduce risk.

2. Intake of formulation and pyraclostrobin

While the mean consumption of diet treated with BAS 500 06 F by wood mice was reduced compared to controls due to lower initial consumption, the overall feeding pattern was similar between treatment and control groups.

Mean ingestion of BAS 500 06 F and pyraclostrobin over the two 4 h periods on each exposure day, as well as the highest intake within a 4 h period on each day, are shown below (Table 3 and 4). The estimated potential intake (predicted dose) for BAS 500 06 F based on the pre-exposure feeding rate for the same mice on day -2 is as well given for comparison.

The actually ingested mean daily dose over the treatment days is 19.0, 148.8, and 216.4 mg BAS 500 06 F/kg b.w./day for the treatment groups of 150, 1500, and 3000 mg formulation/kg diet, respectively (Table 8.1.2.1-6).

The actually ingested mean daily dose for pyraclostrobin over the treatment days is 3.7, 28.8, and 41.9 mg a.s./kg b.w./day for the treatment groups of 150, 1500, and 3000 mg formulation/kg diet, respectively (Table 8.1.2.1-6).

In comparison, the predicted mean daily dose over the treatment days was 4.4 mg a.s./kg b.w. (equivalent to 22.5 mg BAS 500 06 F/kg b.w.) for the 150 mg formulation/kg diet treatment group, 48.0 mg a.s./kg b.w. (equivalent to 247.4 mg BAS 500 06 F/kg b.w.) for 1500 mg formulation/kg diet treatment group and 101.9 mg a.s./kg b.w. (equivalent to 525.6 mg formulation/kg b.w.) for 3000 mg formulation/kg diet treatment group (see Table 8.1.2.1-6).

Table 8.1.2.1-6: Mean total daily intake of BAS 500 06 F and pyraclostrobin over two 4-hour periods in treatments and control on each treatment day (standard errors are in brackets)

Test group [Nominal concen-tration mg BAS 500 0 6 F/kg diet]	**Day 0**			**Day 2**			**Day 4**						
	g diet[1)]	**mg BAS 500 06 F/kg b.w.[1)]**	**mg pyraclo strobin/ kg b.w.[2)]**	**g diet [1)]**	**mg BAS 500 0 6 F/kg b.w.[1)]**	**mg pyraclo strobin/ kg b.w.[2)]**	**g diet[1)]**	**mg BAS 500 06 F/kg b.w.[1)]**	**mg pyraclo strobin/ kg b.w.[2)]**	**Actually ingested mean daily dose of a.s. pyraclo strobin** [mg a.s./kg b.w./day]	**Predicted total daily intake based on day -2 intake rate** [mg pyraclo strobin/kg b.w.][2)]	**Actually ingested mean daily dose of BAS 500 06 F** [mg BAS 500 06 F/kg b.w.]	**Predicted total daily intake based on day -2 intake rate** [mg BAS 500 06 F/kg b.w.][1)]
0	2.94 (±0.23)	0	0	3.19 (±0.26)	0	0	3.16 (±0.30)	0	0	0	0	0	0
150	2.30 (±0.15)	17.6 (±2.6)	3.4	2.40 (±0.22)	18.3 (±2.9)	3.5	2.92 (±0.37)	21.2 (±2.0)	4.1	3.7[3)]	4.4	19.0[3)]	22.5 (±3.0)
1500	1.73 (±0.18)	132.9 (±11.0)	25.8	2.02 (±0.18)	160.4 (±18.6)	31.1	1.98 (±0.28)	153.2 (±22.0)	29.7	28.8[3)]	48.0	148.8[3)]	247.4 (±20.6)
3000	1.29 (±0.12)	204.1 (±21.3)	39.6	1.54 (±0.16)	243.1 (±25.2)	47.1	1.27 (±0.12)	202.0 (±20.4)	39.2	41.9[3)]	101.9	216.4[3)]	525.5 (±43.0)

1) Values for mean total daily intake from Table 2 of the FERA report.
2) Values for mean total daily intake of pyraclostrobin specifically calculated for this study summary from results *for* mg BAS 500 06 F/kg b.w. as presented in Table 2 of the FERA report considering 201 g a.s./L (analysed) and a density of 1.037g/cm^3.
3) Mean value (n=3, from day 0, day 2 and day 4).

The maximum mean intake of pyraclostrobin in any single 4-hour period in the 150 mg BAS 500 06 F/kg diet treatment group was 2.2 mg a.s./kg b.w. (equivalent to 11.1 mg BAS 500 06 F/kg b.w.) on day 2, in the 1500 mg BAS 500 06 F/kg diet treatment group was 16.0 mg a.s./kg b.w. (equivalent to 82.4 mg BAS 500 06 F/kg b.w.) on day 0 and in the 3000 mg BAS 500 06 F/kg diet treatment group was 23.9 mg a.s./kg b.w. (equivalent to 123.5 mg BAS 500 06 F/kg b.w.) on day 0 (see Table 8.1.2.1-7).

Table 8.1.2.1-7: Maximum mean intake of BAS 500 06 F and pyraclostrobin in any single 4-hour period in the treatment groups (standard errors are in brackets)

Test group [Nominal test item concentration [mg BAS 500 06 F/Kg diet]	**Day 0**		**Day 2**		**Day 4**		**Predicted dose** (Estimated 4-hour intake based on maximum day -2[1)] 4-hour intake rate)	
	[mg BAS 500 06 F/kg b.w.][1)]	**[mg pyraclostrobin/kg b.w.][2)]**	**[mg BAS 500 06 F/kg b.w.][1)]**	**[mg pyraclostrobin/kg b.w.][2)]**	**[mg BAS 500 06 F/kg b.w.][1)]**	**[mg pyraclostrobin/kg b.w.][2)]**	**[mg BAS 500 06 F/kg b.w.][1)]**	**[mg pyraclostrobin/kg b.w.][2)]**
150	10.2 (±1.88)	2.0	11.1 (±1.7)	2.2	10.7 (±1.6)	2.1	13.0 (±1.7)	2.5
1500	82.4 (±4.7)	16.0	78.4 (±15.1)	15.2	73.6 (±21)	14.3	154.6 (±22.7)	30.0
3000	123.5 (±15.5)	23.9	122.4 (±21.5)	23.7	99.2 (±13.8)	19.2	307.3 (±37.5)	59.6

1) Values for maximum mean intake from Table 3 of the FERA report.
2) Values for maximum mean intake of pyraclostrobin specifically calculated for this study summary from results for mg BAS 500 06 F/kg b.w. as presented in Table 3 of the FERA report considering 201 g a.s./L (analysed) and a density of 1.037g/cm^3.

The actual ingested dose per day compared with the predicted dose was a maximum of 39% on day 0, 46% on day 2 and 38% on day 4 in the 3000 mg/kg diet treatment, 54% on day 0, 65% on day 2 and 62% on day 4 in the 1500 mg/kg diet treatment and 78% on day 0, 81% on day 2 and 94% on day 3 in the 150 mg/kg diet treatment (see Table 8.1.2.1-8).

Table 8.1.2.1-8: Proportion of actual exposure compared with predicted exposure for BAS 500 06 F[1)]

Test group [Nominal test item concentration]	**150 [mg BAS 500 06 F/kg]**	**1500 [mg BAS 500 06 F/kg]**	**3000 [mg BAS 500 06 F/kg]**
Exposure day			
Day 0	78%	54%	39%
Day 2	81%	65%	46%
Day 4	94%	62%	38%

1) Values from Table 1 of the FERA report

3. Post-trial recovery of diet intake

During the post-administration period a full recovery of food consumption was observed. Mean food consumption values during the recovery period (day 7 and day 9) are presented in Table 8.1.2.1-9 together with consumption values for day -2 (pre-exposure) and exposure days 0, 2 and 4. Consumption rates on day 7 and day 9 (post-exposure) were based on normal (ground) diet ad libitum for a 24h period. In contrast, consumption rates for pre-exposure (day -2) and exposure period (day -2, 0, 2 and 4) are based on restricted feeding for 8 hours (two-times of 4 h) within 24 h on a diet block.
Statistical analysis could not be performed on the recovery data as there was an incomplete data set for Day 9 data (Quantics Report_20140101, p. 7).

Table 8.1.2.1-9: Mean total daily diet intake of woodmice during the study from day -2 to day 9

Test group [Nominal test item concentration [mg BAS 500 06 F/kg diet]]	**Day -2[1)] [g diet]**	**Day 0[1)] [g diet]**	**Day 2[1)] [g diet]**	**Day 4[1)] [g diet]**	**Day 7[2)] [g diet]**	**Day 9[2)] [g diet]**
0	2.87	2.94	3.19	3.16	5.10	5.93[3)]
150	2.96	2.30	2.40	2.92	5.84	8.24[4)]
1500	3.27	1.73	2.02	1.98	4.87	4.39[3)]
3000	3.35	1.29	1.54	1.27	4.39	4.67[3)]

1) Mean total daily intake over two 4-hour periods. Data from Table 2 of the FERA Report.
2) Total consumption in a 24-hour period. Data from Table S1 of the FERA Report.
3) Based on data from only four individual wood mice (n=4). All other data are based on n=6 (see Quantics Report_20140101, p. 17, Appendix I)
4) Based on data from only three individual wood mice (n=3). All other data are based on n=6. (see Quantics Report_20140101, p. 17, Appendix I)

There was no evidence of any sustaining long-term effect of the treatment on the food consumption of the mice during the recovery period.

4. Body weight data

Bodyweight at day -2 was defined as the baseline weight for each woodmouse. Initial body weight on day -2 was found to be a significant factor (p = 0.01) in the longer term fluctuations in body weight and was therefore used as a covariate in all analysis (please refer to Table 6, Table 7 and Figure 4 of Quantics Report_20140101). There was no indication of a significant treatment (p=0.9044) or time by treatment effect (p=0.1933). Across all treatment groups and the control, a significant change in body weight with time (p < 0.0001) could be observed (Quantics Report_20140101, Table 6).

Body weight dropped below baseline levels on day 0 and rose slightly but remained below baseline on day 4. During recovery, when animals had unrestricted access to normal diet, weights peaked above baseline levels on day 7 and stayed around baseline levels on day 9. Please refer to Table 8.1.2.1-10 for mean bodyweight data. Woodmice appear to have over compensated for the initial weight loss and then stabilised. There was no significant difference between treatment groups (Quantics Report_20140101, Table 6) and although body weight does change with time the pattern of change is similar across treatment groups. This suggests that the drop in body weight seen on day 0 and day 4 is a result of the experimental procedure and not a result of exposure to BAS 500 06 F.

Table 8.1.2.1-10: Mean body weight of woodmice during the study from day -2 to day 9[1)]

Nominal test item concentration [mg BAS 500 06 F/kg diet]	Day -2 [g]	Day 0 [g]	Day 4 [g]	Day 7 [g]	Day 9 [g]
0	20.2	18.9	19.5	20.9	20.3
150	20.8	19.9	20.1	21.0	20.2
1500	19.7	18.7	18.9	19.7	19.4
3000	19.3	18.0	18.5	19.8	19.4

1) Values from Table E Appendix 6 of the FERA report.

In conclusion, bodyweight loss was observed during the treatment period (day 0 and 4) was attributed to the experimental procedure and are not considered as a result of exposure to BAS 500 06 F. The pattern of weight change was similar over time across treatment groups and control. There was no indication of any significant treatment or time by treatment effect.

During the post-administration period a recovery of body weights was observed. Body weight reached baseline weights (weights from day -2) on day 7 and day 9, indicating any sustaining long-term effect of the treatment on the body weight of the mice.

5. Time of feeding

There was no impact on the time to start feeding as assessed during the morning and afternoon on day 0, with respect to either treatment or time. Overall, a similar pattern of intake was present in the treatment groups and the control, but initial food consumption was lower in the treatments.

Feeding rate for the control animals peaked in the first 30 min of the first 4 h exposure period (9.0 g diet/kg b.w./15 min) and in 15 min of the second exposure period (20.9 g diet/kg b.w./15 min). Using these figures to predict exposure levels, the highest dose rate (3000 mg formulation/kg diet) would receive the equivalent of 27.1 mg formulation/kg b.w./15 min and 62.7 mg formulation/kg b.w./15 min, respectively. This was much higher than was actually seen, as the 3000 mg formulation/kg diet group received a maximum of 10.5 ± 3.9 mg formulation/kg b.w./15 min in the first and 9.3 ± 6.8 mg formulation/kg b.w./15 min in the second exposure. Therefore, predicting exposure levels from untreated consumption data would over estimate exposure levels.

III. CONCLUSION

There were no apparent significant adverse effects either clinical or behavioural from exposure of wood mice with dietary concentrations up to 3000 mg BAS 500 06 F/kg diet. No mortalities or symptoms of poisoning (e.g. pathological observations in mortalities during the treatment period) were observed.

Dose-dependent reductions in food consumption were observed in all treated test groups consistently during the exposure period.

This reduction in feeding on BAS 500 06 F treated blocks leads to clearly lower actual exposure levels as compared to the exposure levels predicted on pre-exposure consumption data. i.e. the maximum dose of BAS 500 06 F consumed by mice during 8 hours of daily exposure was approximately 243 ± 25 mg formulation/ kg b.w./d, which is a factor of 2.2 less than the predicted total daily intake of 525.5 ± 25 mg formulation/ kg b.w./d recorded during the pre-exposure period on day -2.

Bodyweight loss on day 0 and day 4 was observed as a result of the experimental procedure similarly across treatment groups and control.

During the post-exposure period (day 7 and 9) a full recovery of food consumption and body weights was observed, i.e. body weight was seen to exceed baseline weights on day 7 and day 9 during post-administration.

Report: CA 8.1.2.1/4
Mastitsky S. et al., 2014a
A body burden model to assess the acute dietary risk posed by formulated Pyraclostrobin (BAS 500 06 F) to the common vole, Microtus arvalis
2014/1001603
Guidelines: <none>
GLP: no

Executive Summary

A Tier 1 ecotoxicological risk assessment of the fungicidal product BAS 500 06 F (active substance pyraclostrobin) identified an uncertainty with respect to the acute risk to small herbivorous mammals. This study presents a refined risk assessment based on the body burden modeling approach, one of the higher tier options offered by the EFSA (2009) guidance document on risk assessment for birds and mammals. A body burden model is a mechanistic model that describes the internal content of a chemical in the body over time based on the knowledge about the toxicokinetic properties of that chemical and the foraging behavior of the species of interest. Considering the application timing of BAS 500 06 F in cereals (BBCH ≥ 40), the common vole is recommended by the EFSA guidance as a representative species of the guild of small herbivorous mammals. Thus, the body burden model presented herein focuses on the common vole and the active substance pyraclostrobin applied as a formulated product BAS 500 06 F in cereals.
The model developed in this study was a stochastic simulation model that predicted the internal content of pyraclostrobin in 1000 common voles, which started feeding on the contaminated diet composed of grass and cereal shoots immediately after application of the fungicide. The simulated period lasted for 48 hours. Due to the imposed heterogeneity with respect to their body weight, biokinetic parameters and foraging schedules, the voles demonstrated individual-specific temporal profiles of the internal content of pyraclostrobin, allowing for a quantitative probabilistic assessment of the acute risk. The risk was calculated as the percentage of individuals whose body burden exceeds a certain toxicological threshold. The employed toxicological threshold of 9.6 mg a.s./kg b.w. was derived from an acute oral rat laboratory study with the product BAS 500 06 F (BASF DocID 2007/1053390) by dividing the respective LD_{50} by a safety factor of 10. The latter safety factor was applied in order to cover the uncertainty associated with the interspecies extrapolation.
The selected toxicological threshold was not exceeded by any individual in any of the 2000 model runs (two-dimensional Monte Carlo simulations). This result suggests that the common voles feeding on grass and cereal shoots in a cereal field during 48 hours after treatment with 1.25 L/ha of BAS 500 06 F (corresponding to the rate of 250 g pyraclostrobin per ha) are highly unlikely to experience acute effects. Due to the high level of realism implemented in the foraging submodel, as well direct incorporation of the uncertainties about input model parameters into the calculations, the presented risk estimate can be characterized as robust.

I. MATERIAL AND METHODS

The body burden model presented herein is a stochastic simulation model allowing for conducting a probabilistic risk assessment. The model developed in this study was a stochastic simulation model that predicted the internal content of pyraclostrobin in 1000 common voles, which started feeding on the contaminated diet composed of grass and cereal shoots immediately after application of the fungicide. The simulated period lasted for 48 hours. Due to the imposed heterogeneity with respect to their body weight, biokinetic parameters and foraging schedules, the voles demonstrated individual-specific temporal profiles of the internal content of pyraclostrobin, allowing for a quantitative probabilistic assessment of the acute risk. The risk was calculated as the percentage of individuals whose body burden exceeds a certain toxicological threshold. The employed toxicological threshold of 9.6 mg a.s./kg b.w. was derived from an acute oral rat laboratory study with the product BAS 500 06 F (BASF DocID 2007/1053390, for detailed study summary please refer to chapter M-CP 7.1.1.1) by dividing the respective LD_{50} by a safety factor of 10. The latter safety factor was applied in order to cover the uncertainty associated with the interspecies extrapolation.

II. RESULTS AND DISCUSSION

Probabilistic assessment of the risk
Results show that this threshold was not exceeded in any of the simulated individuals, suggesting that the common voles feeding on a diet composed of grass and cereal shoots in a cereal field during 48 hours after foliar spray with BAS 500 06 F according to the GAP are highly unlikely to experience acute effects.

Sensitivity analysis
The conducted sensitivity analysis showed that most of the input model parameters had a very low or no influence on the calculated endpoints, suggesting that one would obtain stable risk estimates irrespective of the variation of these parameters within the specified ranges. The only (expected) exception was the biokinetic parameter, which is related to the elimination half-life of pyraclostrobin.

Evaluation of the non-modeled uncertainties
As explained in sections 3.2 Uncertainty and variability in the model inputs and 3.3 Two-dimensional Monte Carlo simulation of the study report, the uncertainty about model inputs was explicitly incorporated into the estimate of the acute risk posed by pyraclostrobin to the common vole. However, there were other, non-modeled, sources of uncertainty, which could potentially influence the estimate of risk. Sources deemed particularly important are summarized in Table 5 of the study report.

Validation of the model predictions
Comparing the model outputs with the available independent data suggests that the body burden model presented herein provides realistic predictions with regard to the acute risk posed by pyraclostrobin to the common voles feeding on the BAS 500 06 F-contaminated food.

III. CONCLUSION

This study aimed at conducting a probabilistic assessment of the acute risk posed by the active substance pyraclostrobin (applied as the fungicidal product BAS 500 06 F in cereal fields) to the common vole, a relevant focal species according to EFSA (2009). The assessment conducted using a specially developed body burden model showed that, when applied according to GAP, BAS 500 06 F is highly unlikely to cause acute effects in common voles feeding on the contaminated food for 48 hours after application. Due to a high level of realism with respect to the foraging behavior of common voles implemented in the model, as well as the direct incorporation of uncertainties about input model parameters into the calculations, the presented risk estimate can be characterized as robust. This assessment was conducted using the best data available.

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

Long-term and reproductive toxicity to mammals is discussed in M-CA 5.6. The studies previously evaluated during the Annex I inclusion process are considered to be still valid and sufficient. Therefore, no new studies have been conducted with the active substance.

The following studies are no toxicity studies with mammals. However, these studies are summarised here as they provide data from new studies relevant for the mammalian long-term/reproductive risk assessments with the representative formulation BAS 500 06 F. The field study BASF DocID 2014/1000041 addresses acute and long-term effects of BAS 500 06 F on populations of small mammals. As only low numbers of voles were trapped in the first field effect study, a second field effect study (BASF DocID 2015/1126803) was conducted as follow-up. **The new study also shows no adverse acute and long-term effects of BAS 500 06 F on common vole populations in meadows.** The full summary of this new study has been added to this document (CA 8.1.2.2/2).

Please note that the following studies CA 8.1.2.2/1, CA 8.1.2.2/3 and CA 8.1.2.2/4 were erroneously listed as CP 10.1.2.2/1+3+4 in the Application submitted in January 2014 for the renewal of approval of pyraclostrobin.

Report:	CA 8.1.2.2/1 [redacted], 2014a Field study on the acute and long-term effects of a Pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray in spring to cereals on populations of small mammals (wood mice and common voles) in Central Europe (Germany) 2014/1000041
Guidelines:	<none>
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The study investigated the potential acute and long-term effects of the pyraclostrobin formulation BAS 500 06 F, applied as foliar spray to cereals in spring, on wood mouse and common vole populations. Long-term effects were investigated by comparing species abundance and population development in BAS 500 06 F – in treated winter cereal fields and untreated winter cereal fields over a period of six months.

This study showed no impacts of the fungicide BAS 500 06 F applied to winter cereal under field conditions on resident small mammal populations, of which eight different parameters (captures/individuals in- and off-crop, trapping efficiency, minimum number alive, population growth rate, percentage of reproductively active individuals, percentage of juveniles, percentage of females, and adult body weight) were monitored for the whole growth period of winter cereal in Germany. This could clearly be demonstrated for the resident wood mouse (*Apodemus sylvaticus*) populations. Although sample sizes were low for the common vole (*Microtus arvalis*) populations, no impacts of the fungicide BAS 500 06 F on a low-density population of common voles could be shown.

I. MATERIAL AND METHODS

Study site: The study was conducted between April 2013 and October 2013 in twelve winter cereal fields in the vicinity of the villages of Limburgerhof and Altrip in the federal state of Rhineland-Palatinate in Germany. The size of the study fields ranged between 2.0 ha and 5.5 ha.
Application: Six study fields were treated with the fungicide BAS 500 06 F (treatment fields). The first application was carried out at the early plant growth stage around BBCH stage 25 to 30, and the second application was conducted 20 days after the first application which was according to the use pattern. The remaining six study fields received no BAS 500 06 F treatment (control fields).

Test organisms: Test organisms were free-living, wild populations of the wood mouse (*Apodemus sylvaticus*) and the common vole (*Microtus arvalis*) naturally inhabiting the selected study fields and directly adjacent habitats.
Trapping: Live trapping (capture-mark-recapture) was carried out to compare the abundance and population dynamics of small mammal species in treated and untreated winter cereal fields and adjacent off-crop areas. Eighty Ugglan multiple-capture traps were set up in each study plot, with 60 traps placed inside the cereal field and 20 traps placed in the off-crop area adjacent to the study field. Study plots were defined as the area covered by the small mammal trapping grid. Captured individuals were marked with Passive Integrated Transponders (PIT) for individual identification. A total of ten trapping sessions, each trapping session consisting of three consecutive nights of trapping and one night of pre-baiting, were performed in each study field. Trapping commenced prior to the first application of BAS 500 06 F and continued until October 2013 in accordance with agricultural practice after harvest, crop rotation and the development of the following crop on the study fields. This monitoring method allowed assessing the trapping efficiency and Minimum Number Alive (MNA), the rate of population growth, the rate of reproduction, the proportion of juveniles, the sex ratio and the development of the body weight throughout the Field Phase. To assess whether the treatment with BAS 500 06 F had any effects on mice and vole species, the results of the above mentioned parameters of the six treatment fields were compared with those of the six control fields.

II. RESULTS AND DISCUSSION

a) Wood mouse (Apodemus sylvaticus)
The trapping efficiencies of wood mice in the in-crop habitats were comparable in treatment and control plots over the course of the Field Phase. The trapping efficiencies in the in-crop habitats of the treatment plots experienced a noticeable increase after the second application of BAS 500 06 F (after trapping session 3), while the trapping efficiencies in the in-crop habitats of the control plots increased only gradually. The relatively high trapping efficiencies in the in-crop habitats of treatment and control plots (trapping session 4 until trapping session 8) indicate that wood mice preferentially used the in-crop habitats during the times of advanced crop development. After the study fields were harvested there was a shift in habitat use from the in-crop habitat to the off-crop habitat.
The minimum number of individual wood mice alive (MNA) showed a similar pattern in control and treatment plots over the course of the Field Phase. However, MNAs in the treatment plots increased considerably until the population maximum was reached at trapping session 5 (mid-May). On the other hand, MNAs in the control plots increased only gradually and reached the population maximum at a later stage (trapping session 7; around end of June). Altogether, this also indicates that there are no acute effects of BAS 500 06 F on wood mouse populations, considering that MNAs increased even shortly after each application with BAS 500 06 F. The MNAs in the control plots never reached the maximum of the MNAs in the treatment plots. The population growth rate showed a similar pattern to the MNA values in control and treatment plots.

At the beginning of the trapping period, almost all individuals captured in treatment and control plots were reproductively active. Over the course of the reproductive season of wood mice and after treatments with BAS 500 06 F the percentage of reproductively active individuals in control and treatment plots followed an almost identical development from trapping session 3 until the end of the trapping period.
The mean percentage of juvenile wood mice over the course of the Field Phase followed a similar pattern in treatment and control plots. In treatment and control plots the percentage of juveniles was low during the first trapping sessions (early April), as expected at the beginning of the reproductive season of small mammals in Central Europe.
The percentage of female wood mice followed a similar pattern in control and treatment plots. The sex ratio remained below 50% in control and treatment plots in all trapping sessions except for the trapping session that was conducted after harvest (trapping session 9).
The development of the mean body weight of adult wood mice across all trapping sessions followed a similar pattern in treatment and control plots. Mean body weight decreased in the last two trapping sessions in both control and treatment plots, which reflects the normal development.
The mean recapture rate of marked wood mice was similar in treatment and control plots, indicating no difference in survival of wood mice. From trapping session 2 until 8 was the rate of recapture was in parts very high with almost 80%, but decreased after harvesting of the study fields (trapping session 9 and 10).

b) Common vole (Microtus arvalis)

The number of common voles captured was too low to infer any confident conclusions about abundance and population parameters in the treatment and control plots.

The trapping efficiencies of common voles in the in-crop habitats followed a very similar pattern in the treatment and control plots. Common voles were first trapped in in-crop habitats in trapping session 4 (beginning of May) after the second application of BAS 500 06 F. Thereafter numbers increased steadily until they reached a peak at trapping session 7 (around end of June) which was followed by a continuous decrease until the completion of the Field Phase. On the contrary, trapping efficiencies in the off-crop habitats of both treatment groups were very low, but showed a considerable increase during the last trapping session in October after the study fields were harvested. The low trapping efficiencies of common voles in the off-crop habitats reflect the low number of captured common voles in these habitats and may indicate the potential habitat preferences for this species.

The minimum number of common vole individuals alive (MNA) was similar in control and treatment plots throughout the trapping period. No common voles were captured in the first three trapping sessions in the treatment or in the control plots. The population maximum of common voles was reached at trapping session 7 (around end of June) at the time of high ground cover shortly before harvest. The population growth rate of common voles showed a similar pattern to the MNA values in control and treatment plots.

The mean percentage of reproductively active individuals fluctuated in control and treatment plots probably mainly due to the low number of individuals captured. In control and treatment plots the percentage of reproductively active common voles was highest in trapping session 8, coinciding with the time of highest ground cover. After harvest of the study fields the percentage of reproductively active individuals decreased again potentially reflecting the increased emigration from the study fields.

The mean percentage of juvenile common voles was low over the course of the Field Phase in control and treatment plots. In addition to the low number of juvenile captures, juveniles were not captured in all study fields. Thus no clear understandings of the dynamics of this parameter can be achieved due to the low sample size.

Meaningful statements on the development of the sex ratio of common voles cannot be made due to the sex ratio being based on very small sample sizes in control and treatment plots likewise.

Mean body weight of adult common voles was similar in control and treatment plots across trapping sessions. The body weight decreased slightly towards the end of the trapping period potentially indicating the potential decline in the number of pregnant females.

Some common voles were recaptured despite the overall low number of captured common voles in treatment and control plots.

III. CONCLUSION

In conclusion, this study showed no impacts of the fungicide BAS 500 06 F applied to winter cereal under field conditions on resident small mammal populations, of which eight different parameters (captures/individuals in- and off-crop, trapping efficiency, minimum number alive, population growth rate, percentage of reproductively active individuals, percentage of juveniles, percentage of females, and adult body weight) were monitored for the whole growth period of winter cereal in Germany. This could clearly be demonstrated for the resident wood mouse (*Apodemus sylvaticus*) populations. Although sample sizes were low for the common vole (*Microtus arvalis*) populations, no impacts of the fungicide BAS 500 06 F on a low-density population of common voles could be shown.

Report:	CA 8.1.2.2/2 Ziesemann B., 2016 a Field study on the acute and long-term effects of a Pyraclostrobin formulation (BAS 500 06 F) applied as foliar spray on meadows to populations of common voles in Central Europe (Germany) 2015/1126803
Guidelines:	OECD Principles of Good Laboratory Practice
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The present study aims to investigate potential acute and long-term effects of the fungicide BAS 500 06 F (containing the active substance pyraclostrobin) on common voles (*Microtus arvalis*) in agriculturally managed meadows. Meadows were used as a surrogate for other crop types to ensure sufficient numbers of common voles being exposed to the test item BAS 500 06 F. Acute and long-term effects were investigated by comparing common vole abundance and population parameters in BAS 500 06 F – treated and untreated meadows over a period that covered the reproductive season of common voles.

When applying BAS 500 06 F following the critical use pattern (2 x 250 g pyraclostrobin/ha), no negative effects were detected in any of the many investigated parameters throughout the study, i.e. no adverse acute and long-term effects of the fungicide BAS 500 06 F on common vole populations were found. This second field effect study therefore confirms the outcome of the first field effects study, and the result of the risk assessment presented in M-CP section 10.

I. MATERIAL AND METHODS

The study was conducted in agriculturally used meadows (study fields) in the vicinity of Dornburg in the Limburg-Weilburg district in Hessen, Germany, between May and October 2015. The size of the ten study fields ranged from 1.0 ha to 3.0 ha.

Five study fields were treated twice with BAS 500 06 F at a nominal application rate of 1.25 L product/ha (250 g pyraclostrobin/ha) in a spray volume of 200 L/ha. The first application was carried out on 08-11 June 2015 once common vole populations were large enough to ensure a sufficient part of the population to be exposed to the test item. The second application was conducted approximately three weeks later on 29 June - 04 July 2015. The remaining five study fields served as untreated control fields.

Live trapping (capture-mark-recapture) was carried out to compare abundance, population dynamics, age structure and reproduction of common voles in treated and untreated study fields. A total of 10 trapping sessions, each session consisting of one night of pre-baiting and three consecutive nights of trapping were performed.

Ugglan multiple-capture traps were used to live-trap small mammals. Sixty Ugglan multiple live capture traps were set up in each study field. Captured individuals were marked with Passive Integrated Transponders (PIT) for individual identification. All study fields were mowed before the trapping of common voles commenced and again in late summer (after trapping session 8 at the end of August 2015). This monitoring method allowed assessing the trapping success (number of captured animals and the number of individuals), the minimum number alive (MNA), the recapture rate, the sex ratio, the rate of reproduction, the proportion of juveniles, and the development of the body weight throughout the Field Phase which covered the majority of the reproductive season of common voles. In order to assess whether the treatment with BAS 500 06 F had any effects on common voles, the results of the above mentioned parameters of the five treatment fields were compared with those of the five control fields.

II. RESULTS

Overall, trapping success was high: 9161 captures of common voles were made in all study plots during the Field Phase of this study, including a total of 2495 individually marked animals.

Three abundance parameters were evaluated: number of captures and of individuals, minimum number of individuals alive (MNA) and recapture rate of marked individuals.

The number of individuals of common voles monitored was not statistically significantly different between control and treatment plots in any trapping session.

The minimum number of individual common voles alive (MNA) in the treatment and the control plots showed a similar pattern (Figure 1), and there were no statistically significant differences between the two groups.

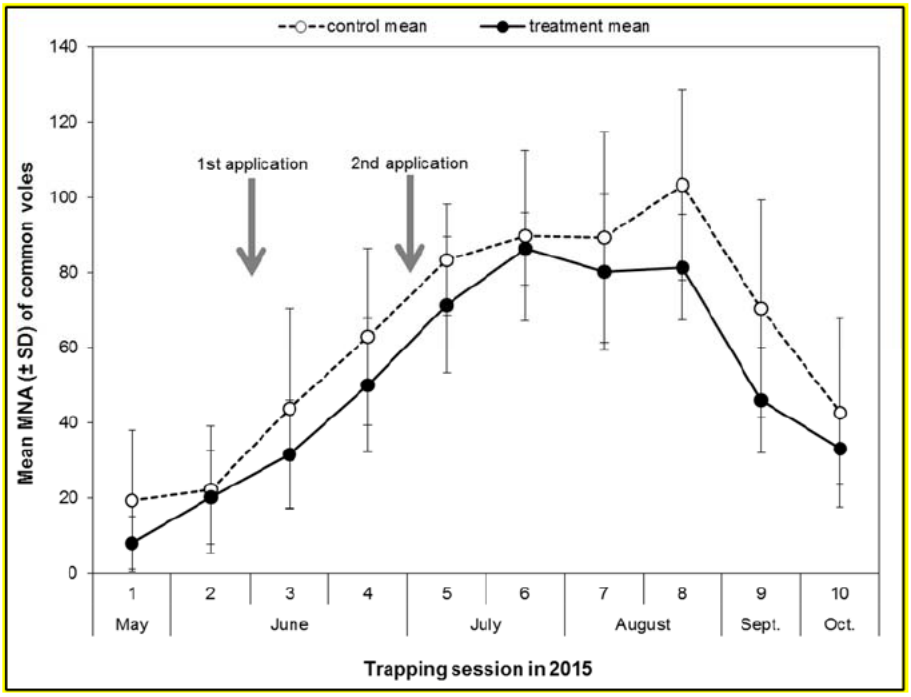

Figure 1: Mean of the minimum number of common voles alive (MNA) in control and treatment plots (the applications of BAS 500 06 F were conducted after trapping session 2 and 4. The error bars represent the standard deviation of the mean (± SD))

The mean recapture rate of marked common voles showed a similar development in treatment and control plots (Figure 2). The recapture rate of individuals exposed to BAS 500 06 F in the treatment plots was not statistically significantly different than the recapture rate of common voles in the control plots.

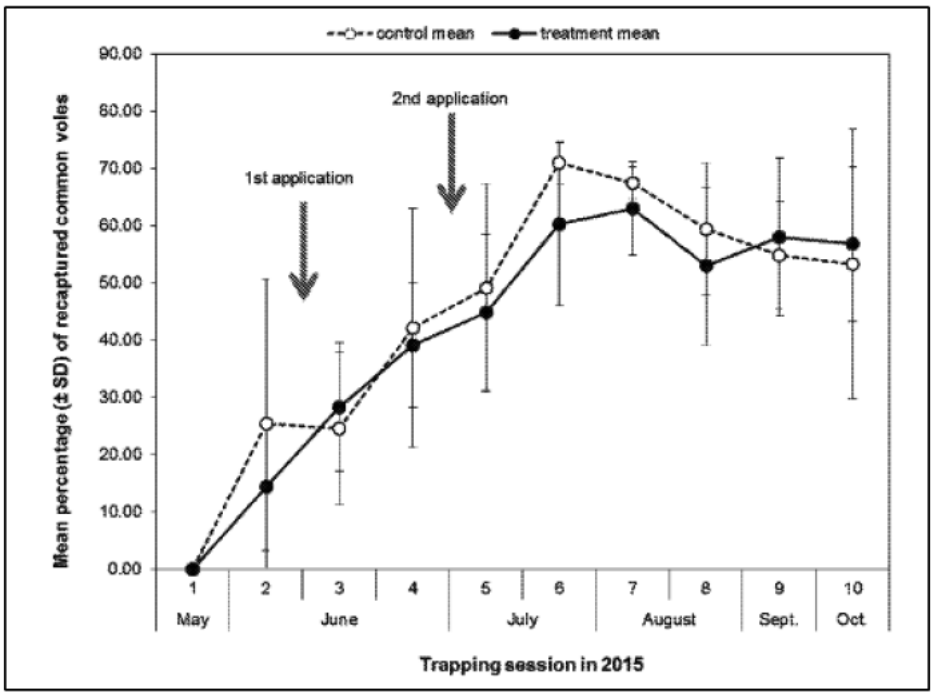

Figure 2: Mean recapture rate (%) of common vole individuals in control and treatment plots (legend as in Figure 1)

Four population parameters were evaluated: sex ratio (proportion of females and males), reproductive activity (proportion of reproductively active animals), age structure (proportion of juveniles), and bodyweight development.

The sex ratio of common voles was similar in treatment and control plots, and the proportion of females fluctuated almost consistently between 50-60% throughout the trapping period. No statistically significant differences in the percentage of female common voles were detected between control and treatment plots.

The proportion of reproductively active common voles followed a similar pattern in the control and treatment plots over the course of the study (Figure 3), with most trapping sessions without statistically significant differences between the two groups. After the second application of BAS 500 06 F, there was a marginal statistically significant difference (p=0.05) in trapping session no. 5, where the proportion of reproducing animals was even higher in the treatment plots than in the controls.

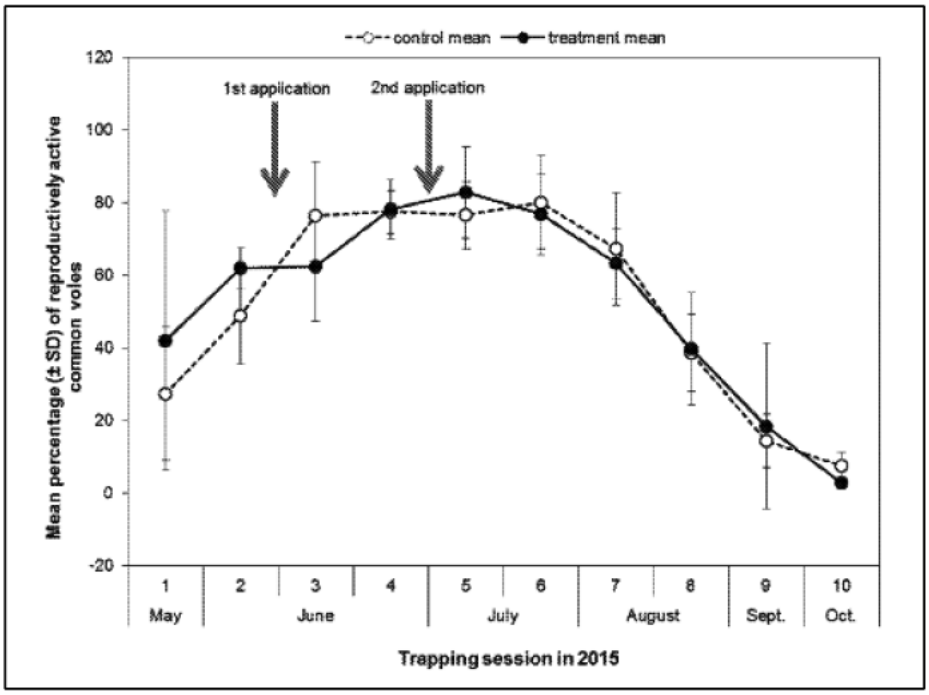

Figure 3: Mean percentage (%) of reproductively active common voles in control and treatment plots (legend as in Figure 1)

The pattern of the proportion of juveniles during the trapping period was similar in the control and treatments plots (Figure 4). The differences observed in trapping session no. 1 (before the application) was unrelated to treatment and likely biased by the low numbers of juveniles present at the early phases of the reproduction season.

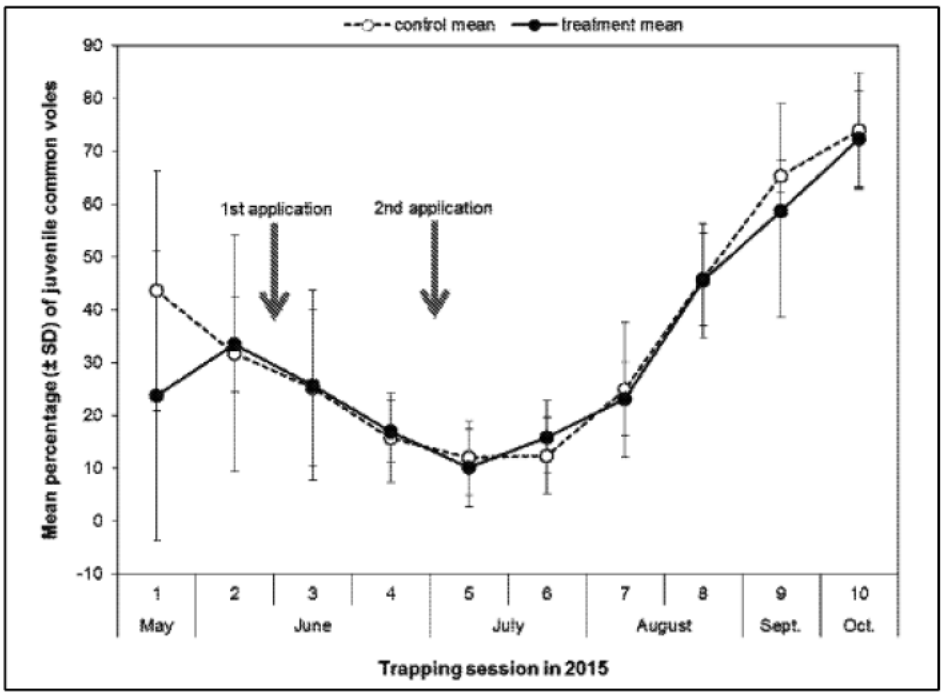

Figure 4: Mean percentage (%) of juvenile common voles in control and treatment plots (legend as in Figure 1)

The development of the mean body weight of adults (females and males) over the course of the trapping period was similar in control and treatment plots (Figure 5). The difference in the mean body weight of females and males was not statistically significant between control and treatment plots throughout the trapping period. The mean body weight of juveniles (females and males) was similar in both experimental groups throughout the trapping period (Figure 6). A decline in the mean body weight of juveniles after the second application of BAS 500 06 F occurred in both, control and treatment plots. Juvenile body weight was not statistically significantly different between control and treatment plots in any trapping session.

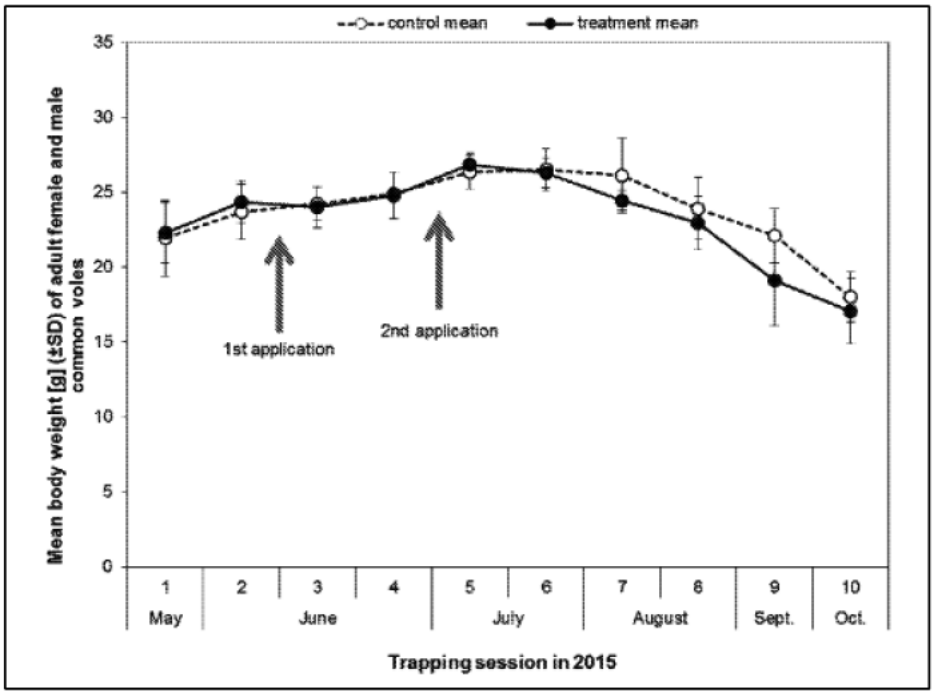

Figure 5: Mean body weight (g) of adult common voles (females and males) in control and treatment plots (legend as in Figure 1)

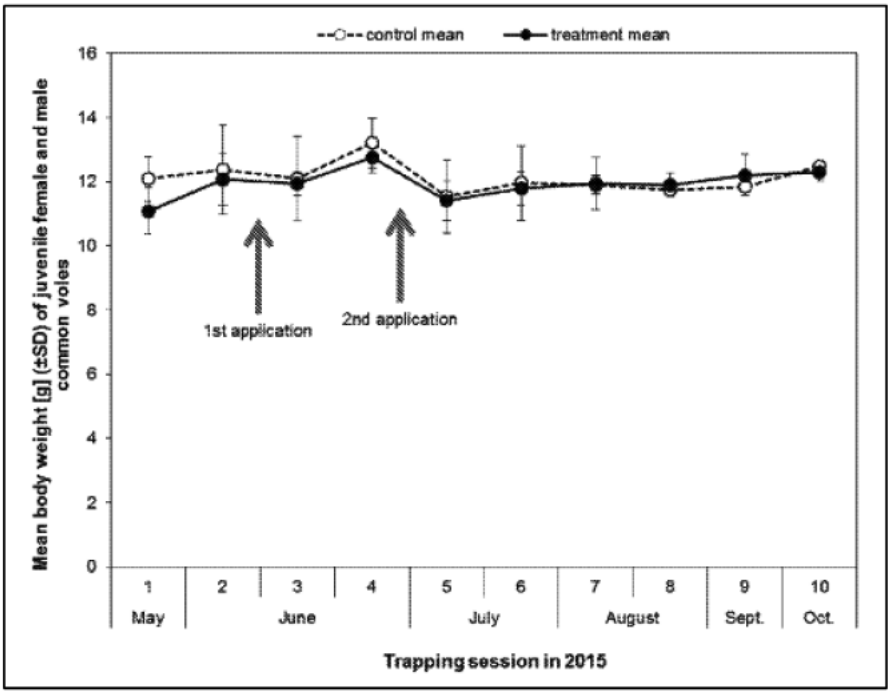

Figure 6: Mean body weight (g) of juvenile common voles (females and males) in control and treatment plots (legend as in Figure 1)

III. CONCLUSION

The study was conducted under realistic worst-case field conditions, i.e. in a preferred common vole habitat with high abundances of common voles. Therefore, the study is well appropriate to assess potential effects of pyraclostrobin (applied as BAS 500 06 F) on common voles on the population level.

When applying BAS 500 06 F following the critical use pattern (2 x 250 g pyraclostrobin/ha), no negative effects were detected in any of the many investigated parameters throughout the study, i.e. no adverse acute and long-term effects of the fungicide BAS 500 06 F (active substance pyraclostrobin) on common vole populations were found. This second field effect study therefore confirms the outcome of the first field effects study (BASF DocID 2014/1000041 shown above), and the result of the risk assessment presented in M-CP section 10.

Report:	CA 8.1.2.2/3 Moreno S., 2013a Study on the residue behaviour of Pyraclostrobin (BAS 500 F) on wheat (young plants) after treatment with BAS 500 06 F under field conditions in North and South Europe, season 2012 2013/1045207
Guidelines:	EEC 87/18 (No. L 15/29) 1986, International guidelines for distribution and pesticides application AEPLA FAO 1985, EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5 Appendix B, EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

Executive Summary

The objective of the study is to determine the magnitude of residues of pyraclostrobin (BAS 500 F) and the analyte 500M07 after one application of BAS 500 06 F carried out on wheat. The selected application rates, frequency and spray interval cover the Good Agricultural Practice (critical GAP).

I. MATERIAL AND METHODS

A. MATERIALS

Test item and application

The trial consisted of a control plot (untreated) and two treated plots (plot 2 and plot 3) without replication. No product containing the test item was used on the test plots during the season 2012.

The test item BAS 500 06 F (EC) was foliar applied on plot 2 at a nominal rate of 250 g pyraclostrobin/ha in a nominal spray volume of 200 L/ha at three leaves unfolded growth stage (BBCH 13) according to Good Laboratory Practice. The test item BAS 500 06 F (EC) was also foliar applied on plot 3 at a nominal rate of 100 g pyraclostrobin/ha in a nominal spray volume of 200 L/ha at three leaves unfolded growth stage (BBCH 13) according to Good Laboratory Practice.

B. STUDY DESIGN

Study site
During the 2012 growing season a total of nine trials were conducted in representative wheat growing areas in Germany, The Netherlands, The United Kingdom, Spain and Italy.

Sampling information
For this study specimens were collected as wheat whole plants without roots 1 hour after application as well as 1, 2, 3, 4, 5, 7, 10, 12 and 14 days thereafter. All specimens were sampled from the untreated, and from both treated plots. Untreated specimens were obtained prior to treated specimens at each sampling occasion. All specimens were transferred to freezer storage on the day of sampling and were then stored frozen (≤ -18ºC).

Residue analysis
All specimens were analyzed for pyraclostrobin (BAS 500 F) and its metabolite 500M07 (BF 500-3) using BASF method no. 535/1 (L0076/01). The method has a limit of quantitation of 0.01 mg/kg for both analyses.

II. RESULTS AND DISCUSSION

Table 8.1.2.2-1: Summary of Residues of pyraclostrobin (BAS 500 F) in Wheat

Plot 2							
Sampling No.	**Portion analyzed**	**DALA[1)]**	**Growth stage**	**n**	**BAS 500 F [mg/kg]**	**500M07 (BF 500-3)[2)] [mg/kg]**	**Sum[3)] [mg/kg]**
1	Wheat, Whole plant no roots	1[4)]	13-14	9	13 - 35	0.036 - 1.5	14 - 36
2		1	13-14	9	2.4 - 26	0.41 - 3.0	2.8 - 27
3		2	13-14	9	4.2 - 25	1.0 - 2.9	5.2 - 28
4		3	13-21	9	2.5 - 18	0.83 - 3.2	3.3 - 22
5		4	13-21	9	1.7 - 22	0.64 - 4.6	2.3 - 26
6		5	13-21	9	1.4 - 19	0.59 - 5.4	2.0 - 24
7		7	13-23	9	0.38 - 12	0.17 - 4.3	0.55 - 17
8		10	13-30	9	0.13 - 10	0.040 - 4.2	0.17 - 14
9		12	13-31	9	0.043 - 5.0	0.021 - 2.7	0.067 - 7.6
10		14	13-31	9	0.028 - 5.5	0.012 - 2.5	0.040 - 8.0
Plot 3							
Sampling No.	**Portion analyzed**	**DALA[1)]**	**Growth stage**	**n**	**BAS 500 F [mg/kg]**	**500M07 (BF 500-3)[2)] [mg/kg]**	**Sum[3)] [mg/kg]**
1	Wheat, Whole plant no roots	1[4)]	13-14	9	4.6 - 11	0.021 - 0.33	4.9 - 12
2		1	13-14	9	3.0 - 10	0.21 - 0.82	3.4 - 11
3		2	13-14	9	2.0 - 9.6	0.41 - 1.3	2.6 - 11
4		3	13-21	9	1.5 - 7.0	0.35 - 1.7	2.0 - 8.7
5		4	13-21	9	0.95 - 8.3	0.28 - 2.0	1.3 - 10
6		5	13-21	9	0.45 - 6.5	0.19 - 2.0	0.64 - 8.5
7		7	13-23	9	0.23 - 4.5	0.091 - 1.7	0.32 - 6.2
8		10	13-30	9	0.079 - 3.3	0.028 - 1.5	0.11 - 4.8
9		12	13-31	9	0.028 - 1.9	< 0.01 - 1.1	0.038 - 3.0
10		14	13-31	9	< 0.01 - 1.8	< 0.01 - 1.0	< 0.02 - 2.8

1) days after last application
2) conversion factor for calculation of 500M07 (BF 500-3) to parent BAS 500 F is 1.084
3) for residues < 0.010 mg/kg, value was set to 0.010 mg/kg for calculation of sum
4) HALA: hours after last application

III. CONCLUSION

In whole plant samples collected from plot 2 directly after the application (BBCH 13-14) residues of pyraclostrobin (BAS 500 F) ranged between 13 and 35 mg/kg. At the last sampling at 14 DALA (BBCH 13-31) residues decreased to a range of 0.028 – 5.5 mg/kg.
In whole plant samples collected from plot 3 directly after the application (BBCH 13-14) residues of pyraclostrobin ranged between 4.6 and 11 mg/kg. At the last sampling at 14 DALA (BBCH 13-31) residues decreased to a range of < 0.01 – 1.8 mg/kg. No residues of pyraclostrobin ≥ 0.01 mg/kg were present in control specimens.

Report:	CA 8.1.2.2/4 Martin T., 2013a Study on the residue behavior of Pyraclostrobin (BAS 500 F) on pea (young plants) after the application of BAS 500 06 F under field conditions in France (North), Germany, United Kingdom, Italy and Spain, 2012 2013/1044539
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 79/117, EEC 91/414, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

Executive Summary

The objective of the study was to determine the residue level of pyraclostrobin (BAS 500 F) in or on young pea plants after one application of BAS 500 06 F.

I. MATERIAL AND METHODS

A. MATERIALS

Test item and application

Each trial consisted of a control plot (untreated) and two treated plots. All applications were made as foliar sprays, using commercial ground equipment or equipment which simulated commercial applications. No product containing the test item was used on the test plots during the year 2012. BAS 500 06 F was the test item used in this study. The actual application rates were within 10% of the nominal values.
The selected application rates, frequency and spray interval cover the Good Agricultural Practice (critical GAP), which will be defined by the label directions.

BAS 500 06 F (200 g/L of pyraclostrobin, EC) was foliar applied once on plot 2 at a rate equivalent to 250 g/ha of pyraclostrobin (1.25 L of formulated product /ha) and once on plot 3 at a rate equivalent to 100 g/ha of pyraclostrobin (0.5 L of formulated product /ha). The spray volume used was 200 L/ha and the application timing was at BBCH 12 - 13.

B. STUDY DESIGN

Study site
During the 2012 growing season, eight trials in peas were conducted in different representative growing areas in France (North), Germany, The United Kingdom, Italy and Spain.

Sampling information
Specimens of whole plant without roots were collected on plot 1 at the day of the application and 5 and 14 days thereafter. Specimens of whole plant without roots were collected on plot 2 and plot 3, 1 hour after the application and 1, 2, 3, 4, 5, 7, 10, 12 and 14 days thereafter.
Control (untreated) specimens were taken at every time point, and were collected prior to collection of the treated specimens to avoid contamination. Generally, the specimens were frozen within 6 hours of being taken, and remained frozen at or below -18°C, including during transportation, until analysis. The maximum storage interval from harvest until analysis was 188 days. Data indicate that residues were stable during the period of frozen storage prior to analysis.

Residue analysis
Specimens were analysed for pyraclostrobin (BAS 500 F) and its metabolite 500M07 (BF 500-3) using BASF method No. 535/1 (L0076/01). The method has a limit of quantitation of 0.01 mg/kg for both analytes.

II. RESULTS AND DISCUSSION

Table 8.1.2.2-2: Summary of Residues of pyraclostrobin (BAS 500 F) and 500M07 in Peas

Plot 2							
Samp. No.	Portion analysed	DALA[1)]	Growth stage	n	Residues expressed as parent equivalents		
					BAS 500 F [mg/kg]	500M07 (BF 500-3)[2)] [mg/kg]	Sum[3)] [mg/kg]
1	Pea, whole plant no roots	1[4)]	12-13	8	11 - 23	0.026 - 0.41	11 - 23
2		1	12-13	8	9.4 - 19	0.53 - 2.5	10 - 22
3		2	12-14	8	2.9 - 11	0.64 - 3.6	3.5 - 15
4		3	12-14	8	3.2 - 8.7	1.2 - 3.7	4.8 - 12
5		4	13-15	8	2.7 - 5.9	1.1 - 2.8	4.1 - 8.0
6		5	13-16	8	2.1 - 5.1	0.83 - 3.1	3.6 - 8.1
7		7	13-17	8	1.4 - 3.6	0.68 - 2.6	2.7 - 6.3
8		10	13-19	8	0.16 - 2.2	0.13 - 1.4	0.29 - 3.6
9		12	14-21	8	0.38 - 2.0	0.23 - 1.4	0.71 - 3.5
10		14	14-23	8	0.14 - 1.4	0.062 - 0.89	0.20 - 2.1
Plot 3							
Samp. No.	Portion analysed	DALA[1)]	Growth stage	n	Residues expressed as parent equivalents		
					BAS 500 F [mg/kg]	500M07 (BF 500-3)[2)] [mg/kg]	Sum[3)] [mg/kg]
1	Pea, whole plant no roots	1[4)]	12-13	8	3.9 - 6.2	0 017 - 0.11	4.0 - 6.3
2		1	12-13	8	1.4 - 5.3	0.12 - 0.78	1.5 5.6
3		2	12-14	8	1.6 - 8.0	0.40 - 1.9	2.1 - 9.9
4		3	12-14	8	1.0 - 2.4	0.46 - 1.1	1.5 - 3.5
5		4	13-15	8	0.95 - 1.5	0.43 - 0.73	1.5 - 2.0
6		5	13-16	8	0.60 - 1.6	0.41 - 0.94	1.1 - 2.4
7		7	13-17	8	0.34 - 0.82	0.25 - 0.62	0.61 - 1.4
8		10	13-19	8	0.24 - 0.69	0.13 - 0.58	0.37 - 1.2
9		12	13-21	8	0.10 - 0.43	0.040 - 0.37	0.14 - 0.80
10		14	14-23	8	0.043 - 0.29	0.023 - 0.27	0.066 - 0.53

1) days after last application
2) conversion factor for calculation of 500M07 (BF 500-3) to parent BAS 500 F is 1.084
3) for residues < 0.010 mg/kg, value was set to 0.010 mg/kg for calculation of sum
4) HALA: hours after last application

III. CONCLUSION

In whole plant samples collected from plot 2 directly after the application (BBCH 12-13) residues of pyraclostrobin (BAS 500 F) ranged between 11 - 23 mg/kg. Residues has been decreasing over the whole trial period and at last sampling at 14 DALA (BBCH 14-23) residues ranged between 0.14 - 1.4 mg/kg.

In whole plant samples collected from plot 3 directly after the application (BBCH 12-13) residues of pyraclostrobin ranged between 3.9 - 6.2 mg/kg. Residues has been decreasing over the whole trial period and at last sampling at 14 DALA (BBCH 14-23) residues ranged between 0.043 - 0.29 mg/kg.

No residues of pyraclostrobin ≥ 0.01 mg/kg were present in control specimens.

Please note, the following studies (CA 8.1.2.2/5 and CA 8.1.2.2/6), presenting the evaluation of residue data, were erroneously not listed in the Application submitted in January 2014 for the renewal of approval of pyraclostrobin. However, the two reports presenting the residue data, i.e. BASF DocID 2013/1045207 and BASF DocID 2013/1044539, were included in the Application (as CP 10.1.2.2/3 and 10.1.2.2/4) and are now submitted as CA 8.1.2.2/3 and CA 8.1.2.2/4.

Report:	CA 8.1.2.2/5 Erzgraeber B., 2013a Dissipation of BAS 500 F - Pyraclostrobin on young plants (wheat and peas) - Trials conducted in the Northern Zone of Europe - Calculation of DT50 / DT90 dissipation times 2013/1078114
Guidelines:	<none>
GLP:	no

Executive Summary

This modelling report provides kinetic analysis and estimation of dissipation times (DT_{50}, DT_{90} values) for pyraclostrobin on young plants.

The decline of pyraclostrobin residues on young plants was well described by this first order kinetics. The overall geometric mean DT_{50} of pyraclostrobin in young plants is 1.8 days (Northern Europe).

I. MATERIAL AND METHODS

Calculation of DT_{50} and DT_{90} values
The concentration time curves were described by a single first order (SFO) kinetic model, and this was fitted against the results of the individual trials using non-linear parameter estimation methods.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures. For visual inspection, the recommended graphical representations of observed and modeled decline curves versus time and the residuals versus time are presented. As goodness-of-fit measures, the χ^2 minimum error level is provided.

II. RESULTS AND DISCUSSION

Table 8.1.2.2-3: Table DT_{50} and DT_{90} values of pyraclostrobin in young wheat plants and peas

Plant	Trial	Plot	DT_{50} [d]	DT_{90} [d]	Kinetic model	χ2 error	r^2
wheat	L120103	P2[1)]	2.67	8.86	SFO	10.3	0.97787
wheat	L120103	P3[2)]	1.37	4.54	SFO	19.9	0.95564
wheat	L120104	P2	1.40	4.67	SFO	11.2	0.98795
wheat	L120104	P3	1.97	6.55	SFO	12.5	0.97825
wheat	L120105	P2	2.18	7.24	SFO	9.3	0.98535
wheat	L120105	P3	1.56	5.19	SFO	10.2	0.98748
wheat	L120106	P2	1.26	4.18	SFO	7.0	0.99658
wheat	L120106	P3	1.25	4.16	SFO	7.9	0.99416
peas	L120073	P2	1.28	4.25	SFO	7.0	0.99504
peas	L120073	P3	1.50	4.97	SFO	9.9	0.98735
peas	L120074	P2	1.91	6.35	SFO	8.4	0.98946
peas	L120074	P3	1.55	5.16	SFO	8.3	0.99175
peas	L120075	P2	3.76	12.50	SFO	5.1	0.99011
peas	L120075	P3	2.50	8.32	SFO	12.6	0.96317
peas	L120076	P2	2.10	6.97	SFO	12.7	0.97268
peas	L120076	P3	2.06	6.84	SFO	16.0	0.95846
geo mean			**1.80**	**5.99**			

[1)] and [2)] In 2012, BASF conducted 16 GLP field residue trials in the Northern Zone of Europe to obtain residue decline data in wheat and peas at early growth stages of the crops [Ref. 3, Ref. 4].
Wheat and peas were treated with either nominally 1.25 L (P2) or 0.5 L (P3) product BAS 500 06 F/ha. BAS 500 06 F contains 200 g pyraclostrobin/L, thus 250 g a.s./ha (P2) or 100 g a.s./ha were applied at BBCH 13 or 13/14 onto wheat and at BBCH 12-13 or 13 onto peas.

III. CONCLUSION

The decline of pyraclostrobin residues on young plants was well described by this first order kinetics. The overall geometric mean DT_{50} of pyraclostrobin in young plants is 1.8 days (Northern Europe).

Report: CA 8.1.2.2/6
Erzgraeber B., 2013b
Dissipation of BAS 500 F - Pyraclostrobin on young plants (wheat and peas) - Trials conducted in the Southern Zone of Europe - Calculation of DT50 / DT90 dissipation times
2013/1291161
Guidelines: <none>
GLP: no

Executive Summary

This modelling report provides kinetic analysis and estimation of dissipation times (DT_{50}, DT_{90} values) for pyraclostrobin on young plants.

The decline of pyraclostrobin residues on young plants was well described by this first order kinetics. The overall geometric mean DT_{50} of pyraclostrobin in young plants is 3.15 days (Southern Europe).

I. MATERIAL AND METHODS

The concentration time curves were described by a single first order (SFO) and first-order multi-compartment (FOMC) kinetics, respectively, and this was fitted against the results of the individual trials using non-linear parameter estimation methods.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures. For visual inspection, the recommended graphical representations of observed and modeled decline curves versus time and the residuals versus time are presented. As goodness-of-fit measures, the χ^2 minimum error level is provided.

II. RESULTS AND DISCUSSION

Table 8.1.2.2-4: Table presenting DT_{50} and DT_{90} values of pyraclostrobin in young wheat plants and peas

Plant	Trial	Plot	DT_{50} [d]	DT_{90} [d]	Kinetic model	χ^2 error	DT_{50} (modeling) [d][3)]
wheat	L120107	P2[1)]	2.50	8.31	SFO	7.1	2.50
wheat	L120107	P3[2)]	3.12	10.37	SFO	1.8	3.12
wheat	L120108	P2	5.20	17.28	SFO	9.9	5.20
wheat	L120108	P3	5.53	18.36	SFO	7.8	5.53
wheat	L120109	P2	3.14	10.44	SFO	11.2	3.14
wheat	L120109	P3	4.27	14.18	SFO	13.0	4.27
wheat	L120110	P2	4.41	14.65	SFO	6.0	4.41
wheat	L120110	P3	3.31	11.00	SFO	7.5	3.31
peas	L120077	P2	1.54	8.64	FOMC	10.9	2.60
peas	L120077	P3	1.52	8.65	FOMC	8.1	2.61
peas	L120078	P2	0.99	8.53	FOMC	6.7	2.57
peas	L120078	P3	1.37	8.06	FOMC	5.5	2.43
peas	L120079	P2	-[4)]	-	-	-	-
peas	L120079	P3	2.59	8.61	SFO	19.2	2.59
peas	L120080	P2	-[4)]	-	-	-	-
peas	L120080	P3	0.16	6.40	FOMC	16.1	1.93
geo mean							**3.15**

[1)] and [2)] In 2012, BASF conducted 16 GLP field residue trials in the Southern Zone of Europe to obtain residue decline data in wheat and peas at early growth stages of the crops [Ref. 3, Ref.4].
Wheat and peas were treated with either nominally 1.25 L (P2) or 0.5 L (P3) product BAS 500 06 F/ha. BAS 500 06 F contains 200 g pyraclostrobin/L, thus 250 g a.s./ha (P2) or 100 g a.s./ha (P3) were applied at BBCH 13 or 13/14 onto wheat and at BBCH 12-13 or 13 onto peas.

[3)] In case of FOMC kinetic, a pseudo-SFO DT_{50} value for modeling is back-calculated from the DT_{90} as $DT_{50} = DT_{90}/3.32$.

[4)] trial discarded, since no acceptable fit could be achieved

III. CONCLUSION

The decline of pyraclostrobin residues on young plants was well described by this first order kinetics. The overall geometric mean DT_{50} of pyraclostrobin in young plants is 3.15 days (Southern Europe).

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

The potential effects of active substance bioconcentration in prey of birds and mammals are addressed according to the guidance document EFSA/2009/1438 with the secondary poisoning risk assessments presented in the documents M-CP 10.1.1 and M-CP 10.1.2. All TER values exceed the trigger of 5 set by Commission regulation (EU) 546/2011 for acceptability of effects with considerable margins of safety. Consequently, no additional study is considered to be necessary.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

According to the data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to amphibians and reptiles shall be addressed. However, there is no guidance or validated regulatory protocol yet available, neither on the type of the necessary regulatory testing nor how to conduct a risk assessment for amphibians and reptiles. Accordingly, specific toxicity tests for amphibian and reptile species are not requested.

Indications of a potential risk to amphibians and reptiles may be derived from findings or reports of adverse impacts and/or from literature data for existing substances.

An extensive literature search for pyraclostrobin and pyraclostrobin-containing plant protection products has not revealed any relevant information with respect to the risk of pyraclostrobin to reptiles. In addition, despite long-term extensive use of the substance worldwide, there has never been any reporting of adverse impacts on reptiles (or on adverse effects on birds and mammals) that the applicant is aware of. There is no indication from 'read across' that reptiles either could be particularly sensitive or would not be covered by the available vertebrate data and risk assessments. Accordingly, it can be concluded that applications of pyraclostrobin according to good agricultural practice will be of low risk to reptiles, too, analogous to birds and mammals.

However, there is literature on amphibians, which indicate that sensitive amphibian life stages may be vulnerable to pyraclostrobin, respectively to some of its formulations.
There are a few studies with aquatic life stages (tadpoles), which roughly reflect the toxicity observed with sensitive fish species. Summaries of these published studies are provided in M-CA 8.2.8). Some investigations indicate also toxicity to terrestrial life stages of amphibians. Summaries of the respective literature are provided below.

In addition, BASF has conducted own GLP laboratory and higher tier semi-field studies in order to assess the potential impact of pyraclostrobin applications to amphibians. These studies were conducted with the formulated product BAS 500 06 F (an EC-formulation considered to reflect the worst-case with respect to potential toxicity). The studies and a subsequent thorough risk assessment are provided in M-CP 10.1.3.

The following study investigates the acute toxicity of different fungicide formulations to juvenile Great Plains toad (*Bufo cognatus*) tadpoles and juveniles. In the following summary only the results for the pyraclostrobin containing formulation Headline® are presented.

Report:	CA 8.1.4/1 Belden J. et al., 2010a Acute toxicity of fungicide formulations to amphibians at environmentally relevant concentrations - Short communication 2014/1143801
Guidelines:	<none>
GLP:	no

Executive Summary

In a 72 h acute laboratory study tadpoles and juveniles of the Great Plains toad (*Bufo cognatus*) were exposed to Headline®, a fungicide with the active substance pyraclostrobin, at the following rates: 0.015, 0.15 and 1.50 mg a.s./L for tadpoles and 0.00022, 0.0022 and 0.0220 mg a.s./cm^2 for juveniles. Additionally, a dilution water control was set up for both tests. Each treatment consisted of three replicates. 9 - 10 juveniles per replicate were directly treated with an aerosol spray of formulation while contained in aquaria, whereas 20 tadpoles per replicate were exposed in water (static system).

Concentration of the active ingredient was measured within the spray mix used for juveniles and within the water used for tadpole testing at the beginning and end of the test. The mean concentration in the spray mix was between 89% and 102%.
For the highest treatment group no analysis of the concentration within the test water for tadpoles was done after 72 hours, because all individuals were dead within 24 hours. After 3 hours the mean measured value for pyraclostrobin within the test water for tadpoles was 80% of nominal. The following biological results are based on nominal concentrations of the active substance.

All aquatic concentrations resulted in 100% mortality of *B. cognatus* tadpoles. The medium and highest concentrations resulted in significant toxicity to juveniles ($F_{3,8} = 37.9$; $p < 0.001$). Most of the mortality occurred within the first 24 h (>90%) for both juveniles and tadpoles.

Acute laboratory studies with tadpoles (aquatic exposure) and juveniles (terrestrial exposure) of *Bufo cognatus* exposed to the formulation Headline® resulted in 100% mortality of tadpoles at concentrations equivalent to 0.015, 0.15 and 1.50 mg a.s./L in an aquatic system. Terrestrial exposure showed 100% juvenile mortality at 22 µg a.s./cm^2 and about 70% mortality at 2.2 µg µg a.s./cm^2 while 0.22 µg a.s./cm^2 caused no significant effect.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Headline® (EPA Reg. No. 7969-186; purchased from a local distributor) containing the active substance pyraclostrobin.

B. STUDY DESIGN

Test species: Great Plains toad (*Bufo cognatus*) tadpoles and juveniles, tadpoles: obtained from in-house culture, 7 days old, parents were wild caught; juveniles: wild caught near wetlands in western Texas, age: ≈ 60 d post-metamorphosis.

Test design: Test duration: 72 h, 3 test item concentrations plus a water control; each treatment consisted of three replicates; tadpoles: water exposure, static system, 20 tadpoles per aquarium and replicate; juveniles: directly treated with an aerosol spray of formulation (while contained in aquaria without water); 9 - 10 juveniles per aquarium and replicate; assessment of mortality every 12 hours; water quality was tested at test initiation and termination.

Endpoints: Mortality for tadpoles and juveniles of Great Plains toad (*Bufo cognatus*).

Test concentrations: Application rate to test chambers (spray mix): water control, 0.00022, 0.0022 and 0.022 mg a.s./cm^2;
Concentration in water: water control, 0.015, 0.15 and 1.50 mg a.s./L.

Test conditions: Aquaria (400 cm^2 floor surface area) for both life stages; juveniles: 1 cm sterilized soil; tadpoles: 16 cm carbon-filtered water, test volume: 6 L; temperature: 26 ± 1 C; pH: 7.0 - 7.2; dissolved oxygen: > 5.5 mg/L; photoperiod 13 h light : 11 h dark.

Analytics: Analytical verification of test item concentrations was conducted using a GC-method with MS detection.

Statistics: Descriptive statistics, one-way ANOVA, Tukey´s multiple range test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Concentration of the active ingredient was measured within the spray mix used for juveniles and within the water used for tadpole testing at the beginning and end of the test. The mean concentration in the spray mix was between 89% and 102% of nominal with standard deviations of less than 13%.
For the highest treatment group no analysis of the concentration within the test water for tadpoles was done after 72 hours, because all individuals were dead within 24 hours. After 3 hours the mean measured value for pyraclostrobin within the test water for tadpoles was 80% of nominal. The following biological results are based on nominal concentrations of the active substance.

Biological results: With respect to the aquatic exposure test, all concentrations resulted in 100% mortality of *B. cognatus* tadpoles ($F_{3,8} = 42.3$; $p < 0.001$). Within the terrestrial exposure test an application rate of 22 µg a.s./cm^2 resulted in 100% juvenile mortality and 2.2 µg µg a.s./cm^2 caused about 70% mortality while at 0.22 µg a.s./cm^2 no significant effect was observed. Most of the observed mortality occurred within the first 24 h (>90%) for both juveniles and tadpoles.

III. CONCLUSION

Acute laboratory studies with tadpoles (aquatic exposure) and juveniles (terrestrial exposure) of *Bufo cognatus* exposed to the formulation Headline® resulted in 100% mortality of tadpoles at concentrations equivalent to 0.015, 0.15 and 1.50 mg a.s./L in an aquatic system. Terrestrial exposure showed 100% juvenile mortality at 22 µg a.s./cm^2 and about 70% mortality at 2.2 µg µg a.s./cm^2 while 0.22 µg a.s./cm^2 caused no significant effect.

The following publication received only a reduced reliability index. For example, no analytical confirmation of test substances was conducted and no statistical analysis of the data was performed (i.e. the text indicates 20% mortality as a substance related effect, while similar mortality was observed in some of the control runs, and the same effect was seen at a factor of 10 different rates; for more details, see the literature evaluation). Thus, a reliability index of RI = 3 was assigned based on an evaluation according to Klimisch et al. 1997 (high reliability score = 1, not reliable = 4). Nonetheless, this publication is considered here, because it contains relevant information, which is in line with data from Belden et al. (2010). In the following summary only the results for the two pyraclostrobin containing formulations are presented (Headline® and a product coded BAS 500 18 F).

Report:	CA 8.1.4/2 Bruehl C. et al., 2013a Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline? 2014/1143855
Guidelines:	<none>
GLP:	no

Executive Summary

The effects of the pyraclostrobin containing formulations Headline® and BAS 500 18 F on juvenile European common frogs (*Rana temporaria*) were tested in 7 day laboratory studies using direct overspray of young metamorphs. The test organisms were exposed to 0.1 x, 1 x and 10 x label rate. All test item concentrations had 3 or 5 replicates (depending on mortality within 24 h) with one frog per replicate container and the water control had 10 replicates. Assessment of mortality was performed one, two, and four hours after application and afterwards daily until the end of the study.

Mortality of juvenile European common frogs differed largely between the two pyraclostrobin formulations. The formulation Headline®, (naphta content 67%) caused 100% mortality just after 1 h at the test concentration of 880 ml formulation/ha. This test concentration corresponds to the maximum label rate for the product for commercial use. No effect was observed at 0.1x this rate. BAS 500 18 F, the formulation with the lower naphta content (<25%), revealed 20% mortality at 880 ml and at 8800 ml formulation/ha, i.e. the same level as the corresponding control run. In the four control runs mortality ranged from 0% to 20%. No statistical comparisons were carried out.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: 1. Headline® (EC-formulation with 250 g pyraclostrobin/L);
2. BAS 500 18 F (CS-formulation with 250 g pyraclostrobin/L)

B. STUDY DESIGN

Test species: European common frog (*Rana temporaria*), juveniles, wild caught from the nature protection area "Tal" between CH-4467 Rothenfluh and CH-4469 Anwil.

Test design: Test duration: 7 d; application: test organisms were confined to containers the floor of which covered by some sterilized soil substrate; applications were conducted with a laboratory track sprayer using commercial flat fan nozzles; spray volume of 200 L/ha on the soil substrate taking the interception of the wire cage into account; stepwise test design: first, three juvenile frogs were exposed individually to the 0.1x field rate; if they did not show any treatment effects after 24 h, two additional individuals were exposed; if the first three test organisms died, no further testing was conducted, 10x higher rates were tested accordingly; four parallel control with 10 individuals per run sprayed with water only; assessment of mortality: one, two and four hours after application and afterwards daily until the end of the study.

Endpoints: Mortality of juvenile European common frogs (*Rana temporaria*).

Test concentrations: Water control, 88 mL formulation/ha (equivalent to 0.1x of the max. label rate), 880 mL formulation/ha (1x max. label rate)

Test conditions: Macrolon containers Type II (area: 375 cm²), covered with a mesh, 2 cm layer of sterilized standard soil (Soil 2.3, LUFA Speyer, Germany), soil was kept moist containers were kept in climate chamber; temperature: 20 ± 2°C; relative humidity: 75 ± 15%; photoperiod: 16 h light : 8 h dark; feeding: every two days with fruit flies (*Drosophila hydei*) and crickets (*Acheta domestica*); Application equipment: laboratory track sprayer (Schachtner Fahrzeug- and Gerätetechnik, Germany); commercial flat fan nozzles (TeeJet 8001 EVS, Tee-Jet Spraying Systems Co., USA).

Analytics: No analytics were carried out.

Statistics: Descriptive statistics only, no statistical analysis of the data.

II. RESULTS AND DISCUSSION

Analytical measurements: No analytics were carried out.

Biological results: Mortality of juvenile European common frogs differed largely between the two pyraclostrobin formulations. The formulation Headline®, (naphta content 67%) caused 100% mortality just after 1 h at the test concentration of 880 ml formulation/ha. This test concentration corresponds to the maximum label rate for the product for commercial use. No effect was observed at 0.1x this rate. BAS 500 18 F, the formulation with the lower naphta content (<25%), revealed 20% mortality after 5 days at 880 ml formulation/ha and after 6 days at 8800 ml formulation/ha (*i.e*. the same level as the corresponding control run 'D'). In the four control runs mortality ranged from 0% to 20%. No statistic comparisons were carried out. The results are summarized in Table 8.1.4-1.

Table 8.1.4-1: Effects of Headline® (active substance: pyraclostrobin) and BAS 500 18 F (active substance: pyraclostrobin) on survival of juvenile *Rana temporaria*

Treat-ment	Nominal appli-cation rate	Corres-ponding control run	n*	Mortality (%)									
				1h	2h	4h	24h	2d	3d	4d	5d	6d	7d
Control Run A	--	--	10	0	0	0	0	0	0	0	0	0	0
Control Run B	--	--	10	0	0	0	0	0	0	10	10	10	10
Control Run C	--	--	10	0	0	0	0	0	0	0	0	0	0
Control Run D	--	--	10	0	0	0	10	20	20	20	20	20	20
Headline®													
0.1 x Label rate	88 mL/ha	D	5	0	0	0	0	0	0	0	0	0	0
Label rate	880 mL/ha	D	3	100	100	100	100	100	100	100	100	100	100
BAS 500 18 F													
0.1 x Label rate	88 mL/ha	B	5	0	0	0	0	0	0	0	0	0	0
Label rate	880 mL/ha	C	5	0	0	0	0	0	0	0	20	20	20
10 x Label rate	8800 mL/ha	D	5	0	0	0	0	0	0	0	0	20	20

* n = sample number

Considering the significant differences in toxicity of the two formulations containing the same level of the active substance pyraclostrobin the authors speculate that other chemicals in the formulation may play a major role in the effect size of pesticides.

III. CONCLUSION

The formulation Headline® (naphta content 67%) caused 100% mortality under laboratory conditions at an application rate of 880 ml formulation/ha. No effect was observed at 0.1x this rate. BAS 500 18 F, the formulation with the lower naphta content (<25%), revealed 20% mortality both at 880 ml formulation/ha and at 8800 ml formulation/ha, which is at the same level as the corresponding control run.

CA 8.1.5 Endocrine disrupting properties

An assessment of all available data in mammalian toxicology has come to the conclusion that there is no indication that pyraclostrobin has any potential to influence known endocrine mechanisms (for more detailed information please see M-CA 5.8.3).

Furthermore, neither the avian reproductive studies nor any studies conducted with fish give any indication for an endocrine activity of pyraclostrobin (for more detailed information please see M-CA 8.1.1.1, M-CP 10.1.1 and M-CP 10.2).

REFERENCES

Belden, J., McMurry, S., Smith, L., & Reilley, P. (2010). Acute toxicity of fungicide formulations to amphibians at environmentally relevant concentrations. Environmental Toxicology and Chemistry, 29(11), 2477-2480.

Bruehl, C. A., Schmidt, T., Pieper, S., & Alscher, A. (2013). Terrestrial pesticide exposure of amphibians: An underestimated cause of global decline?. Scientific reports, 3.

CA 8.2 Effects on aquatic organisms

Since Annex I inclusion of the active substance pyraclostrobin (BAS 500 F), new studies on aquatic organisms have been performed with the active substance and its major metabolites. As a result, there are new endpoints, which are considered in the aquatic risk assessment. Summaries of these new studies are provided below. For completeness this includes some older studies that had not been submitted during the previous Annex I inclusion process (e.g. because there is no respective data requirement in the EU) and also EU agreed studies that have been amended in the meantime (e.g. due to recalculations of endpoints or a new evaluation according to current guidelines). In addition, summaries are provided for peer-reviewed scientific literature that were considered to be of relevance (and reliable) for the aquatic risk assessment of pyraclostrobin.

Further information on the EU agreed studies, which have been already evaluated within the previous Annex I inclusion process of pyraclostrobin, can be found in the Monograph (Vol. 3, Annex B.9, August 2001) and the EU Review Report (SANCO/1420/2001-final, September 2004). Furthermore, the reports of these studies will be provided to RMS, co-RMS and EFSA and are available upon request for other member states.

References used within the following chapter, but not provided with the dossier, are given at the end of M-CA 8.2.

In general, different code numbers of different numbering systems are assigned to the metabolites of pyraclostrobin. In this chapter all metabolites are identified by the BF-code and synonym metabolite codes are given in brackets only where deemed to be helpful.

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.2-1.

Table 8.2-1: List of studies and endpoints with aquatic organisms and the active substance pyraclostrobin (BAS 500 F) and its major metabolites

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)	EU agreed
Active substance: pyraclostrobin				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	0.00616	1999/11414	yes
O. mykiss [1)]	96 h LC_{50}	0.00620	2000/5034	no (new data; requested by US-EPA)
Cyprinodon variegatus [1), 2)]	96 h LC_{50}	0.0769	2000/5032	no (new data; requested by US-EPA)
Cyprinus carpio	96 h LC_{50}	0.0177 [3)] (> 0.0121 < 0.0252)	1998/11580	yes
Danio rerio [4)]	96 h LC_{50}	0.0619 [5)] (0.0558 - 0.0680)	1999/11834	yes
Lepomis macrochirus	96 h LC_{50}	0.0254 [5)] (0.0233 - 0.0275)	1998/10951	yes

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)	EU agreed
L. macrochirus [1)]	96 h LC_{50}	0.0114	2000/5033	no (new data; requested by US-EPA)
Leuiscus idus melanotus [4)]	96 h LC_{50}	0.0191 [3)] (> 0.0135 < 0.0270)	1999/11835	yes
Oryzias latipes [4)]	96 h LC_{50}	0.0533 [5)] (0.0444 - 0.0622)	1999/11821	yes
Pimephales promelas [4)]	96 h LC_{50}	0.0161 [5)] (0.0141 - 0.0181)	1999/11833	yes
O. mykiss	28 d NOEC	0.00464	1999/11249	yes
O. mykiss	98 d NOAEC (ELS study)	0.00235	1999/11343	yes
C. variegatus [1), 2)]	36 d NOEC (ELS study)	0.0108	2000/5247	no (new data; requested by US-EPA)
P. promelas [1)]	36 d NOEC (ELS study)	0.00414	2000/5053	no (new data; requested by US-EPA)
L. macrochirus	BCF (whole fish; unchanged parent)	379 - 507	1999/11348	yes
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	0.0157	1999/10444 + amendment: 1999/10739	yes
Americamysis bahia [1), 2)] (former name: *Mysidopsis bahia*)	48 h LC_{50}	> 0.00597 [6)]	2000/5031	no (new data; requested by US-EPA)
Crassostrea virginica [1), 2)]	96 h EC_{50}	0.0125	2000/5042	no (new data; requested by US-EPA)
D. magna	21 d NOEC	0.0040	1999/11864	yes
A. bahia [1), 2)]	28 d NOEC (LC study)	0.00050	2004/5000004	no (new data; requested by US-EPA)
A. bahia [1), 2)]	31 d NOEC (LC study)	0.000365	2013/7002075	no (new data; requested by US-EPA)
Sediment dwelling aquatic invertebrates				
Leptocheirus plumulosus [1), 2)]	10 d LC_{50} (spiked sediment)	4.412 mg/kg dry sediment	2013/7000055	no (new data; requested by US-EPA)
Chironomus riparius	28 d NOEC (spiked water)	0.040	2000/1000010	yes
C. riparius [1)]	28 d NOEC (spiked sediment)	1.37 mg/kg dry sediment	2012/1185699	no (new study)

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)	EU agreed
Algae				
Pseudokirchneriella subcapitata (syn. Selenastrum capricornutum)	72 h E_rC_{50}	> 0.843 [9]	1999/11020 + supplement: 2009/1037148	yes, but recalculation of endpoints
	72 h E_yC_{50}	0.148 [7]		
Anabaena flos-aquae [1]	96 h E_rC_{50}	1.41 [9), 10]	2000/5036	no (new data; requested by US-EPA)
	96 h E_bC_{50}	0.367 [9), 10]		
Navicula pelliculosa [1]	72 h E_rC_{50}	0.0158 [8]	2000/5046	no (new data; requested by US-EPA)
	72 h E_bC_{50}	0.00165 [8]		
Skeletonema costatum [1), 2]	72 h E_rC_{50}	0.0962 [8), 10]	2000/5035	no (new data; requested by US-EPA)
	72 h E_bC_{50}	< 9.73 [8), 10]		
Aquatic macrophytes				
Lemna gibba [1]	14 d E_bC_{50}	> 1.72 [11] / 1.72 [12]	2000/5037	no (new data; requested by US-EPA)
Higher tier studies				
O. mykiss [1]	96 h LC_{50} (TTE study)	> 0.0270 (0.5 h) [13]	2000/1014919	no (but data used in previous product registrations)
		0.0220 (2 h) [13]		
		0.0150 (8 h) [13]		
O. mykiss	97 d NOEC (ELS study with multiple exposure)	0.005	1999/11537	yes
Outdoor mesocosm (multiple applications) [4]	6 mo NOEC	0.0080	2000/1000011 + supplement: 2012/1357084	yes, plus additional new evaluation according to current guidance
	6 mo NOEAEC	> 0.0080 < 0.0240		
Metabolite: BF 500-3 (Reg. No. 340266; 500M07)				
Fish				
O. mykiss [1]	96 h LC_{50}	> 0.0948	2007/1010836	no (new study; requested by Canadian authorities)
Aquatic invertebrates				
D. magna [1]	48 h EC_{50}	> 0.100	2006/1038907	no (new study; requested by Canadian authorities)
Sediment dwelling organisms				
C. riparius [1]	28 d NOEC (spiked sediment)	≥ 16.0 mg/kg dry sediment	2013/1237446	no (new study)

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)	EU agreed
Algae				
P. subcapitata [1)]	72 h E_rC_{50} / E_yC_{50}	> 1.17	2006/1038445 [8)]	no (new study; requested by Canadian authorities)
Metabolite: BF 500-5 (Reg. No. 298327; 500M04)				
Fish				
O. mykiss [1)]	96 h LC_{50}	11.3	2013/1349200	no (new study)
Aquatic invertebrates				
D. magna [1)]	48 h EC_{50}	> 10.0	2013/1349201	no (new study)
Algae				
P. subcapitata [1)]	72 h E_rC_{50}	5.33	2013/1349202	no (new study)
	72 h E_yC_{50}	2.03		
Metabolite: BF 500-6 (Reg. No. 364380; 500M01) [14)]				
Sediment dwelling organisms				
C. riparius [1)]	28 d NOEC (spiked sediment)	1.2 mg/kg dry sediment	2014/1001481	no (new study)
Metabolite: BF 500-7 (Reg. No. 369315; 500M02) [14)]				
Sediment dwelling organisms				
C. riparius [1)]	28 d NOEC (spiked sediment)	≥ 123.5 mg/kg dry sediment	2014/1001482	no (new study)
Metabolite: BF 500-11 (Reg. No. 411847; 500M60)				
Fish				
O. mykiss	96 h LC_{50}	> 100	1999/11909	yes
Aquatic invertebrates				
D. magna	48 h EC_{50}	> 100	1999/11917	yes
Algae				
Scenedesmus subspicatus	72 h E_rC_{50} / E_bC_{50}	> 100	1999/11918	yes
Metabolite: BF 500-13 (Reg. No. 412785; 500M62)				
Fish				
O. mykiss	96 h LC_{50}	> 50 < 100	1999/11913	yes
Aquatic invertebrates				
D. magna	48 h EC_{50}	> 100	1999/11921	yes
Algae				
S. subspicatus	72 h E_rC_{50}	> 100	1999/11922	yes
	72 h E_bC_{50}	66.0		

Organism	Endpoint	Value [mg/L]	Reference (BASF DocID)	EU agreed
Metabolite: BF 500-14 (Reg. No. 413039; 500M76)				
Fish				
O. mykiss	96 h LC_{50}	> 39.4 < 82.6	1999/11837	yes
Aquatic invertebrates				
D. magna	48 h EC_{50}	> 60.9	1999/11910	yes
Algae				
S. subspicatus	72 h E_rC_{50}	> 100	1999/11914	yes
	72 h E_bC_{50}	46.6		

US-EPA = United States Environmental Protection Agency; ELS = early life stage; NO(E)AEC = No observed (ecologically) adverse effect concentration; LC = life cycle; TTE = Time-To-Effect/Event

1) Study has not been submitted during the Annex I inclusion process of pyraclostrobin. A study summary is provided below.
2) marine / saltwater species
3) Geometric mean plus corresponding LC_0 (>) and LC_{100} (<) values given in brackets.
4) Study was performed with the solo-formulation BAS 500 00 F (containing 250 g pyraclostrobin/L, nominally).
5) Spearman-Karber estimate of LC_{50} plus confidence limits given in brackets.
6) The 48-h LC_{50} obtained in the 96 h study is used in the risk assessment according to EU Regulation 283/2013 on the data requirements for active substances and the EFSA Aquatic Guidance (EFSA, 2013).
7) In accordance to recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints of the EU agreed study on the green alga have been (re-)calculated from original data. The re-calculated values are presented here and are used in the aquatic risk assessment; for details on these calculations please refer to the supplement. A summary of this study and the re-calculations is provided below.
8) In accordance to recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints obtained in the 96 h / 120 h alga studies are considered as relevant endpoint and are presented here
9) In this study the 24, 48, and 72 hour effective concentrations were not calculated because sufficient growth had not yet occurred in any of the test groups to allow for these calculations; thus the 96 h endpoints obtained in the 120 h alga studies are considered as relevant endpoint and are presented here.
10) Due to the reasoning given below, the study on this alga species is considered to be not valid.
11) based on frond numbers
12) based on dry weight
13) Endpoint obtained in the TTE study from respective exposure duration, i.e. the value in brackets (0.5, 2 or 8 hours exposure time).
14) The pyraclostrobin metabolites BF 500-6 and BF 500-7 do not occur to any significant extent in water; they are thus considered to be of relevance only in sediment and therefore, and also for animal welfare reasons, only studies on the sediment dwelling insect *C. riparius* have been performed.

CA 8.2.1 Acute toxicity to fish

An acute toxicity study with rainbow trout was already evaluated during the previous Annex I inclusion process. The following additional (flow through) study with rainbow trout (*Oncorhynchus mykiss*) performed with the active substance pyraclostrobin was conducted due to U.S. data requirements and is provided for completeness.

Report:	CA 8.2.1/1 [redacted].et al., 2000a Flow-through acute toxicity of BAS 500F to the rainbow trout, Oncorhynchus mykiss 2000/5034
Guidelines:	EPA 72-1(c), EPA 850.1075
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour flow-through acute toxicity laboratory study, juvenile rainbow trout were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0013, 0.0022, 0.0036, 0.0060 and 0.010 mg pyraclostrobin/L (corresponding to mean measured concentrations of 0.0014, 0.0022, 0.0036, 0.0053 and 0.0090 mg a.s./L) in groups of 10 animals in glass aquaria containing 15 L water. Fish were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality was observed in the dilution water control, the solvent control and at test item concentrations of up to and including 0.0036 mg a.s./L, whereas 20% mortality was observed at 0.0053 mg a.s./L. At the highest tested concentration, all fish were dead after 96 hours of exposure.

In a flow-through acute toxicity study with rainbow trout the LC_{50} (96 h) of pyraclostrobin was 0.0062 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0036 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C, purity: 97.09%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*), juveniles; mean body length of control fish: 39.2 mm; mean wet weight of control fish: 0.55 g; supplied by Thomas Fish Company, Anderson, California, USA.

Test design: Flow through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control, 2 replicates per treatment; 10 fish per aquarium (loading 0.37 g fish/L); assessment of mortality and sublethal effects directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.0013, 0.0022, 0.0036, 0.0060 and 0.010 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.0014, 0.0022, 0.0036, 0.0053 and 0.0090 mg a.s./L.

Test conditions: 20 L glass aquaria, test volume: 15 L; dilution water: deionized, carbon filtered and aerated water; flow rate: 6.1 volume additions per 24 hours on average in each test vessel; hardness: 40 mg $CaCO_3$/L; temperature: 11.1 - 13.0°C; pH 7.3 - 7.9; oxygen content: 10.4 mg/L - 10.6 mg/L; conductivity: 130 - 140 µmhos/cm; photoperiod 16 h light : 8 h dark; light intensity: approx. 52 foot candles; no aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics; binomial/ nonlinear interpolation for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of pyraclostrobin concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of pyraclostrobin ranged from 86 to 115% of nominal at test initiation and from 88 to 111% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control, the solvent control and at test item concentrations of up to and including 0.0036 mg a.s./L, whereas 20% mortality was observed at 0.0053 mg a.s./L. At the highest tested concentration, all fish were dead after 96 hours of exposure. Sub-lethal effects (i.e. change in coloration) were found at 0.0053 mg a.s./L after 96 hours. The results are summarized in Table 8.2.1-1.

Table 8.2.1-1: Acute toxicity (96 h) of pyraclostrobin to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0013**	**0.0022**	**0.0036**	**0.0060**	**0.010**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.0014**	**0.0022**	**0.0036**	**0.0053**	**0.0090**
Mortality [%] (96 h)	0	0	0	0	0	20	100
Symptoms (after 96 h) *	none	none	none	none	none	C	n.d.
Endpoints [mg pyraclostrobin/L] (mean measured)							
LC_{50} (96 h)	**0.0062** (95% confidence limits: 0.0053 - 0.0090)						
NOEC (96 h)	0.0036						

n.d. = not determined; all fish dead
* Symptoms after 96 h: C = change in coloration.

III. CONCLUSION

In a flow-through acute toxicity study with rainbow trout the LC_{50} (96 h) of pyraclostrobin was 0.0062 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0036 mg a.s./L.

The following acute toxicity study with bluegill sunfish (*Lepomis macrochirus*) performed with the active substance pyraclostrobin is not required for registration in the EU and it has not been evaluated previously on EU level. However, the study was conducted due to U.S. data requirements and is provided for completeness.

Report:	CA 8.2.1/2 [redacted] et al., 2000b Flow-through acute toxicity of BAS 500F to the bluegill sunfish, Lepomis macrochirus 2000/5033
Guidelines:	EPA 72-1(a), EPA 850.1075
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour flow-through acute toxicity laboratory study, juvenile bluegill sunfish were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0065, 0.011, 0.018, 0.030 and 0.050 mg pyraclostrobin/L (corresponding to mean measured concentrations of 0.0061, 0.0095, 0.0159, 0.0269 and 0.0451 mg a.s./L) in groups of 10 animals in glass aquaria containing 15 L water. Fish were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality was observed in the dilution water control, the solvent control and at the lowest test item concentration of 0.0061 mg a.s./L, whereas 15% mortality was observed at 0.0095 mg a.s./L. At the three highest test item concentrations all fish were dead after 96 hours of exposure.

In a flow-through acute toxicity study with bluegill sunfish the LC_{50} (96 h) of pyraclostrobin was 0.0114 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0061 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C, purity: 97.09%.

B. STUDY DESIGN

Test species: Bluegill sunfish (*Lepomis macrochirus*), juveniles; mean body length of control fish: 23.8 mm; mean wet weight of control fish: 0.11 g; supplied by "Osage Catfisheries", Osage Beach, Missouri, USA.

Test design: Flow through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control, 2 replicates per treatment; 10 fish per aquarium (loading 0.073 g fish/L); assessment of mortality and sublethal effects directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.0065, 0.011, 0.018, 0.030 and 0.050 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.0061, 0.0095, 0.0159, 0.0269 and 0.0451 mg a.s./L.

Test conditions: 20 L glass aquaria, test volume: 15 L; dilution water: deionized, carbon filtered and aerated water, flow rate: 6.3 volume additions per 24 hours on average in each test vessel; hardness: 48 mg $CaCO_3$/L; temperature: 21.2°C - 22.5°C; pH 7.3 - 7.9; oxygen content: 8.7 mg/L - 9.4 mg/L; conductivity: 130 - 160 µmhos/cm; photoperiod: 16 h light : 8 h dark; light intensity: approx. 52 foot candles; no aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; binomial/nonlinear interpolation for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of pyraclostrobin concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of pyraclostrobin ranged from 85 to 93% of nominal at test initiation and from 85% to 94% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control, the solvent control and at the lowest test item concentration of 0.0061 mg a.s./L, whereas 15% mortality was observed at 0.0095 mg a.s./L. At the three highest test item concentrations, all fish were dead after 96 hours of exposure. After 96 hours of exposure, sub-lethal effects (i.e. lethargy and loss of equilibrium) were found at 0.0095 mg a.s./L. The results are summarized in Table 8.2.1-2.

Table 8.2.1-2: Acute toxicity (96 h) of pyraclostrobin to bluegill sunfish (*Lepomis macrochirus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0065**	**0.011**	**0.018**	**0.030**	**0.050**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.0061**	**0.0095**	**0.0159**	**0.0269**	**0.0451**
Mortality [%] (96 h)	0	0	0	15	100	100	100
Symptoms (after 96 h) *	none	none	none	L	n.d.	n.d.	n.d.
Endpoints [mg pyraclostrobin/L] (mean measured)							
LC_{50} (96 h)	**0.0114** (95% confidence limits: 0.0095 - 0.0159)						
NOEC (96 h)	0.0061						

n.d. = not determined; all fish dead
* Symptoms after 96 h: L = lethargy and loss of equilibrium.

III. CONCLUSION

In a flow-through acute toxicity study with bluegill sunfish the LC_{50} (96 h) of pyraclostrobin was 0.0114 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0061 mg a.s./L.

The following acute toxicity study with the marine fish species sheepshead minnow (*Cyprinodon variegatus*) performed with the active substance pyraclostrobin is not required for registration in the EU and it has not been evaluated previously on EU level. However, the study was conducted due to U.S. data requirements and is provided for completeness.

Report: CA 8.2.1/3
[redacted]., 2000c
Flow-through acute toxicity of BAS 500F to the sheepshead minnow, Cyprinodon variegatus
2000/5032
Guidelines: EPA 72-3(a), EPA 850.1075
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour flow-through acute toxicity laboratory study, juvenile sheepshead minnow were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.013, 0.022, 0.036, 0.060 and 0.10 mg pyraclostrobin/L (corresponding to mean measured concentrations of 0.0108, 0.0188, 0.0302, 0.0535 and 0.0879 mg a.s./L) in groups of 10 animals in glass aquaria containing 15 L water. Fish were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality was observed in the dilution water control, the solvent control and at test item concentrations of up to and including 0.0535 mg a.s./L, whereas 75% mortality was observed at the highest tested concentration of 0.0879 mg a.s./L.

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of pyraclostrobin was 0.0769 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0535 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C, purity: 97.09%.

B. STUDY DESIGN

Test species:	Sheepshead minnow (*Cyprinodon variegatus*), juveniles; mean body length of control fish: 21.0 mm; mean wet weight of control fish: 0.16 g; supplied by "Aquatic BioSystems", Fort Collins, Colorado, USA.
Test design:	Flow through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control; 2 replicates per treatment; 10 fish per aquarium (loading 0.11 g fish/L); assessment of mortality and sublethal effects directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.013, 0.022, 0.036, 0.060 and 0.10 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.0108, 0.0188, 0.0302, 0.0535 and 0.0879 mg a.s./L.
Test conditions:	20 L glass aquaria, test volume: 15 L; dilution water: filtered natural seawater mixed with deionized water, salinity: 15 - 16 ‰; flow rate: 5.7 volume additions per 24 hours on average per test vessel; temperature: 21.8°C- 22.8°C; pH 7.8 - 7.9; oxygen content: 7.4 mg/L - 8.1 mg/L; photoperiod 16 h light : 8 h dark; light intensity: approx. 53 foot candles; no aeration; no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.
Statistics:	Descriptive statistics; binomial/nonlinear interpolation for calculation of the LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of pyraclostrobin concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of pyraclostrobin ranged from 82 to 89% of nominal at test initiation and from 83 to 89% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control, the solvent control and at test item concentrations of up to and including 0.0535 mg a.s./L, whereas 75% mortality was observed at the highest tested concentration of 0.0879 mg a.s./L. No sub-lethal effects were found in the control groups and in all test item treatments after 96 hours. The results are summarized in Table 8.2.1-3.

Table 8.2.1-3: Acute toxicity (96 h) of pyraclostrobin to sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.013**	**0.022**	**0.036**	**0.060**	**0.10**
Concentration [mg a.s./L] (mean measured)	**Control**	**Solvent control**	**0.0108**	**0.0188**	**0.0302**	**0.0535**	**0.0879**
Mortality [%] (96 h)	0	0	0	0	0	0	75
Symptoms (after 96 h)	none	none	none	none	none	none	none
Endpoints [mg pyraclostrobin/L] (mean measured)							
LC_{50} (96 h)	**0.0769** (95% confidence limits: 0.0535 - 0.0879)						
NOEC (96 h)	0.0535						

III. CONCLUSION

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of pyraclostrobin was 0.0769 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0535 mg a.s./L.

The following acute toxicity study on rainbow trout (*Oncorhynchus mykiss*) performed with the metabolite BF 500-3 was requested by Canadian authorities and has not been evaluated previously on EU level.

Report:	CA 8.2.1/4 [redacted] 2007a Reg.No. 340266 (metabolite BF 500-3 of BAS 500 F): Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in a static system over 96 hours 2007/1010836
Guidelines:	OECD 203, EEC 92/69 A V C 1, EPA 72-1, EPA 850.1075
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, juvenile rainbow trout were exposed to BF 500-3 (metabolite of pyraclostrobin) in two replicates per concentration with 10 animals in aquaria containing 50 L water at nominal concentrations of 0.0050, 0.010, 0.022, 0.050 and 0.10 mg/L (corresponding to mean measured concentrations of 0.0034, 0.0073, 0.018, 0.042 and 0.095 mg/L). Additionally, a solvent (acetone) control and a dilution water control were set up. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and after 4, 24, 48, 72 and 96 hours of exposure.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality or other symptoms of toxicity were observed including the highest test concentrations, which is at or even slightly above the maximum solubility of this metabolite.

In a static acute toxicity study with rainbow trout no mortality or other effects were observed up to the solubility limits of the test substance. The LC_{50} (96 h) of BF 500-3 (metabolite of pyraclostrobin) was determined to be > 0.0948 mg/L based on mean measured concentrations. The NOEC was ≥ 0.0948 mg/L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-3 (Reg. No. 340 266, synonym: 500M07; metabolite of pyraclostrobin), batch no. 01311-150, purity: 100.0%.

B. STUDY DESIGN

Test species:	Rainbow trout (*Oncorhynchus mykiss*), juveniles, approx. 4 months old; body length 5.2 cm (4.0 - 6.0 cm); body weight 1.24 g (0.66 - 1.93 g); supplied by 'Forellenzucht Trostadt GbR', Trostadt, Germany.
Test design:	Static system (96 hours); 5 test item concentrations plus a dilution water control and a solvent control, 2 replicates per treatment; 10 fish per aquarium (loading 0.2 g fish/L); assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), solvent control (0.1 mL acetone/L), 0.0050, 0.010, 0.022, 0.050 and 0.10 mg BF 500-3/L (nominal); corresponding to mean measured concentrations of 0.0034, 0.0073, 0.018, 0.042 and 0.095 mg/L.
Test conditions:	Glass aquaria with stainless steel frame (0.60 x 0.35 x 0.40 m); water volume: 50 L; dilution water: non chlorinated charcoal filtered tap water mixed with deionized water; temperature: 11°C; pH 7.5 -7.8; oxygen content: 9.0 mg/L - 9.1 mg/L; total hardness: 100 mg $CaCO_3$/L; conductivity: approx. 250 µS/cm; acid capacity: approx. 2.5 mmol/L; photoperiod 16 h light : 8 h dark; light intensity: 36 - 191 lux; no aeration, no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each replicate at the beginning of the test, after 48 hours and at the end of the test. Measured values for BF 500-3 ranged from 107 to 116% of nominal at test initiation and from 36 to 56% at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality or other symptoms of toxicity were observed including the highest test concentrations, which is at or even slightly above the maximum solubility of this metabolite. The results are summarized in Table 8.2.1-4.

Table 8.2.1-4: Acute toxicity (96 h) of BF 500-3 (metabolite of pyraclostrobin) to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**Control**	**Solvent control**	**0.0050**	**0.010**	**0.022**	**0.050**	**0.10**
Concentration [mg/L] (mean measured)	**--**	**--**	**0.0034**	**0.0073**	**0.018**	**0.042**	**0.095**
Mortality [%] (96 h)	0	0	0	0	0	0	0
Symptoms (after 96 h)	none	none	none	none	none	none	none
Endpoints [mg BF 500-3/L] (mean measured)							
LC_{50} (96 h)	**> 0.0948**						
NOEC (96 h)	≥ 0.0948						

III. CONCLUSION

In a static acute toxicity study with rainbow trout no mortality or other effects were observed up to the solubility limits of the test substance. The LC_{50} (96 h) of BF 500-3 (metabolite of pyraclostrobin) was determined to be > 0.0948 mg/L based on mean measured concentrations. The NOEC was ≥ 0.0948 mg/L.

The following acute toxicity study on rainbow trout (*Oncorhynchus mykiss*) performed with the metabolite BF 500-5 is provided in support of the aquatic risk assessment. Due to new data requirements this new metabolite was identified and tested and has not been evaluated previously on EU level.

Report:	CA 8.2.1/5 [redacted] 2014a Acute toxicity of Reg.No. 298327 (BF 500-5, metabolite of Pyraclostrobin) to rainbow trout (Oncorhynchus mykiss) in a 96-hour static test 2013/1349200
Guidelines:	OECD 203 (1992), EC 440/2008 C.1 Acute Toxicity for Fish
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In a static acute toxicity laboratory study, juvenile rainbow trout were exposed to BF 500-5 (metabolite of pyraclostrobin) at nominal concentrations of 1, 2, 4, 8 and 16 mg/L and a water control in one replicate per concentration with 7 animals in aquaria containing 14 L water. Fish were observed for survival and symptoms of toxicity 2, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on nominal concentrations. After 96 hours of exposure no mortality was observed in the control and at test item concentrations of up to and including 8 mg BF 500-5/L, whereas 100% mortality occurred at the highest tested concentration of 16 mg/L.

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of BF 500-5 (metabolite of pyraclostrobin) was 11.3 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 8 mg/L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-5 (Reg.No. 298 327, synonym: 500M04, metabolite of pyraclostrobin); batch no.: L84-174; purity: 99.6% (± 1.0%)

B. STUDY DESIGN

Test species:	Rainbow trout (*Oncorhynchus mykiss*); juveniles; average body length 5.39 cm ± 0.4 cm; average body weight: 1.57 ± 0.29 g; supplied by "Forellenzuchtbetrieb Störk", Bad Saulgau, Germany.
Test design:	Static system (96 hours); 7 fish per aquarium (loading: 0.79 g fish/L) and per concentration, assessments of mortality and symptoms of toxicity 2, 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	0 (control), 1, 2, 4, 8 and 16 mg BF 500-5/L (nominal).
Test conditions:	16 L glass aquaria; test volume: 14 L; deionized water with analytical grade salts (ISO Medium); temperature: 13.1°C - 13.5°C; pH 7.1 - 7.9; oxygen saturation: 85% - 100%; hardness: 250.0 mg $CaCO_3$/L; photoperiod: 16 h light: 8 h dark, light intensity: 480 - 1080 lux, gentle aeration, no feeding.
Analytics:	Analytical verification of the test item was conducted using an HPLC-method with MS/MS-detection
Statistics:	Descriptive statistics. Fisher`s Exact Binomial Test with Bonferroni Correction for determination of the NOEC; probit analysis for determination of the EC_{50} value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The mean analyzed contents of BF 500-5 ranged from 82 to 120% of nominal at test initiation and from 85 to 105% of nominal at test termination. As the initially measured concentrations confirmed the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 96 hours of exposure no mortality was observed in the control and at test item concentrations of up to and including 8 mg BF 500-5/L, whereas 100% mortality occurred at the highest tested concentration of 16 mg/L. After 96 hours of exposure, sub-lethal effects were observed at test item concentrations of 4 mg/L (i.e. mucus secretion) and 8 mg/L (i.e. tumbling during swimming, fish lying at the side or back, distended abdomen and dark coloration). Statistically significant differences in the mortality rates compared to the control were observed at the highest test item concentrations of 16 mg/L (Fisher's Exact Test, $\alpha = 0.05$). For results see Table 8.2.1-5.

Table 8.2.1-5: Acute toxicity (96 h) of BF 500-5 (metabolite of pyraclostrobin) on rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] (nominal)	**Control**	**1**	**2**	**4**	**8**	**16**
Mortality [%] (96 h)	0	0	0	0	0	100 *
Symptoms (96 h) $	none	none	none	MS	DA, DC, SB, TS	n.d.
Endpoints [mg BF 500-5/L] (nominal)						
LC_{50} (96 h) #	11.3 (95% confidence limits: 8 - 16)					
NOEC (96 h)	8					

n.d. = not determined; all fish dead

* Statistically significantly different compared to the control (Fisher's Exact Test, $\alpha = 0.05$)

$ Symptoms after 96 h: MS = mucus secretion, DA = distended abdomen; DC = dark coloration; SB = fish lying at the side or back, TS = tumbling during swimming

\# The LC_{50} was calculated as the geometric mean of the highest concentration causing no mortality and the lowest concentration resulting in 100% mortality.

III. CONCLUSION

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) of BF 500-5 (metabolite of pyraclostrobin) was 11.3 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 8 mg/L (nominal).

To strengthen the ecotoxicological profile of pyraclostrobin and to justify the potential use of time weighted average concentrations (respectively the consideration of short-term exposure pulses), an acute time to effect study was conducted with different exposure pulses and rainbow trout. The study has been used in previous end-use product registrations, but has not been evaluated during the previous Annex I evaluation process.
This study had erroneously not been listed in the application submitted for renewal of approval.

Report:	CA 8.2.1/6 [redacted] 2000a BAS 500 F - Acute toxicity study on the rainbow trout (Oncorhychus mykiss) after short time exposure over 0.5, 2 and 8 hours in a flow-through system followed up by a post exposure period 2000/1014919
Guidelines:	EPA 72-1, EEC 92/69 A V C 1, OECD 203
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The acute toxicity of pyraclostrobin to rainbow trout (*Oncorhynchus mykiss*) was evaluated after short time exposure over 0.5, 2 and 8 hours in a flow-through test system followed by a post-exposure period until 96 h after start of exposure. Fish were exposed to a water control and at nominal test item concentrations of 0.0080, 0.012, 0.018 and 0.027 mg a.s./L in groups of 30 animals per concentration in glass aquaria containing approximately 100 L water. After 0.5 hours one third, after 2 hours half and after 8 hours the rest of the surviving fish were transferred into new chambers with pure dilution water for the post-exposure period. Fish were observed for survival and symptoms of toxicity at test initiation, at the end of respective exposure periods, during the post-exposure period directly after transfer, in the earlier transferred exposure groups at each time when another exposure group was transferred and at 24, 48, 72 and 96 hours after study start.

The biological results are based on nominal concentrations of the test item. No mortality occurred in the controls and in all test item concentrations of the 0.5 h exposure group over the whole study period. After peak exposure over 2 hours, 2 out of 20 fish (10%) were found dead at the highest test item concentration of 0.027 mg a.s./L. After 8 hours of exposure, 8 out of 10 fish (80%) were found dead at the second highest test item concentration of 0.018 mg a.s./L and all fish were dead at the highest tested concentration. At the end of the post-exposure period, 1 out of 10 fish (10%) and 8 out of 9 fish (89%) had died in the 2 h exposure group at the two highest test item concentrations, respectively. In the 8 h exposure group, 1 out of 10 fish (10%) was found dead at 0.012 mg a.s./L whereas no further mortality occurred at 0.018 mg a.s./L during the post-exposure period.

In a flow-through acute toxicity study with rainbow trout, the LC_{50} values (96 h) of pyraclostrobin were > 0.027, 0.022 and 0.015 mg a.s./L after exposure durations of 0.5, 2 and 8 hours followed by a post-exposure period until 96 hours after study start (nominal). In general, shorter exposure times lead to a marked reduction of toxicity in comparison to the results of the 96 hours standard test with *O. mykiss*.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. CP 029 053, purity: 99.0%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*), age: 4 - 5 months; mean body length: 5.6 cm (5.2 - 5.9 cm); mean body weight: 1.5 g (1.1 - 2.0 g); supplied by "Forellenhof Fredelsloh", Moringen, Germany.

Test design: Flow through system (96 h); 3 peak exposure groups: exposure over 0.5, 2 or 8 hours followed by a post-exposure period until 96 h after start of exposure; 4 test item concentrations plus a dilution water control, 1 replicate per treatment; 30 fish per concentration for the first (0.5 h) exposure period (loading: max. 0.45 g fish/L during exposure period, max. 1.67 g fish/L during post exposure period); after 0.5 hours one third, after 2 hours half and after 8 hours the rest of the surviving fish were transferred into new test chambers with pure dilution water for the post exposure period; assessment of mortality and sublethal effects each time, when fish were transferred to post exposure chambers, during post exposure period directly after transfer, in the earlier transferred exposure groups at each time when another exposure group was transferred and at 24, 48, 72 and 96 hours after study start.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), 0.0080, 0.012, 0.018 and 0.027 mg pyraclostrobin/L (nominal).

Test conditions:	Exposure period: glass aquaria (80 cm x 35 cm x 46 cm), test volume: approximately 100 L; flow rate: 20 L/hour/test vessel; no aeration; temperature: 13°C; pH 8.0 - 8.1; oxygen content: 10.2 mg/L - 10.7 mg/L. Post-exposure period: stainless steel aquaria (29 cm x 21 cm x 22 cm), dilution water volume: approximately 9 L; flow rate of dilution water: 6.5 L/hour/vessel; no aeration; temperature: 12°C; pH 8.0 - 8.1; oxygen content: 10.3 mg/L - 11.0 mg/L. Dilution water: carbon filtered and aerated non-chlorinated drinking water; hardness: approximately 250 mg $CaCO_3$/L. Photoperiod: 16 h light : 8 h dark.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.
Statistics:	Descriptive statistics; probit analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of pyraclostrobin concentrations was conducted in each concentration at the beginning and at the end of the 8 hour peak exposure period. The analyzed contents of pyraclostrobin ranged from 93.7 to 101.1% of nominal concentrations at test initiation and from 86.7 to 97.2% of nominal after 8 hours. As the measured concentrations confirmed the correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: No mortality occurred in the controls and in all test item concentrations of the 0.5 h exposure group over the whole study period. After peak exposure over 2 hours, 2 out of 20 fish (10%) were found dead at the highest test item concentration of 0.027 mg a.s./L. At this concentration, only 9 instead of 10 fish could thus be transferred to the post-exposure chambers and only 9 fish remained in the exposure chamber for further exposure. After 8 h peak exposure, 8 out of 10 fish (80%) were found dead at the second highest test item concentration of 0.018 mg a.s./L and all fish were dead at the highest tested concentration.
At the end of the post-exposure period, 1 out of 10 fish (10%) and 8 out of 9 fish (89%) had died in the 2 h peak treatment at the two highest test item concentrations, respectively. In the 8 h exposure group, 1 out of 10 fish (10%) was found dead at 0.012 mg a.s./L whereas no further mortality occurred at 0.018 mg a.s./L during the post-exposure period.
At the end of 0.5 h peak exposure, sub-lethal effects (*i.e.* swimming at the bottom, tottering and swimming at the surface) were observed at the highest test item concentration of 0.027 mg a.s./L. In the 2 h exposure group, swimming at the bottom, tottering and apathy were observed at the three highest test item concentrations and in the 8 h exposure group, tottering and apathy were observed at 0.012 and 0.018 mg a.s./L at the end of the respective exposure peaks. After the post-exposure period, no sub-lethal effects were observed at up to and including the highest test item concentration in all peak exposure treatments.

The results are summarized in Table 8.2.1-6.

Table 8.2.1-6: Effect of pyraclostrobin on rainbow trout (*Oncorhynchus mykiss*) in an acute toxicity study after peak exposure over 0.5, 2 and 8 hours followed by a post-exposure period

Concentration [mg a.s./L] (nominal)		**Control**	**0.0080**	**0.012**	**0.018**	**0.027**
Mortality during exposure[1)]	0.5 h peak exposure	0 / 30	0 / 30	0 / 30	0 / 30	0 / 30
	2 h peak exposure	0 / 20	0 / 20	0 / 20	0 / 20	2 / 20 [2)]
	8 h peak exposure	0 / 10	0 / 10	0 / 10	8 / 10	9 / 9
Mortality at end of post-exposure period (96 h)[3)]	0.5 h peak exposure	0 / 10	0 / 10	0 / 10	0 / 10	0 / 10
	2 h peak exposure	0 / 10	0 / 10	0 / 10	1 / 10	8 / 9
	8 h peak exposure	0 / 10	0 / 10	1 / 10	0 / 2	--
Symptoms during exposure *	0.5 h peak exposure	none	none	none	none	T, D, C
	2 h peak exposure	none	none	D, T	N, T	N
	8 h peak exposure	none	none	N, T	N	n.d.
Symptoms at end of post-exposure period (96 h)	0.5 h peak exposure	none	none	none	none	none
	2 h peak exposure	none	none	none	none	none
	8 h peak exposure	none	none	none	none	n.d.
Endpoints [mg pyraclostrobin/L] (nominal)						
LC_{50} (96 h)	0.5 h peak exposure	> 0.027				
	2 h peak exposure	0.022				
	8 h peak exposure	0.015				
NOEC (96 h)	0.5 h peak exposure	≥ 0.027				
	2 h peak exposure	0.012				
	8 h peak exposure	0.0080				

n.d. = not determined; all fish dead
1) Number of dead fish / total number of exposed fish
2) Only 9 surviving fish could be transferred to post-exposure chambers of the 8 h peak treatment
3) Number of dead fish / total number of fish transferred to post-exposure chambers
* Symptoms: D = swimming at the bottom, T = tottering, N = apathy, C = swimming at the surface

III. CONCLUSION

In a flow-through acute toxicity study with rainbow trout, the LC_{50} values (96 h) of pyraclostrobin were > 0.027, 0.022 and 0.015 mg a.s./L after exposure durations of 0.5, 2 and 8 hours followed by a post-exposure period until 96 hours after study start (nominal). In general, shorter exposure times lead to a marked reduction of toxicity in comparison to the results of the 96 hours standard test with *O. mykiss*.

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

The following early life-stage test on fathead minnow (*Pimephales promelas*) performed with the active substance pyraclostrobin is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and is provided for completeness.

Report:	CA 8.2.2.1/1 [redacted].et al., 2000i Early life stage toxicity of BAS 500 F to the fathead minnow, Pimephales promelas 2000/5053
Guidelines:	EPA 72-4(a), EPA 850.1400
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The chronic toxicity of pyraclostrobin to fathead minnow (*Pimephales promelas*) was evaluated in a 36-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0014, 0.0026, 0.0049, 0.010 and 0.020 mg pyraclostrobin/L (corresponding to mean measured concentrations of 0.000944, 0.00218, 0.00414, 0.00837 and 0.0161 mg a.s./L). Hatchability, survival rate and behavior of fathead minnow embryos and fry were assessed throughout the study. Individual fish lengths and weights were measured at test termination.

The results are based on mean measured concentrations. Egg hatch was complete on day 4 in the control groups and all test item treatments. Mean survival rates at hatch (day 4) and at test end (32 days post-hatch) were ≥ 90% in both the dilution water control, the solvent control and at test item concentrations up to and including 0.00837 mg a.s./L, whereas all fish had died before hatch (day 4) at the highest tested concentration of 0.0161 mg a.s./L. No statistically significant effects on survival, time to hatch, time to first feeding, total length, wet weight or dry weight compared to the pooled control were observed at concentrations of up to and including 0.00414 mg a.s./L. A slight sublethal effect on live, normal fathead minnow at 0.00414 mg/L was apparent on only 4% of the fish on one day (day 4), and although statistically significant, the effect was not considered to be of biological relevance.

In an early life-stage study with fathead minnow (*Pimephales promelas*) the overall NOEC (36 d) for pyraclostrobin was determined to be 0.00414 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F, Reg. No. 304 428), lot no. 27882/191/C, purity: 97.09%, dissolved in dimethylformamide (lot no. BV921).

B. STUDY DESIGN

Test species: Fathead minnow (*Pimephales promelas*), eggs less than 24 hours at test initiation, source: "Aquatic BioSystems, Inc.", Fort Collins, Colorado, USA.

Test design: Flow-through system (36 d); 5 test item concentrations plus a dilution water control and a solvent control; 4 replicate test chambers per treatment with 20 fertilized eggs in each; a proportional diluter system was used for intermittent introduction of the solutions to the test chambers. During the embryo stage, the developing embryos were incubated in glass cups. At the end of hatch (day 4), fish were released into the test chamber and randomly thinned to 10 fish per vessel. Daily assessment of hatch, survival, signs of toxicity and abnormal behavior. At test termination surviving animals were sacrificed and measured for length and weight.

Endpoints: NOEC values based on hatchability, survival, toxic signs and growth rates.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.0014, 0.0026, 0.0049, 0.010 and 0.020 mg a.s./L (nominal), corresponding to mean measured concentrations of 0.000944, 0.00218, 0.00414, 0.00837 and 0.0161 mg a.s./L.

Test conditions: Test vessels: 9 L glass aquaria (approx.15 x 30 x 20 cm) with a test volume of approx. 8.0 L; 4 replicate test chambers; glass incubation cups (used during embryo stage) closed on one end with Nitex® screen; two incubation cups per test chamber; dilution water: filtered deionized water sterilized with UV and aerated; temperature: 23.0°C - 26.4°C; pH 7.4 - 7.8; oxygen content: 8.0 mg/L - 9.4 mg/L; total hardness: 40 - 44 mg $CaCO_3$/L; conductivity: 130 - 180 µmhos/cm; light intensity: approx. 45 foot candles; photoperiod: 16 hours light : 8 hours dark; flow rate: approx. 6.6 volume additions per 24 hours per vessel; feeding: fish were fed 2-3 times daily *ad libitum* freshly hatched *Artemia salina* nauplii from day 5 onwards until 1 day before study termination; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; t-test for comparison of the dilution water control and solvent control data; ANOVA followed by Bonferroni's test or William's test to calculate NOEC values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in all concentrations at test initiation, at regular intervals during the study and test end, except for the highest test item concentration, where analytical measurements were only conducted on days 0 and 7. Recoveries of pyraclostrobin ranged from 74.5 to 90.0% of nominal concentrations in all treatments at test initiation and from 80.9 to 88.5% of nominal in the 0.0026, 0.0049 and 0.010 mg/L (nominal) treatments at test termination. Measured contents in samples from the lowest test item treatment were below the limit of quantification (LOQ = 0.50 µg/L) on days 35 and 36; this is believed to be due to an unobserved diluter malfunction on test days 35 and 36. All analysis at this concentration prior to that time resulted in recoveries ranging from 75 to 84% of nominal. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Egg hatch was complete on day 4 in the control and all test item treatments. Mean survival rates at hatch (day 4) and at test end (32 days post-hatch) were ≥ 90% in both the dilution water control and solvent control and at test item concentrations up to and including 0.00837 mg a.s./L, whereas all fish had died before hatch (day 4) in the highest tested concentration of 0.0161 mg a.s./L. No statistically significant effects on survival, time to hatch, time to first feeding, total length, wet weight or dry weight compared to the pooled control were observed at concentrations of up to and including 0.00414 mg a.s./L (Bonferroni's test / William's test). Sublethal effects, observed as fish exhibiting lethargy and/or a loss of equilibrium or change in coloration, were noted at 0.0161 mg a.s./L on day 3 (complete mortality occurred on day 4 at this concentration), at 0.00837 mg a.s./L on days 4 - 6, 8 - 12, and on day 34, and at 0.00414 mg a.s./L on day 4. These effects were not observed at any other time during the test. As the effect on live, normal fathead minnow at 0.00414 mg/L was apparent on only 4% of the fish on one day (day 4), and although statistically significant, the effect was not considered to be of biological relevance. The results are summarized in Table 8.2.2.1-1.

Table 8.2.2.1-1: Chronic toxicity of pyraclostrobin to fathead minnow (*Pimephales promelas*) in a fish early life-stage test (36 d)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0014**	**0.0026**	**0.0049**	**0.010**	**0.020**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.000944**	**0.00218**	**0.00414**	**0.00837**	**0.0161**
Embryo survival at hatch on day 4 [%]	98	99	99	99	94	90	0
Survival of larvae 32 days post hatch [%]	100	98	95	95	100	90	0
Percent live normal at hatch on day 4 [%]	98	99	99	99	90[#]	80	0
Percent live normal 32 days post hatch [%]	100	98	95	95	100	90	0
Mean total length [mm]	23.3	23.0	23.3	22.8	23.0	22.3	n.d.
Mean wet weight [mg]	110.5	108.8	108.5	106.9	107.0	96.8	n.d.
Mean dry weight [mg]	23.6	23.0	23.2	22.7	23.3	22.4	n.d.
Endpoints [mg pyraclostrobin/L] (mean measured)							
$NOEC_{overall}$ (36 d)	0.00414						

n.d. = not determined; no fish survived at the concentration above 0.00837 mg a.s./L.

[#] Because the effect on live, normal fathead minnow at 0.00414 mg a.s/L was apparent on only 4% of the fish on one day (day 4), the effect was not considered to be of biological significance.

III. CONCLUSION

In an early life-stage study with fathead minnow (*Pimephales promelas*) the overall NOEC (36 d) for pyraclostrobin was determined to be 0.00414 mg a.s./L based on mean measured concentrations.

The following fish early life-stage test on sheepshead minnow (*Cyprinodon variegatus*) performed with the active substance pyraclostrobin is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and is provided for completeness.

Report:	CA 8.2.2.1/2 [redacted] et al., 2001j Early life stage toxicity of BAS 500 F to the Sheepshead Minnow, Cyprinodon variegatus 2000/5247
Guidelines:	EPA 72-4
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The chronic toxicity of pyraclostrobin to sheepshead minnows (*Cyprinodon variegatus*) was evaluated in a 36-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0034, 0.0065, 0.013, 0.025 and 0.050 mg pyraclostrobin/L (corresponding to mean measured concentrations of 0.00291, 0.00557, 0.0108, 0.0240 and 0.0445 mg a.s./L). Hatchability, survival rate and behavior of sheepshead minnow embryos and fry were assessed throughout the study. Individual fish lengths and weights were measured at test termination.

The results are based on mean measured concentrations. Egg hatch was complete on day 4 in the control groups and the three lowest test item treatments. At 0.0240 mg a.s./L, egg hatch was completed on day 5. In the highest test item concentration of 0.0445 mg a.s./L, all animals were dead before hatch (day 4). Mean survival rates at hatch (day 4) and at test end (32 days post-hatch) were ≥ 95% in both the dilution water control and the solvent control and in the test item concentrations of up to and including 0.0108 mg a.s./L. At 0.0240 mg a.s./L mean survival was 29% at hatch on day 4 and 55% on day 32 post-hatch. No statistically significant effects on survival, time to hatch, time to first feeding, total length, wet weight or dry weight compared to the pooled control were observed at concentrations of up to and including 0.0108 mg a.s./L.

In an early life stage study with sheepshead minnows (*Cyprinodon variegatus*) the overall NOEC (36 d) for pyraclostrobin was determined to be 0.0108 mg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F, Reg. No. 304 428), lot no. N68, purity: 93.5%, dissolved in dimethylformamide (lot no. BW660).

B. STUDY DESIGN

Test species:	Sheepshead minnow (*Cyprinodon variegatus*); eggs less than 24 hours before test initiation, source: "Aquatic BioSystems, Inc.", Fort Collins Colorado, USA.
Test design:	Flow-through system (36 d); 5 test item concentrations plus a dilution water control and a solvent control; 4 replicate test chambers per treatment with 20 fertilized eggs in each; a proportional diluter system was used for intermittent introduction of the solutions to the test chambers. During the embryo stage, the developing embryos were incubated in glass cups. At the end of hatch (day 4 and 5), fish were released into the test chamber and randomly thinned to 10 fish per vessel. Daily assessment of hatch, swim-up, survival, signs of toxicity and abnormal behavior. On day 36 surviving animals were sacrificed and measured for length and weight.
Endpoints:	NOEC values based on hatch rate, post-hatch survival, sublethal effects, growth and time spans to hatch.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L) and 0.0034, 0.0065, 0.013, 0.025 and 0.050 mg pyraclostrobin/L (nominal); corresponding to mean measured concentrations of 0.00291, 0.00557, 0.0108, 0.0240 and 0.0445 mg a.s./L.
Test conditions:	Test vessels: 9 L glass aquaria (15 x 30 x 20 cm) with a test volume of approx. 7.0 L; 4 replicate test chambers; glass incubation cups (used during embryo stage) with 8.5 cm diameter closed on one end with Nitex® screen; two incubation cups per test chamber; dilution water: filtered natural seawater diluted with deionized water,; water temperature: 29.0°C - 30.8°C; pH 7.5 - 8.1; oxygen content: 5.4 mg/L - 7.9 mg/L; salinity: 15 - 16 ppt; light intensity: approx. 42 foot candles; photoperiod: 16 hours light : 8 hours dark; flow rate: approx. 6.7 volume additions per 24 hours per vessel; feeding: fish were fed 2-3 times daily *ad libitum* freshly hatched *Artemia salina* nauplii from day 6 onwards until 1 day before study termination; no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.
Statistics:	Descriptive statistics; t-test for comparison of the dilution water control and solvent data ($\alpha = 0.05$); ANOVA followed by Bonferroni's test, William's test or Kruskal & Wallis's test to calculate NOEC values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in all concentrations at test initiation, at regular intervals during the study and at test end, except for the highest test item concentration, where analytical measurements were only conducted on days 0 and 7. Recoveries of pyraclostrobin ranged from 75.3 to 103.1% of nominal concentrations at test initiation and from 79.4 to 84.0% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Egg hatch was complete on day 4 in the control groups and the three lowest test item treatments. At 0.0240 mg a.s./L egg hatch was completed on day 5. In the highest test item concentration of 0.0445 mg a.s./L, all animals were dead before hatch (day 4). Mean survival rates at hatch (day 4) and at test end (32 days post-hatch) were $\geq$ 95% in both the dilution water control and the solvent control and in test item concentrations of up to and including 0.0108 mg a.s./L. At 0.0240 mg a.s./L, mean survival was 29% at hatch and 55% on day 32 post-hatch, respectively. No statistically significant effects on survival, time to hatch, time to first feeding, total length, wet weight or dry weight compared to the pooled control were observed at concentrations of up to and including 0.0108 mg a.s./L (Bonferroni's test / William's test / Kruskal & Wallis's test). Sublethal effects (i.e., lethargy and/or erratic swimming) were noted at 0.0108 mg a.s./L on day 4 (at hatch) and at 0.0240 mg a.s./L on days 4 and 5. These effects were not observed at any other time during the test. No other sublethal effects (other than delayed hatch) were observed at any test concentration at any time during the test. The results are summarized in Table 8.2.2.1-2.

Table 8.2.2.1-2: Chronic toxicity of pyraclostrobin to sheepshead minnow (*Cyprinodon variegatus*) in a fish early life stage test (36 d)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0034**	**0.0065**	**0.013**	**0.025**	**0.050**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.00291**	**0.00557**	**0.0108**	**0.0240**	**0.0445**
Embryo survival at hatch on day 4 [1] [%]	100	95	98	99	99	29	0
Survival of larvae 32 days post hatch [%]	95	100	95	95	98	55	0
Percent live normal at hatch on day 4 [%]	100	95	98	99	98	9	0
Percent live normal 32 days post hatch [%]	95	100	95	95	98	55	0
Mean total length [mm]	17.7	18.1	18.5	19.0	18.6	18.7	-- [2]
Mean wet weight [mg]	85.0	85.5	97.5	98.6	98.3	108.7	-- [2]
Mean dry weight [mg]	20.4	20.8	21.8	23.8	22.3	23.2	-- [2]
Endpoint [mg pyraclostrobin/L] (mean measured)							
$NOEC_{overall}$ (36 d)	0.0108						

[1] at 0.0240 and 0.0445 mg a.s./L hatch was complete on day 5.
[2] not determined; no fish survived at the concentration above 0.0240 mg a.s./L.

III. CONCLUSION

In an early life stage study with sheepshead minnows (*Cyprinodon variegatus*) the overall NOEC (36 d) for pyraclostrobin was determined to be 0.0108 mg a.s./L based on mean measured concentrations.

CA 8.2.2.2 Fish full life cycle test

As shown before the chronic toxicity to fish is fully addressed in a number of studies (partly already evaluated during the previous Annex I inclusion process, partly new studies performed for registration in the U.S.). No additional fish full life cycle study is required and no (new) study has been conducted.

CA 8.2.2.3 Bioconcentration in fish

Bioconcentration in fish was addressed in respective studies, which were evaluated already during the previous Annex I inclusion process. No further studies are required or were conducted.

CA 8.2.3 Endocrine disrupting properties

Based on the physical, chemical and structural characteristics of the active substance pyraclostrobin as well as based on results of available studies there is no indication of endocrine disrupting properties of this active substance. Thus, no studies are required.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

The following acute toxicity study on *Daphnia magna* performed with the metabolite BF 500-3 is provided in support of the aquatic risk assessment. The study was requested by Canadian authorities and has not been evaluated previously on EU level.

Report:	CA 8.2.4.1/1 Bergtold M., Janson G., 2006a Acute toxicity of Reg.No. 340 266 (Metabolite of BAS 500 F) to Daphnia magna STRAUS in a 48 hour static test 2006/1038907
Guidelines:	OECD 202, EPA 850.1010
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute limit test, water flea neonates were exposed to BF 500-3 (metabolite of pyraclostrobin) at a single nominal concentration of 0.100 mg/L (limit test at a concentration above standard water solubility of this metabolite), a dilution water control and a solvent control (acetone) in 4 replicates per concentration containing 5 daphnids each. The test item was dissolved and tested with and without centrifugation (to check potential impact of potentially undissolved test item) of the test water prior to the addition of the daphnids. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. No mortality or other symptoms of toxicity were observed in the control, the solvent control and the test item treatments after 48 hours of exposure.

In a 48-hour static acute limit test with *Daphnia magna* the EC_{50} of BF 500-3 (metabolite of pyraclostrobin) was determined to be > 0.100 mg/L (concentration just above water solubility) based on nominal concentrations. The NOEC was ≥ 0.100 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-3 (Reg. No. 340 266; synonym: 500M07; metabolite of pyraclostrobin), batch no. L74-118, purity: 99.9%.

B. STUDY DESIGN

Test species:	Water flea (*Daphnia magna* STRAUS), neonates from in-house culture (originally obtained from Institute National de Recherché Chimique Appliquée, France), > 2 < 24 hours old at test initiation.
Test design:	Static system (48 hours), limit test: 1 test concentration plus a control and a solvent control; the test item was dissolved and tested with and without centrifugation of the test water prior to the addition of the daphnids; 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.
Endpoints:	EC_{50} and NOEC based on immobility of daphnids.
Test concentrations:	Control (dilution water), solvent control (acetone), and 0.100 mg BF 500-3/L (nominal).
Test conditions:	Glass vessels, test volume 50 mL, dilution water: "M4" (Elendt medium); temperature: 20.6°C - 21.8°C; pH 7.92 - 8.00; oxygen content: 8.2 mg/L - 8.9 mg/L; total hardness: 2.39 mmol/L; conductivity: 665 µS/cm; photoperiod: 16 hours light : 8 hours dark; light intensity: 260 - 320 lux; no feeding and no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in the controls and the test item treatment (standard and centrifuged test water) at the beginning and at the end of the test. Mean measured values for BF 500-3 in the non-centrifuged treatment were 101.8% of nominal at test initiation and 91.0% of nominal at test termination. In the centrifuged treatment mean measured concentrations of 95.7% of nominal at test initiation and 88.2% of nominal at test termination were determined. Hence, centrifugation had only a minor effect on the recovery of the test item though the test item was applied at a concentration just above its standard water solubility. As measured concentrations confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No mortality or other symptoms of toxicity were observed in the control, the solvent control and at the test item treatments after 48 hours of exposure. For results see Table 8.2.4.1-1.

Table 8.2.4.1-1: Effect of BF 500-3 (metabolite of pyraclostrobin) on *Daphnia magna* mobility

Concentration [mg/L] (nominal)	Control	Solvent control	0.100 (a)	0.100 (b)
Immobility (24 h) [%]	0	0	0	0
Immobility (48 h) [%]	0	0	0	0
Endpoints [mg BF 500-3/L] (nominal)				
EC_{50} (48 h)	> 0.100			
NOEC (48 h)	≥ 0.100			

(a) standard test water (non-centrifuged treatment); (b) centrifuged test water

III. CONCLUSION

In a 48-hour static acute limit test with *Daphnia magna* the EC_{50} of BF 500-3 (metabolite of pyraclostrobin) was determined to be > 0.100 mg/L (concentration just above water solubility) based on nominal concentrations. The NOEC was ≥ 0.100 mg/L (nominal).

The following acute toxicity study on *Daphnia magna* performed with the metabolite BF 500-5 is provided in support of the aquatic risk assessment. The study was conducted due to findings of this metabolite in new required e-fate studies and has not been evaluated previously.

Report:	CA 8.2.4.1/2 Kuhl R., Frank C., 2014b Acute toxicity of Reg.No. 298327 (BF 500-5, metabolite of Pyraclostrobin) to Daphnia magna in a static 48-hour immobilization test 2013/1349201
Guidelines:	OECD 202 (2004), (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part C.2
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to BF 500-5 (metabolite of pyraclostrobin) at nominal concentrations of 0 (control), 0.625, 1.25, 2.5, 5.0 and 10.0 mg/L in 4 replicates per concentration containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. After 48 h of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 5.0 mg/L, whereas 15% of the daphnids were immobile at the highest test item concentration of 10.0 mg/L. Statistically significant effects on mobility of daphnids after 48 h of exposure were observed at the highest test item concentration.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of BF 500-5 (metabolite of pyraclostrobin) was determined to be > 10.0 mg/L based on nominal concentrations. The NOEC was 5.0 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-5 (Reg. No. 298 327, synonym: 500M04, metabolite of pyraclostrobin,), batch no. L84-174, purity: 99.6% (± 1.0%).

B. STUDY DESIGN

Test species:	Water flea (*Daphnia magna* STRAUS), neonates from in-house culture, < 24 hours old at test initiation and not first brood progeny.
Test design:	Static system (48 hours), 5 test concentrations plus dilution water control, 4 replicates with 5 daphnids in each; assessment of immobility after 24 and 48 hours.
Endpoints:	EC_{50} and NOEC based on immobility of daphnids.
Test concentrations:	Control (dilution water), 0.625, 1.25, 2.5, 5.0 and 10.0 mg BF 500-5/L (nominal).
Test conditions:	100 mL glass beakers, test volume 60 mL, dilution water: "M4" (Elendt medium); temperature: 19.3°C - 20.2°C; pH 7.7 - 7.8; oxygen content: 7.8 mg/L - 8.6 mg/L; photoperiod: 16 hours light : 8 hours dark; light intensity: 950 - 1100 lux; no feeding; no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using an LC-method with MS/MS detection.
Statistics:	Descriptive statistics; Williams t-test for determination of the NOEC.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at the beginning and at the end of the test. Mean measured values for BF 500-5 ranged from 89 to 98% of nominal concentrations at test initiation and from 86 to 99% at test termination. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: After 48 h of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 5.0 mg/L, whereas 15% of the daphnids were immobile at the highest test item concentration of 10.0 mg/L. Statistically significant effects on mobility of daphnids after 48 h of exposure were observed at the highest test item concentration (Williams t-test, $\alpha = 0.05$). For results see Table 8.2.4.1-2.

Table 8.2.4.1-2: Effect of BF 500-5 (metabolite of pyraclostrobin) on *Daphnia magna* immobility

Concentration [mg/L] (nominal)	Control	0.625	1.25	2.5	5.0	10.0
Immobility (24 h) [%]	0	0	0	0	0	5
Immobility (48 h) [%]	0	0	0	0	0	15 *
	Endpoints [mg BF 500-5/L] (nominal)					
EC_{50} (48 h)	> 10.0					
NOEC (48 h)	5.0					

* Statistically significantly different compared to the control (Williams t-test, $\alpha = 0.05$)

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of BF 500-5 (metabolite of pyraclostrobin) was determined to be > 10.0 mg/L based on nominal concentrations. The NOEC was 5.0 mg/L (nominal).

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

The following acute toxicity study on the saltwater mysid *Americamysis bahia* performed with the active substance pyraclostrobin is not required for registration in the EU and it has not been evaluated previously on EU level. However, the study was conducted due to U.S. data requirements and it is provided for completeness.
The 48-h LC_{50} obtained in the 96 h study on *A. bahia* is used for the risk assessment of pyraclostrobin according to EU Regulation 283/2013 (European Commission, 2013) which describes the data requirements for active substances. Also the recent EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013) advises to use the 48-h endpoint, which allows an easy comparison to the other standard acute invertebrate tests. Therefore, only the 48-h results are shown.

Report:	CA 8.2.4.2/1 Boeri R.L. et al., 2000d Flow-Through Acute Toxicity of BAS 500 F to the Mysid, Americanysis bahia 2000/5031
Guidelines:	EPA 850.1035, EPA 72-3(b)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a flow-through acute toxicity laboratory study, saltwater mysids were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0010, 0.0018, 0.0029, 0.0048 and 0.0080 mg pyraclostrobin/L (nominal) (corresponding to mean measured concentrations of 0.00079, 0.0014, 0.0021, 0.0036 and 0.0060 mg a.s./L) in two replicates per treatment containing 10 mysids each. Saltwater mysids were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 48 hours of exposure no mortality and no other toxic effects were observed in the control, the solvent control and in concentrations of up to and including 0.0036 mg/L, whereas 20% mortality was observed at the highest test item concentrations of 0.0060 mg a.s./L. After 48 hours, no sub-lethal effects were found.

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h) for pyraclostrobin was determined to be > 0.00597 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. No. 304 428), batch no. 27882/191/C, purity: 97.09%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Americamysis bahia*), juveniles, age: less than 24 hours old; average wet weight of control mysids: 0.88 mg; source: in-house cultures.

Test design: Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates per treatment; 10 mysids per replicate (loading 0.00059 g mysid/L); assessment of mortality and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50} (48 h), mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L) and 0.0010, 0.0018, 0.0029, 0.0048 and 0.0080 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.00079, 0.0014, 0.0021, 0.0036 and 0.0060 mg pyraclostrobin/L.

Test conditions: Glass aquaria (20 L), test volume 15 L; test chambers: glass cylinders (8 cm in height and 8 cm in diameter) with mesh screen attached to the bottom; dilution water: filtered, sterilized and aerated natural seawater mixed with deionized water; flow rate: 6.5 volume additions per 24 hours on average; salinity: 16 - 17‰; temperature: 21.2°C - 22.9°C; pH 7.9 - 8.1; oxygen content: 7.9 - 8.3 mg/L; photoperiod 16 h light : 8 h dark with a 15 minute transition period between dark and light; light intensity: 55 foot-candles; feeding: juvenile mysids were fed daily with brine shrimps (*Artemia salina*); no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; binomial/nonlinear interpolation method for calculation of the LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analytically determined concentrations of pyraclostrobin ranged from 66.8 to 78.0% of nominal concentrations at test initiation and from 66.8 to 83.2% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 48 hours of exposure no mortality and no other toxic effects were observed in the control, the solvent control and in concentrations of up to and including 0.0036 mg/L, whereas 20% mortality was observed at the highest test item concentrations of 0.0060 mg a.s./L. After 48 hours, no sub-lethal effects were found. The results are summarized in Table 8.2.4.2-1.

Table 8.2.4.2-1: Acute toxicity of pyraclostrobin to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0010**	**0.0018**	**0.0029**	**0.0048**	**0.0080**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.00079**	**0.0014**	**0.0021**	**0.0036**	**0.0060**
Mortality [%] (48 h)	0	0	0	0	0	0	20
Symptoms after 48 h	none	none	none	none	none	none	none.
Endpoints [mg pyraclostrobin/L] (mean measured)							
LC_{50} (48 h)	> 0.00597						

III. CONCLUSION

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h) for pyraclostrobin was determined to be > 0.00597 mg a.s./L.

The following acute toxicity study on the eastern oyster (*Crassostrea virginica*) performed with the active substance pyraclostrobin is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and it is provided for completeness.

Report:	CA 8.2.4.2/2 Boeri R.L. et al., 2000e Flow-through mollusc shell deposition test with BAS 500 F 2000/5042
Guidelines:	EPA 850.1025, EPA 72-3(c)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour acute toxicity laboratory study the effect of pyraclostrobin on shell deposition of eastern oysters was investigated under flow-through conditions. The eastern oysters were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0033, 0.0055, 0.0090, 0.015 and 0.025 mg pyraclostrobin/L (corresponding to mean measured concentrations of 0.0027, 0.0041, 0.0065, 0.0128 and 0.0215 mg a.s./L) in groups of 10 oysters per replicate with two replicates per treatment. Eastern oysters were observed for survival and symptoms of toxicity daily during the exposure period. Measurements of shell deposition for each oyster were made after 96 hours.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, survival of oysters was 95 and 100% in the control and the solvent control, respectively. No mortality occurred at test item concentrations of up to and including 0.0065 mg pyraclostrobin/L, whereas 5 and 10% mortality was observed at 0.0128 mg a.s./L and 0.0215 mg a.s./L, respectively. Statistically significant inhibition of shell growth compared to the pooled control / solvent control was observed at the three highest tested concentrations.

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the LC_{50} was > 0.0215 mg a.s./L; the EC_{50} (96 h) for pyraclostrobin was 0.0125 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.00406 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C; purity: 97.09%.

B. STUDY DESIGN

Test species:	Eastern oyster (*Crassostrea virginica*), juveniles, height: 30 - 50 mm; source: "P. Cummins Oyster Company", Baltimore, Maryland, USA.
Test design:	Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates for each test item concentration and the controls with 10 oysters per replicate (20 animals per treatment); initially and daily assessment of mortality and symptoms of toxicity; measurements of shell deposition 96 hours after start of exposure.
Endpoints:	EC_{50} and NOEC for shell growth inhibition, mortality and symptoms of toxicity.
Test concentrations:	Control (dilution water: unfiltered seawater), solvent control (0.1 mL dimethylformamide/L); 0.0033, 0.0055, 0.0090, 0.015 and 0.025 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.0027, 0.0041, 0.0065, 0.0128 and 0.0215 mg a.s./L.
Test conditions:	20 L glass aquaria, test volume 15 L, natural unfiltered seawater, flow rate: average of 8.7 volume additions per 24 hours in each test vessel, 0.54 L per oyster per hour; salinity: 34‰; temperature: 20.2°C - 22.0°C; pH 7.8 - 8.1; oxygen content: 5.5 mg/L - 8.5 mg/L; photoperiod 16 h light : 8 h dark with a 15 minute transition period between dark and light; light intensity: 50 foot candles; no aeration; live marine phytoplankton as supplement to existing food in unfiltered seawater used as dilution water.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.
Statistics:	Descriptive statistics; t-test (a = 0.05) for comparison of shell deposition data in the control groups; standard statistical techniques for calculation of EC_{50}, ANOVA followed by Bonferroni's test for shell deposition data of the test item treatments.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation and at test termination. Mean measured concentrations for pyraclostrobin ranged from 73 to 89% of nominal concentrations at test initiation and from 72 to 85% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, survival of oysters was 95 and 100% in the control and the solvent control, respectively. No mortality occurred at test item concentrations of up to and including 0.0065 mg pyraclostrobin/L, whereas 5 and 10% mortality was observed at 0.0128 mg a.s./L and 0.0215 mg a.s./L, respectively. No sublethal effects were noted during the exposure period in the controls and the test item treatments. Control and solvent control oysters deposited an average of 2.3 and 2.5 mm of new shell during the test, respectively. No statistically significant difference in shell deposition was observed between the control groups (t-test, $\alpha = 0.05$). Subsequent statistical analyses were performed by comparing the pooled control and solvent control data to the treatment data. Statistically significant inhibition of shell growth compared to the pooled control / solvent control was observed at the three highest tested concentrations (Bonferroni's test). The results are summarized in Table 8.2.4.2-2.

Table 8.2.4.2-2: Acute toxicity (96 h) of pyraclostrobin to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0033**	**0.0055**	**0.0090**	**0.015**	**0.025**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.0027**	**0.0041**	**0.0065**	**0.0128**	**0.0215**
Mortality after 96 h [%]	5	0	0	0	0	5	10
Shell growth after 96 h [% of control]	--	109	100	117	70 *	52 *	35 *
Endpoints [mg pyraclostrobin/L] (mean measured)							
LC_{50} (96 h)	LC_{50} was > 0.0215						
EC_{50} (96 h)	0.0125 (95% confidence limits: 0.0092 - 0.0171)						
NOEC (96 h)	0.00406						

* Statistically significant difference compared to the pooled control / solvent control (Bonferroni's test).

III. CONCLUSION

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the LC_{50} was > 0.0215 mg a.s./L; the EC_{50} (96 h) for pyraclostrobin was 0.0125 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.00406 mg a.s./L.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

The reproductive and development toxicity to *Daphnia magna* has been addressed and evaluated already during the previous Annex I inclusion process. No further study is required or was conducted.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

The following life-cycle test on the saltwater mysid *Americamysis bahia* performed with the active substance pyraclostrobin is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and it is provided for completeness.
Note: This study had apparent deficiencies and has therefore not been accepted as valid by the US EPA.

Report:	CA 8.2.5/1 Wyskiel D.C. et al., 2004a BAS 500 F: A flow-through life-cycle toxicity test with the Saltwater Mysid Americamysis bahia 2004/5000004
Guidelines:	EPA 72-4 (b)
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The chronic toxicity of pyraclostrobin to saltwater mysids (*Americamysis bahia*) was evaluated in a 28-day full-life cycle test under flow-through conditions. Mysids were exposed to a dilution water control, a solvent control and to nominal test item concentrations of 0.28, 0.52, 1.0, 2.0 and 4.0 µg pyraclostrobin/L (corresponding to mean measured concentrations of 0.27, 0.50, 0.93, 1.9 and 3.6 µg a.s./L). Survival, reproductive success and symptoms of toxicity were assessed throughout the study. Length, dry weight and wet weight of surviving mysids were determined at test termination.

The biological results are based on mean measured concentrations of the test item. Survival of unaffected saltwater mysids was statistically significantly affected compared to the pooled control at the three highest test item concentrations of 0.93, 1.9 and 3.6 µg pyraclostrobin/L. Reproductive success and mean total length was statistically significantly different to the pooled control data at the two highest test item concentrations. Mean dry and wet weight of surviving mysids at the test item concentrations of up to and including 1.9 µg a.s./L showed no statistically significant differences compared to the pooled control data.

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for pyraclostrobin was determined to be 0.50 µg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F, Reg. no. 304 428), batch no. WF19407, purity: 99.7%.

B. STUDY DESIGN

Test species: Saltwater mysids (*Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house cultures, original culture obtained from "Aquatic BioSystems, Inc.", Fort Collins, Colorado, USA.

Test design: Flow-through system (28 days); 5 test concentrations plus control and solvent control, 2 replicates per test item concentration; 30 mysids per test vessel; 15 mysids per retention chamber; on day 14 when the sex of mysids could be determined, up to ten females were segregated from the population in each glass aquarium and paired with a male in the test chambers, unpaired mysids were sexually differentiated and placed in separate test chambers; daily assessment of survival and symptoms of toxicity, assessment of reproduction (number of offspring produced by each female) from day 17 on; determination of length, dry weight and wet weight of surviving mysids at test termination.

Endpoints: NOEC based on survival, reproductive success, length, dry and wet weight.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L) and 0.28, 0.52, 1.0, 2.0 and 4.0 µg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.27, 0.50, 0.93, 1.9 and 3.6 µg a.s./L.

Test conditions: 20 L glass aquaria (21 cm x 40 cm x 25 cm) containing two test chambers, test volume up to 7 L; test chambers: glass petri dishes with a 12 cm high collar of Nitex screen attached (from test initiation until day 14: petri dishes of 10 cm diameter, from day 14 on: petri dishes of 6 cm diameter); dilution water: filtered, aerated, sterilized and diluted seawater; flow rate: 13.6 volume additions per 24 hours on average; salinity: 15 - 17‰; temperature: 23.0°C - 26.7°C; pH 7.5 - 8.9; oxygen content: 5.0 - 7.9 mg/L; photoperiod 16 h light : 8 h dark with a 15 minute transition period between dark and light; light intensity: approx. 38 foot candles; feeding: newly hatched *Artemia salina nauplii* three times per day, no feeding during the final 24 hours; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics, Bonferroni's or Williams's test for determination of NOEC values ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation, on days 7, 14, 21, and at test termination on day 28. Measured concentrations for pyraclostrobin were between 61.5 and 100.0% of nominal at test initiation. Measured concentrations after 7, 14, and 21 days ranged from 107.1 to 125.0%, from 80.0 to 119.2% and from 83.0 to 107.7% of nominal, respectively. At test termination, measured concentrations were between 73.1 and 85.0% of nominal. The following biological results are based on mean measured concentrations.

Biological results: The data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Survival of unaffected (not lethargic) saltwater mysids was statistically significantly affected compared to the pooled control at the three highest test item concentrations of 0.93, 1.9 and 3.6 µg pyraclostrobin/L (Bonferroni's test / Williams's test; $\alpha = 0.05$). Reproductive success and mean total length was statistically significantly different to the pooled control data at the two highest test item concentrations (Bonferroni's test / Williams's test; $\alpha = 0.05$). Mean dry and wet weight of surviving mysids at the test item concentrations of up to and including 1.9 µg a.s./L showed no statistically significant differences compared to the pooled control data. The results are summarized in Table 8.2.5-1.

Table 8.2.5-1: Chronic toxicity (28 d) of pyraclostrobin to saltwater mysids (*Americamysis bahia*)

Concentration [µg a.s./L] (nominal)	**Control**	**Solvent control**	**0.28**	**0.52**	**1.0**	**2.0**	**4.0**
Concentration [µg a.s./L] (mean measured)	**--**	**--**	**0.27**	**0.50**	**0.93**	**1.9**	**3.6**
Survival (unaffected mysids) on day 28 [%]	88	80	83	82	73 *	52 *	0 *
Production of young per female by day 28 §	7.5	7.1	6.0	11.4	7.6	2.0 *	0 *
Mean total length on day 28 [mm]	7.4	7.3	7.4	7.2	7.3	7.1 *	-- *
Mean wet weight on day 28 [mg]	3.58	3.48	3.55	3.46	3.41	3.19	-- *
Mean dry weight on day 28 [mg]	0.74	0.76	0.78	0.76	0.77	0.75	-- *
	Endpoints [µg pyraclostrobin/L] (mean measured)						
$NOEC_{overall}$ (28 d)	0.50						

§ Defined as the sum of the total number of young each day divided by the number of surviving females on the respective day
* Statistically significant differences compared to pooled control (Bonferroni's test / Williams's test; $\alpha = 0.05$).

III. CONCLUSION

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOEC (28 d) for pyraclostrobin was determined to be 0.50 µg a.s./L based on mean measured concentrations.

Note: This study had apparent deficiencies and has therefore not been accepted as valid by the US EPA.

The following life-cycle test on the saltwater mysid *Americamysis bahia* performed with the active substance pyraclostrobin is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements following deficiencies in the first chronic mysid study. It is provided for completeness.

Report:	CA 8.2.5/2 Dinehart S., 2013a BAS 500 F: Life-cycle toxicity test of the saltwater mysid, Americamysis bahia, conducted under flow-through test conditions 2013/7002075
Guidelines:	EPA 850.1000, EPA 850.1350, EPA 72-4
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The chronic toxicity of pyraclostrobin to saltwater mysids (*Americamysis bahia*) was evaluated in a 31-day full-life cycle test under flow-through conditions. Mysids were exposed to a dilution water control, a solvent control and to nominal test item concentrations of 0.25, 0.50, 1.0, 2.0, and 4.0 µg pyraclostrobin/L (corresponding to mean measured concentrations of 0.198, 0.365, 0.676, 1.28 and 2.57 µg a.s./L). Survival, reproductive success and symptoms of toxicity were assessed throughout the study. For the males and females of the parental generation, body length was determined after 14 days of exposure and at test termination. Additionally, dry weight was determined at test termination. The body length of the males and females of the filial generation was determined after 10 days of exposure

The biological results are based on mean measured concentrations. Survival of F_0-generation mysids was statistically significantly affected compared to the control at the highest test item concentration of 2.57 µg pyraclostrobin/L after 7 days of exposure. However, this effect was considered to be not biologically relevant, because the magnitude of the difference in percent survival was very small (i.e. 100% survival in the control compared to 97% survival at 2.57 µg a.s./L). There were no statistically significant differences among survival rates of the F_0-generation after 13, 14, 21, and 31 days of exposure compared to the control. There was a statistically significant reduction in the mean number of young produced per female at the highest test item concentration compared to the control. Length of F_0-males and females exposed for 14 days, length of F_0-males exposed for 31 days and weight of F_0-females exposed for 31 days was statistically significantly reduced at the highest test item concentration only. There was a slight but statistically significantly effect on the weight of F_0-males at the three highest test item concentrations. However, there was no impact on female weight and no impact on fecundity/reproductive performance (except for the highest treatment level). Therefore, though statistically significant, this impact is not of biological relevance and thus not considered an adverse effect. No statistically significant adverse effects on survival and growth of the F_1-generation mysids were detected in any of the test item concentrations tested.

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOAEC (31 d) for pyraclostrobin was determined to be 1.28 µg a.s./L based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. CD-001236, purity: 99.02%.

B. STUDY DESIGN

Test species:	Saltwater mysid (*Americamysis bahia*), juveniles, age: less than 24 hours old; source: in-house culture.
Test design:	Flow-through system (31 d); 5 test item concentrations plus a control and a solvent control, 3 replicates for each test item concentration, the control and the solvent control, two retention baskets per test vessel: one containing mysids for reproduction observations and one for growth observations. F_0-generation: 15 mysids per retention basket; 30 mysids per test vessel; pairing of mysids on day 13 by transferring male-female pairs from retention baskets into brood baskets (7 pairs per replicate); remaining mysids (after isolation of male-female pairs) were transferred to a single brood basket; determination of dry weight on day 31; measurement of body length after 14 days of exposure for mysids in the growth-retention baskets and on day 31 for paired mysids in brood baskets; assessment of survival on days 7, 13 (pre-pairing) and on days 14, 21 and 31 (post-pairing); reproduction (days to first brood release and number of young per female). F_1-generation: started with the first 15 post-larval F1-mysids; post-larval F1-mysids were assigned to retention baskets within the same test vessels as used for F0-mysid exposure; F1-mysids were exposed for 10 days; assessment of survival 4, 7 and 10 days after start of exposure; measurement of body length after 10 days of exposure.
Endpoints:	NOEC based on survival, reproductive success, body length and dry weight.
Test concentrations:	Control (dilution water), solvent control (approximately 25 µL dimethylformamide/L), 0.25, 0.50, 1.0, 2.0, and 4.0 µg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.198, 0.365, 0.676, 1.28 and 2.57 µg pyraclostrobin/L.

Test conditions:	Test vessels: glass aquaria (test volume: 20 L) with a glass pane in the middle of the tank containing two holes near the bottom; retention baskets: glass petri dish base (approximately 1.5 x 15 cm) with a nylon screen collar (mesh size: 355 µm); brood baskets: glass Petri dish base (approximately 1.5 x 10 cm) with a nylon screen collar (mesh size: 355 µm). Dilution water: artificial saltwater; salinity: 18.2 - 20.3 ‰; temperature: 24.1 - 25.0°C; pH 7.3 - 8.1; oxygen content: 4.1 mg/L - 7.3 mg/L. Flow rate: until day 5: approximately 5.3 volume exchanges/aquarium/24 h; from day 6 on: approximately 7.5 volume exchanges/aquarium/24 h. Photoperiod: 14 h light : 10 h dark with two 30-minute transition periods of lower light intensity; light intensity: 315 - 390 lux. Feeding: brine shrimp nauplii (*Artemia* sp.) supplemented with a mixture of commercially available enrichment products at least twice daily, saltwater rotifers once daily, F1 mysids on the last day of the study were fed only once. Aeration: from day 17 on.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics; Williams's test for determination of NOEC values ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation, on days 9, 14, 21, and at test termination on day 31. Measured concentrations for pyraclostrobin were between 63 and 74% of nominal at test initiation. Measured concentrations after 9, 14, and 21 days ranged from 65 to 99%, from 62 to 84% and from 53 to 71% of nominal, respectively. At test termination, measured concentrations were between 67 and 76% of nominal. The following biological results are based on mean measured concentrations.

Biological results: Survival of F_0-generation mysids was statistically significantly affected compared to the control at the highest test item concentration of 2.57 µg pyraclostrobin/L after 7 days of exposure (William's test, $\alpha = 0.05$). However, this effect was considered to be not biologically relevant, because the magnitude of the difference in percent survival was very small (i.e. 100% survival in the control compared to 97% survival at 2.57 µg a.s./L). There were no statistically significant differences among survival rates of the F_0-generation after 13, 14, 21, and 31 days of exposure compared to the control. There was a statistically significant reduction in the mean number of young produced per female at the highest test item concentration compared to the control. Length of F_0-males and females exposed for 14 days, length of F_0-males exposed for 31 days and weight of F_0-females exposed for 31 days was statistically significantly reduced at the highest test item concentration (William's test, $\alpha = 0.05$). There was a slight but statistically significantly effect on the weights of F_0-males at the three highest test item concentrations (William's test, $\alpha = 0.05$). However, there was no impact on female weight and no impact on fecundity/reproductive performance (except for the highest treatment level). Therefore, though statistically significant, this impact is not of biological relevance and thus not considered an adverse effect. No statistically significant adverse effects on survival and growth of the F_1-generation mysids were detected in any of the test item concentrations tested. The results are summarized in Table 8.2.5-2.

Table 8.2.5-2: Chronic toxicity (31 d) of pyraclostrobin to saltwater mysids (*Americamysis bahia*)

Concentration [µg a.s./L] (nominal)			**Control**	**Solvent control**	**0.25**	**0.50**	**1.0**	**2.0**	**4.0**
Concentration [µg a.s./L] (mean measured)			**--**	**--**	**0.198**	**0.365**	**0.676**	**1.28**	**2.57**
F_0-generation	Survival (pre-pairing)	survival after 7 days of exposure [%]	100	91	99	100	98	98	97 * a)
		survival after 13 days of exposure [%]	98	88	99	97	98	93	92
	Survival (post-pairing)	survival after 14 days of exposure [%]	100	100	100	100	98	98	100
		survival after 21 days of exposure [%]	98	100	100	100	98	98	100
		survival after 31 days of exposure [%]	97	98	100	93	93	90	100
	Growth	length of males exposed for 31 days [mm] #	6.11 ± 0.122	6.14 ± 0.0709	6.08 ± 0.0842	6.19 ± 0.114	5.95 ± 0.132	6.01 ± 0.206	5.83 ± 0.193 *
		length of females exposed for 31 days [mm] #	6.23 ± 0.0515	6.32 ± 0.150	6.44 ± 0.0565	6.45 ± 0.118	6.08 ± 0.384	6.26 ± 0.118	5.98 ± 0.149
		weight of males exposed for 31 days [mg] #	1.14 ± 0.121	1.07 ± 0.020	1.07 ± 0.900	1.12 ± 0.0402	0.989 ± 0.0939 d*	0.972 ± 0.0812 d*	0.937 ± 0.0982 *
		weight of females exposed for 31 days [mg] #	1.51 ± 0.141	1.44 ± 0.0336	1.39 ± 0.108	1.46 ± 0.110	1.30 ± 0.283	1.37 ± 0.146	1.01 ± 0.109 *
	Reproduction	days to first brood release #	17.9 ± 2.15	18.2 ± 1.10	17.7 ± 0.850 b)	18.5 ± 3.32	19.0 ± 3.37	21.8 ± 4.41	17.0
		mean young per female #, c)	12.8 ± 4.33	14.1 ± 7.64	3.19 ± 0.577 b)	11.3 ± 0.247	14.6 ± 11.5	8.00 ± 6.61	0.191 ± 0.330 *

Concentration [µg a.s./L] (nominal)			**Control**	**Solvent control**	**0.25**	**0.50**	**1.0**	**2.0**	**4.0**
Concentration [µg a.s./L] (mean measured)			**--**	**--**	**0.198**	**0.365**	**0.676**	**1.28**	**2.57**
F_1-generation	Survival	survival after 4 days of exposure [%]	100	100	100	100	96	88	100
		survival after 7 days of exposure [%]	100	98	100	98	96	88	100
		survival after 10 days of exposure [%]	100	93	100	98	96	88	100
	Growth	length of males exposed for 10 days [mm] #	4.17 ± 0.137	4.41 ± 0.0782	4.43 ± 0.0382	4.48 ± 0.154	4.39 ± 0.0531	4.30 ± 0.247	4.29
		length of females exposed for 10 days [mm] #	4.18 ± 0.0566	4.57 ± 0.206	4.68 ± 0.0487	4.69 ± 0.194	4.42 ± 0.174	4.53 ± 0.209	4.34
Endpoint [mg pyraclostrobin/L] (mean measured)									
$NOAEC_{overall}$ (31 d)					1.28				

***** Statistically significant differences compared to the control (William's test, $\alpha = 0.05$).
# Values represent mean and standard deviation from all replicates.
a) The statistically significant difference in F0-mysid survival rate in the 2.57 µg/L treatment on day 7 was judged not to be biologically meaningful because of the magnitude of the difference in mean percent survival between the control and the solvent control.
b) The 0.198 µg/L treatment was excluded from statistical analyses for reproduction endpoints because of low reproductive output from F_0-female mysids in this treatment which did not appear to reflect a test substance treatment-related effect.
c) Paired females that produced no young were excluded from the mean calculation.
d) Slight impact on weight only without impact on length, on females or on reproduction; thus not considered to be a biologically relevant adverse effect.

III. CONCLUSION

In a flow-through chronic toxicity study with saltwater mysids (*Americamysis bahia*), the overall NOAEC (31 d) for pyraclostrobin was determined to be 1.28 µg a.s./L based on mean measured concentrations.

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

A spiked water toxicity study with *Chironomus riparius* was already evaluated during the previous Annex I inclusion process. The following additional spiked sediment toxicity study with *C. riparius* was also performed with the active substance pyraclostrobin. It is provided in support of the aquatic risk assessment covering the exposure via sediment, and has not been evaluated previously on EU level.

Report:	CA 8.2.5.3/1 Kuhl R., Wydra V., 2013a Effects of BAS 500 F (Pyraclostrobin) on the development of sediment dwelling larvae of Chironomus riparius in a sediment-water system - Exposed via spiked sediment 2012/1185699
Guidelines:	OECD 218 (2004)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In a 28-day static spiked sediment study, non-biting midge larvae (*Chironomus riparius*) were exposed to pyraclostrobin at nominal concentrations of 0.3, 0.6, 1.2, 2.4 and 4.8 mg a.s./kg dry sediment. Additionally, a solvent (acetone) control and a water control were set up. All test item concentrations and the water control had 4 replicates, whereas 6 replicates were tested for the solvent control. 20 larvae were added to each test vessel.

The biological results are based on nominal concentrations of the test item. Additionally, biological endpoints based on mean measured values are given. In the control and the solvent control mean emergence rates of 78.8 and 71.7% and mean development rates of 0.057 and 0.055 were observed, respectively. In the test item concentrations of up to and including 4.8 mg a.s./kg dry sediment, between 60 and 76% of the test animals emerged until day 28. Statistically significant differences compared to the pooled control were found for the emergence rates at the highest test item concentration. No statistically significant effect on the development was observed in any treatment group.

In a 28-day static sediment test with *Chironomus riparius* the NOEC values of pyraclostrobin were determined to be 2.4 mg a.s./kg dry sediment (nominal; equivalent to the mean measured concentration of 1.37 mg a.s./kg dry sediment) based on emergence rate and ≥ 4.8 mg a.s./kg dry sediment (nominal; equivalent to the mean measured concentration of ≥ 2.83 mg a.s./kg dry sediment) based on development rate.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F, Reg. No. 304 428), batch no. 10-510009, purity: 100% (analytical).

B. STUDY DESIGN

Test species: Non-biting midge (*Chironomus riparius*), first instar larvae, 3 days old at test initiation; source: in-house culture.

Test design: Static system (28 days); 5 test concentrations plus a solvent (acetone) control and a water control, 4 replicates per test item concentration and for the water control, 6 replicates for the solvent control; 20 larvae were added to each test vessel; assessment of emergence rate and development rate.

Endpoints: NOEC and EC_{50} (regarding emergence rate and development rate).

Test concentrations: Solvent (acetone) control, water control, 0.3, 0.6, 1.2, 2.4 and 4.8 mg a.s./kg dry sediment (nominal), corresponding to mean measured concentrations of 0.66, 1.37 and 2.83 mg a.s./kg dry sediment in the 1.2, 2.4 and 4.8 a.s./kg dry sediment treatments.

Test conditions: 600 mL glass vessels with 100 g spiked wet artificial sediment (according to OECD 218), 400 mL M4 water (Elendt medium) corresponding to a water layer of about 6.4 cm; pH 7.6 - 8.6; oxygen 62% to 100%; total hardness: 284.8 - 320.4 mg $CaCO_3$/L at test initiation and 311.5 - 329.3 mg $CaCO_3$/L at test termination; conductivity: 588 µS/cm; ammonia: 1.2 mg/L at test initiation and 2.0 mg/L at test termination; water temperature: 20°C - 21°C; light intensity: 620 - 720 lux; photoperiod: 16 h light : 8 h dark; continuous gentle aeration; food: TetraMin (days 0-10: 0.5 mg food/larva/day, days 11-27: 0.5 - 1.0 mg food/larva/day).

Analytics: Analytical verification of test item concentrations was conducted using a LC-MS/MS-method.

Statistics: Descriptive statistics, Student-t-test ($p < 0.05$) for comparison of the emergence and development rates in the control groups; ANOVA followed by one-sided Williams' t-test for determination of the NOEC based on emergence and development rate ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in the overlying water, the pore water and the sediment was conducted in the test concentrations of 1.2 and 4.8 mg a.s./kg dry sediment at the beginning and the end of the test. Recoveries in the sediment were in a range between 86 and 101% of the nominal concentrations at test initiation, and < LOQ (limit of quantification = 0.57 mg a.s./kg dry sediment) and 17% of nominal at test termination. Pyraclostrobin concentrations found in the overlaying water ranged from < LOQ (LOQ = 0.001 mg/L) to 0.4% of nominal concentrations on day 0 and from < LOQ to 0.03% of nominal on day 28. Measured pore water concentrations were between 0.2% and 0.9% of nominal on test start and < LOQ (LOQ = 0.001 mg/L) in both test treatments on test end. The following biological results are based on nominal sediment concentrations. Additionally, biological endpoints based on mean measured values are given.

Biological results: In the control and the solvent control mean emergence rates of 78.8% and 71.7% and mean development rates of 0.057 and 0.055 were observed, respectively. In the test item concentrations of up to and including 4.8 mg a.s./kg dry sediment, between 60% and 76% of the test animals emerged until day 28. No statistically significant difference was observed between the controls (Student-t-test, $p < 0.05$). Hence, the controls were pooled and used as the reference in all evaluations. Statistically significant differences compared to the pooled control were found for the emergence rates at the highest test item concentration (Williams Multiple t-test, $\alpha = 0.05$). No statistically significant effect on the development was observed in any treatment group (Williams Multiple t-test, $\alpha = 0.05$). The results are summarized in Table 8.2.5.3-1.

Table 8.2.5.3-1: Effects of pyraclostrobin on emergence and development of Chironomus riparius

Concentration [mg a.s./kg dry sediment] (nominal)	**Control**	**Solvent control**	**0.3**	**0.6**	**1.2**	**2.4**	**4.8**
Emergence rate (ER) [% emerged midges] #	78.8 ± 19.7	71.7 ± 11.7	66.3 ± 13.1	75.0 ± 4.1	73.8 ± 12.5	76.3 ± 9.5	60.0 * ± 10.8
Development rate per day (DR) #	0.057 ± 0.001	0.055 ± 0.001	0.057 ± 0.003	0.056 ± 0.001	0.056 ± 0.002	0.059 ± 0.003	0.058 ± 0.002
	Endpoints [mg pyraclostrobin/kg dry sediment] (nominal)						
$EC_{50\ emergence\ rate}$ (28 d)	> 4.8 (mean measured: > 2.83)						
$NOEC_{emergence\ rate}$ (28 d)	2.4 (mean measured: 1.37)						
$NOEC_{development\ rate}$ (28 d)	≥ 4.8 (mean measured: ≥ 2.83)						

Values represent mean and standard deviation from all replicates, each with 20 larvae.
* Statistically significant difference compared to the pooled control (Williams Multiple t-test, $\alpha = 0.05$).

III. CONCLUSION

In a 28-day static sediment test with *Chironomus riparius* the NOEC values of pyraclostrobin were determined to be 2.4 mg a.s./kg dry sediment (nominal; equivalent to the mean measured concentration of 1.37 mg a.s./kg dry sediment) based on emergence rate and ≥ 4.8 mg a.s./kg dry sediment (nominal; equivalent to the mean measured concentration of ≥ 2.83 mg a.s./kg dry sediment) based on development rate.

The following spiked sediment toxicity study on non-biting midge larvae (*Chironomus riparius*) performed with the metabolite BF 500-3 is provided in support of the aquatic risk assessment of a sediment metabolite and has not been evaluated previously.

Report:	CA 8.2.5.3/2 Kuhl R., Wydra V., 2013b Effects of Reg.No. 340266 (metabolite of BAS 500 F (Pyraclostrobin), synonymous: 500M07, BF 500-3) on the development of sediment dwelling larvae of Chironomus riparius in a sediment-water system - exposed via spiked sediment 2013/1237446
Guidelines:	OECD 218 (2004)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In a 28-day static spiked sediment study, non-biting midge larvae (*Chironomus riparius*) were exposed to BF 500-3 (metabolite of pyraclostrobin) at nominal test item concentrations of 1.0, 2.0, 4.0, 8.0 and 16.0 mg/kg dry sediment. Additionally, a solvent (acetone) control and a water control were set up. All test item concentrations and the water control had 4 replicates, whereas 6 replicates were tested for the solvent control. 20 larvae were added to each test vessel.

The biological results are based on nominal concentrations of the test item. In the control and the solvent control mean emergence rates of 90.0 and 88.3% were observed, respectively. The mean development rate was 0.059 in both control groups. In test item concentrations of up to and including 16.0 mg/kg dry sediment, between 87.5 and 95.0% of the test animals emerged until day 28. No statistically significant differences were found for the emergence and development rates at any test item concentration when compared to the pooled control.

In a 28-day static sediment test with *Chironomus riparius* the NOEC of BF 500-3 (metabolite of pyraclostrobin) was determined to be ≥ 16.0 mg a.s./kg dry sediment based on emergence and development rate (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-3 (Reg. No. 340 266; synonym: 500M07; metabolite of pyraclostrobin), batch no. L74-118, purity: 99.9%.

B. STUDY DESIGN

Test species: Non-biting midge (*Chironomus riparius*), first instar larvae, 3 days old at test initiation; source: in-house culture.

Test design: Static system (28 days); 5 test concentrations plus a solvent (acetone) control and a water control, 4 replicates per test item concentration and for the water control, 6 replicates for the solvent control; 20 larvae were added to each vessel; assessment of emergence rate and development rate.

Endpoints: NOEC and EC_{50} (regarding emergence rate and development rate).

Test concentrations: Solvent control, water control, 1.0, 2.0, 4.0, 8.0 and 16.0 mg/kg dry sediment (nominal).

Test conditions: 600 mL glass vessels with 100 g spiked wet artificial sediment (according to OECD 218), 280 mL M4 water (Elendt medium) corresponding to a water layer of about 6.0 cm; pH 8.1 - 8.7; oxygen saturation 86% - 102%; total hardness: 302.6 - 311.5 mg $CaCO_3$/L at test initiation and 302.6 - 329.3 mg $CaCO_3$/L at test termination; conductivity: 620 µS/cm; ammonia: 0.8 mg/L at test initiation and 0.6 - 0.8 mg/L at test termination; water temperature: 19°C - 21°C; light intensity: 670 - 870 lux; photoperiod: 16 h light : 8 h dark; continuous gentle aeration; food: TetraMin on workdays, 0-10 days: 0.5 mg food/larva/day, 11-27 days: 0.5 - 1.0 mg food/larva/day.

Analytics: Analytical verification of test item concentrations was conducted using a LC-MS/MS-method.

Statistics: Descriptive statistics, Student's t-test ($p < 0.05$) for comparison of the emergence and development rates in the control groups; ANOVA followed by Williams' Multiple Sequential t-test procedure for determination of the NOEC based on emergence and development rate ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in the overlying water, the pore water and the sediment was conducted in the test concentrations of 2.0 and 16.0 mg/kg dry sediment at the beginning and the end of the test. Mean recoveries in the sediment were in a range between 96 and 101% of the nominal concentrations at test initiation and between 75 and 81% of nominal at test termination. Overlying water concentrations ranged from 2.18 to 11.23 µg/L at test start (= insertion of larvae) and < LoQ (limit of quantification) to 2.13 µg/L at test end. The pore water concentrations ranged from 8.62 to 66.58 µg/L at test start and from 2.51 to 13.62 µg/L at test termination. Since the analytical measurements of initial sediment concentrations confirmed the correct application of the test item, the following biological results are based on nominal sediment concentrations.

Biological results: In the control and the solvent control mean emergence rates of 90.0 and 88.3% were observed, respectively. The mean development rate was 0.059 in both control groups. In the test item concentrations of up to and including 16.0 mg/kg dry sediment between 87.5 and 95.0% of the test animals emerged until day 28. No statistically significant differences were observed between the control groups (Student's t-test, $p < 0.05$). Hence, the controls were pooled and used as the reference in all evaluations. No statistically significant differences were found for the emergence and development rates at any test item concentration when compared to the pooled control (Williams Multiple Sequential t-test procedure, $\alpha = 0.05$). The results are summarized in Table 8.2.5.3-2.

Table 8.2.5.3-2: Effects of BF 500-3 (metabolite of pyraclostrobin) on emergence and development of *Chironomus riparius*

Concentration [mg BF 500-3/kg dry sediment] (nominal)	**Control**	**Solvent control**	**1.0**	**2.0**	**4.0**	**8.0**	**16.0**
Emergence rate (ER) [% emerged midges] (28 d) #	90.0 ± 7.1	88.3 ± 9.8	87.5 ± 6.5	95.0 ± 4.1	90.0 ± 9.1	87.5 ± 2.9	91.3 ± 7.5
Development rate per day (DR) (28 d) #	0.059 ± 0.001	0.059 ± 0.003	0.060 ± 0.001	0.060 ± 0.001	0.060 ± 0.002	0.061 ± 0.002	0.059 ± 0.002
Endpoints [mg BF 500-3/kg dry sediment] (nominal)							
$EC_{50\ emergence\ rate}$ (28 d)	> 16.0						
$NOEC_{emergence\ \&\ development\ rate}$ (28 d)	≥ 16.0						

Values represent mean and standard deviation from all replicates, each with 20 larvae.

III. CONCLUSION

In a 28-day static sediment test with *Chironomus riparius* the NOEC of BF 500-3 (metabolite of pyraclostrobin) was determined to be ≥ 16.0 mg a.s./kg dry sediment based on emergence and development rate (nominal).

The following spiked sediment toxicity study on non-biting midge larvae (*Chironomus riparius*) performed with the metabolite BF 500-6, which has not been evaluated previously, is provided in support of the aquatic risk assessment of a soil metabolite potentially contaminating sediments.

Report:	CA 8.2.5.3/3 Backfisch K., 2014b Chronic toxicity of Reg. No. 364380 (BF 500-6; metabolite of Pyraclostrobin) to the non-biting midge Chironomus riparius - A spiked sediment study 2014/1001481
Guidelines:	OECD 218 (2004)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 28-day static spiked sediment study, non-biting midge larvae (*Chironomus riparius*) were exposed to BF 500-6 (metabolite of pyraclostrobin) at nominal test item concentrations of 2.0, 6.0, 18.0, 54.0 and 162.0 mg/kg dry sediment (corresponding to mean measured concentrations of 2.0, 6.5, 18.3, 48.7 and 151.5 mg/kg dry sediment). Even the highest test concentration did not cause 50% effect on emergence or development rate. However, no clear NOEC was reached in the first experiment. Therefore, a second test was conducted with lower nominal concentrations of 0.5, 1.0 and 2.0 mg/kg dry sediment (corresponding to mean measured concentrations of 0.6, 1.2 and 2.8 mg/kg dry sediment). Additionally, for each trial a solvent (acetone) control and a water control were set up. In the first experiment all test item concentrations and the water control had 4 replicates, whereas 6 replicates were tested for the solvent control. For the second experiment 4 replicates for each test item concentration and three replicates for each control were used. 20 larvae were added to each test vessel.

The biological results are based on mean measured concentrations of the test item. In the control and the solvent control of the first trial mean emergence rates of 0.9375 and 0.9583 were observed, respectively. The mean development rate was 0.0625 in the water control and 0.0617 in the solvent control. No statistically significant differences were found for the development rates at test item concentrations of up to and including the highest tested concentration when compared to the pooled control. The emergence rate was slightly, but statistically significantly reduced in all tested concentration groups. Therefore, a second test was added including some lower concentrations in order to derive a clear NOEC. Mean emergence rate in the second experiment was 0.8333 and 0.9333 in the control and the solvent control, respectively. The mean development rate was 0.0638 in the water control and 0.0626 in the solvent control. As expected, no statistically significant effect on the development rates was observed in any of the lower treatments whereas the emergence rate was significantly reduced at the highest test concentration of 2.8 mg/kg dry sediment.

In a 28-day spiked sediment test with *Chironomus riparius* the NOEC of BF 500-6 (metabolite of pyraclostrobin) was determined to be 1.2 mg/kg dry sediment based on emergence rate and ≥ 151.5 mg/kg dry sediment based on development rate (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-6 (Reg. No. 346 380; synonym: 500M01, metabolite of pyraclostrobin); batch no. 01311-142; purity: 99.2%.

B. STUDY DESIGN

Test species: Non-biting midge (*Chironomus riparius*), first instar larvae from in-house culture (non-GLP), originally obtained from "the Zoological Institute of the J.W. Goethe University", Frankfurt, Germany.

Test design: Static system (28 days); first experiment: 5 test concentrations plus a solvent (acetone) control and a water control, 4 replicates per test item concentration and for the water control, 6 replicates for the solvent control; a second test was conducted with lower concentrations since no clear NOEC was reached in the first experiment: 3 test concentrations with four replicates plus a solvent (acetone) control and a water control with three replicates. 20 larvae were added to each vessel; assessment of emergence rate and development rate.

Endpoints: NOEC, EC_{50} values (regarding emergence rate and development rate).

Test concentrations: First experiment: solvent control, water control, 2.0, 6.0, 18.0, 54.0 and 162.0 mg/kg dry sediment (nominal); corresponding to mean measured concentrations of 2.0, 6.5, 18.3, 48.7 and 151.5 mg/kg dry sediment;
Second experiment: solvent control, water control, 0.5, 1.0 and 2.0 mg/kg dry sediment (nominal); corresponding to mean measured concentrations of 0.6, 1.2 and 2.8 mg/kg dry sediment.

Test conditions: 600 mL glass vessels with 100 g spiked wet artificial sediment (according to OECD 218), 400 mL M4 water (Elendt medium) corresponding to a water layer of about 8.0 cm; pH 7.52 - 8.17; oxygen content: 6.99 - 8.60 mg/L; total hardness: 2.48 mmol/L (1st trial) / 2.47 mmol/L (2nd trial); conductivity: 634 / 657 µS/cm; ammonia: 0.2 / 0.8 mg/L at test initiation and 0.5 / 2.0 mg/L at test termination; water temperature: 19.7°C - 20.6°C; light intensity: 361 - 461 lux; photoperiod: 16 h light : 8 h dark; continuous gentle aeration; food: finely ground and suspended TetraMin, 0.25 - 1.0 mg food/larva/day (i.e. 5 - 20 mg/vessel/day).

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.

Statistics: Descriptive statistics, Probit analysis using linear maximum likelihood regression for determination of the EC_x values; ANOVA followed by Chi-square test with Bonferroni correction or Dunnett's Multiple t-test for determination of the NOEC based on emergence and development rate ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in the overlying water, the pore water and the sediment was conducted in all test concentrations at the beginning and the end of the test for both trials. Mean recoveries in the sediment in the first experiment were in a range between 89% and 114% of the nominal concentrations at test initiation and between 92% and 109% of nominal at test termination. Overlying water concentrations ranged from < LoQ (limit of quantification = 1 µg/L) to 163.0 µg/L at test start (= insertion of larvae) and < LoQ to 2.42 µg/L at test end. The very high measurement in the overlying water in the highest treatment group at start of the test is likely due to some contamination of the sample (with sediment during sampling). The pore water concentrations ranged from < LoQ to 4.49 µg/L at test start and from < LoQ to 8.59 µg/L at test termination. Mean recoveries in the sediment during the second trial were between 120% and 141% of the nominal concentrations at test start and between 112% and 137% of nominal at test end. Mean measured test item concentrations in the overlying water and the pore water were < LoQ in all treatment groups at test start and at test end. The following biological results are based on mean measured sediment concentrations.

Biological results: In the control and the solvent control of the first trial mean emergence rates of 0.9375 and 0.9583 were observed, respectively. The mean development rate was 0.0625 in the water control and 0.0617 in the solvent control. No statistically significant differences were observed between the control groups (Chi-square test with Bonferroni correction, Dunnett's Multiple t-test procedure, $\alpha = 0.05$). Hence, the controls were pooled and used as the reference in all evaluations. No statistically significant differences were found for the development rates at test item concentrations of up to and including the highest tested concentration when compared to the pooled control (Chi-square test with Bonferroni correction, Dunnett's Multiple t-test procedure, $\alpha = 0.05$). The emergence rate was slightly, but statistically significantly reduced reduced in all tested concentration groups ($\alpha = 0.05$). Therefore, a second test was added including some lower concentrations in order to derive a clear NOEC. Mean emergence rate in the second experiment was 0.8333 and 0.9333 in the control and the solvent control, respectively. The mean development rate was 0.0638 in the water control and 0.0626 in the solvent control. Since there were no statistically significant differences observed between the control groups ($\alpha = 0.05$), the control data were pooled and used as the reference in all evaluations. As expected, no statistically significant effect on the development rates was observed in any of the lower treatments whereas the emergence rate was significantly reduced at the highest test concentration of 2.8 mg/kg dry sediment ($\alpha = 0.05$). The results are summarized in Table 8.2.5.3-3.

Table 8.2.5.3-3: Effects of BF 500-6 (metabolite of pyraclostrobin) on emergence and development of *Chironomus riparius* (trial 1 and 2)

Trial 1							
Concentration [mg/kg dry sediment] (nominal)	**Control**	**Solvent control**	**2.0**	**6.0**	**18.0**	**54.0**	**162.0**
Concentration [mg/kg dry sediment] (mean measured)	**Control**	**Solvent control**	**2.0**	**6.5**	**18.3**	**48.7**	**151.5**
Emergence rate (ER) (28 d) #	0.9375 ± 0.025	0.9583 ± 0.0376	0.8250 ± 0.0289 *	0.7875 ± 0.025 *	0.8500 ± 0.0913 *	0.7625 ± 0.1315 *	0.7125 ± 0.1436 *
Development rate (DR) (28 d) #	0.0625 ± 0.0013	0.0617 ± 0.0012	0.0634 ± 0.0011	0.0617 ± 0.0022	0.0623 ± 0.0023	0.0625 ± 0.0017	0.0643 ± 0.0025

Trial 2					
Concentration [mg/kg dry sediment] (nominal)	**Control**	**Solvent control**	**0.5**	**1.0**	**2.0**
Concentration [mg/kg dry sediment] (mean measured)	**Control**	**Solvent control**	**0.6**	**1.2**	**2.8**
Emergence rate (ER) (28 d) #	0.8333 ± 0.0289	0.9333 ± 0.0764	0.8375 ± 0.0629	0.8500 ± 0.0707	0.7000 ± 0.1155 *
Development rate (DR) (28 d) #	0.0638 ± 0.0001	0.0626 ± 0.0012	0.0625 ± 0.0012	0.0650 ± 0.0024	0.0646 ± 0.0019

Overall endpoints [mg BF 500-6/kg dry sediment] (mean measured)	
$EC_{50\ emergence\ \&\ development\ rate}$ (28 d)	> 151.5
$NOEC_{\ emergence\ rate}$ (28 d)	1.2
$NOEC_{\ development\ rate}$ (28 d)	≥ 151.5

Values represent mean and standard deviation from all replicates, each with 20 larvae.
* Statistically significant difference compared to the pooled control (Chi-square test with Bonferroni correction, Dunnett's Multiple t-test procedure, $\alpha = 0.05$)

III. CONCLUSION

In a 28-day spiked sediment test with *Chironomus riparius* the NOEC of BF 500-6 (metabolite of pyraclostrobin) was determined to be 1.2 mg/kg dry sediment based on emergence rate and ≥ 151.5 mg/kg dry sediment based on development rate (mean measured).

The following spiked sediment toxicity study on non-biting midge larvae (*Chironomus riparius*) performed with the metabolite BF 500-7, which has not been evaluated previously, is provided in support of the aquatic risk assessment of a soil metabolite potentially contaminating sediments.

Report:	CA 8.2.5.3/4 Backfisch K., 2014a Chronic toxicity of Reg.No. 369315 (BF 500-7; Metabolite of Pyraclostrobin) to the non-biting midge Chironomus riparius - A spiked sediment study 2014/1001482
Guidelines:	OECD 218 (2004)
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 28-day static spiked sediment study, non-biting midge larvae (*Chironomus riparius*) were exposed to BF 500-7 (metabolite of pyraclostrobin) at nominal test item concentrations of 2.0, 6.0, 18.0, 54.0 and 162.0 mg/kg dry sediment (corresponding to mean measured concentrations of 0.96, 4.55, 13.83, 47.15 and 123.5 mg/kg dry sediment). Additionally, a solvent (acetone) control and a water control were set up. All test item concentrations and the water control had 4 replicates, whereas 6 replicates were tested for the solvent control. 20 larvae were added to each test vessel.

The biological results are based on mean measured concentrations of the test item. Mean emergence rates in both controls were above 90%. The mean development rates were 0.0650 and 0.0644 for the water control and the solvent control, respectively. No statistically significant differences were found for the emergence and development rates at any test item concentration when compared to the water control and the solvent control.

In a 28-day static sediment test with *Chironomus riparius* the NOEC of BF 500-7 (metabolite of pyraclostrobin) was determined to be ≥ 123.5 mg a.s./kg dry sediment based on emergence and development rate (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-7 (Reg. No. 369 315; metabolite of pyraclostrobin), batch no. L83-168, purity: 96.2%.

B. STUDY DESIGN

Test species:	Non-biting midge (*Chironomus riparius*), first instar larvae from in-house culture, originally obtained from "the Zoological Institute of the J.W. Goethe University" Frankfurt, Germany.
Test design:	Static system (28 days); 5 test concentrations plus a solvent (acetone) control and a water control, 4 replicates per test item concentration and for the water control, 6 replicates for the solvent control; 20 larvae were added to each vessel; assessment of emergence rate and development rate.
Endpoints:	NOEC and EC_{50} (regarding emergence rate and development rate).
Test concentrations:	Solvent control, water control, 2.0, 6.0, 18.0, 54.0 and 162.0 mg BF 500-7/kg dry sediment (nominal), corresponding to mean measured concentrations of 0.96, 4.55, 13.83, 47.15 and 123.5 mg/kg dry sediment.
Test conditions:	600 mL glass vessels with 100 g spiked wet artificial sediment (according to OECD 218), 400 mL M4 water (Elendt medium) corresponding to a water layer of about 8.0 cm; pH 7.41 - 8.11; oxygen content: 7.35 - 8.45 mg/L (saturation > 60%); total hardness: 2.53 mmol/L at test initiation; conductivity: 617 µS/cm at test initiation; ammonia: 0.8 mg/L; water temperature: 19.3°C - 20.1°C; light intensity: 397 - 592 lux; photoperiod: 16 h light : 8 h dark; continuous gentle aeration except during addition of the larvae and about 24 hours afterwards; food: TetraMin, until DAI 23: 0.25 - 1.0 mg food/larva/day.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with MS-detection.
Statistics:	Descriptive statistics, Dunnett's Multiple t-test procedure ($p < 0.05$) for comparison of the water control, the solvent control and the treatment groups; ANOVA followed by Chi-square test with Bonferroni correction for determination of the NOEC based on emergence and development rate ($\alpha = 0.05$), Probit and Weibull analysis using linear max. likelihood regression for determination of EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations in the overlying water, the pore water and the sediment was conducted in the controls and in each concentration at the beginning and the end of the test. Recoveries in the sediment were in the range between 66 and 87% of the nominal concentrations at test initiation and between 46 and 86% of nominal at test termination. Overlying water concentrations ranged from < LoQ (limit of quantification) to 5.32 µg/L at test start (= insertion of larvae) and from < LoQ to 2.7 µg/L at test end. The pore water concentrations ranged from < LoQ to 1.95 µg/L at test start and from < LoQ to 2.31 µg/L at test termination. The following biological results are based on the mean measured sediment concentrations.

Biological results: Mean emergence rates in both controls were above 90%. The mean development rates were 0.0650 and 0.0644 for the water control and the solvent control, respectively. No statistically significant differences were found for the emergence and development rates at any test item concentration when compared to the water control and the solvent control (Chi-square test with Bonferroni correction, $\alpha = 0.05$). The results are summarized in Table 8.2.5.3-4.

Table 8.2.5.3-4: Effects of BF 500-7 (metabolite of pyraclostrobin) on emergence and development of *Chironomus riparius*

Concentration [mg BF 500-7/kg dry sediment] (nominal)	**Control**	**Solvent control**	**2.0**	**6.0**	**18.0**	**54.0**	**162.0**
Concentration [mg BF 500-7/kg dry sediment] (mean measured)	**--**	**--**	**0.96**	**4.55**	**13.83**	**47.15**	**123.5**
Emergence rate (ER) (28 d) [#]	0.9375 ± 0.0479	0.9333 ± 0.0516	0.9250 ± 0.0500	0.9000 ± 0.0816	0.9000 ± 0.1225	0.9000 ± 0.1080	0.8500 ± 0.1080
Development rate per day (DR) (28 d) [#]	0.0650 ± 0.0008	0.0644 ± 0.0007	0.0635 ± 0.0014	0.0639 ± 0.0012	0.0633 ± 0.0005	0.0636 ± 0.0034	0.0633 ± 0.0034
Endpoints [mg BF 500-7/kg dry sediment] (mean measured)							
$EC_{50\ emergence\ rate}$ (28 d)	> 123.5						
$NOEC_{emergence\ \&\ development\ rate}$ (28 d)	≥ 123.5						

[#] Values represent mean and standard deviation from all replicates, each with 20 larvae.

III. CONCLUSION

In a 28-day static sediment test with *Chironomus riparius* the NOEC of BF 500-7 (metabolite of pyraclostrobin) was determined to be ≥ 123.5 mg a.s./kg dry sediment based on emergence and development rate (mean measured).

CA 8.2.5.4 Sediment dwelling organisms

The following acute spiked sediment toxicity study on marine amphipods (*Leptocheirus plumulosus*) performed with the active substance pyraclostrobin is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and is provided for completeness.

Report:	CA 8.2.5.4/1 Gaertner K., 2013a BAS 500 F: Whole sediment acute toxicity to a marine amphipod (Leptocheirus plumulosus) 2013/7000055
Guidelines:	EPA 850.1740
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 10-day static acute spiked sediment study, marine amphipods (*Leptocheirus plumulosus*) were exposed to pyraclostrobin at nominal concentrations of 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./kg dry sediment (corresponding to mean measured concentrations of 0.771, 1.51, 2.74, 5.05 and 11.3 mg a.s./kg dry sediment). Additionally, a solvent control and a dilution water control were set up. All test item concentrations and the control groups had 8 replicates consisting of 20 amphipods per replicate. Assessment of survival and general health and behavior of the amphipods was performed at test end.

The biological results are based on mean measured sediment concentrations. After 10 days of exposure, the mean survival was 99 and 98% in the dilution water control and the solvent control, respectively. Survival rates at the test item concentrations of 0.771, 1.51, 2.74, 5.05 and 11.3 mg a.s./kg dry sediment were 98, 100, 97, 25 and 0.6%, respectively. Statistically significant differences compared to the dilution water control were observed at the two highest tested concentrations.

In a 10-day static acute sediment test with *Leptocheirus plumulosus*, the LC_{50} of pyraclostrobin was determined to be 4.412 mg a.s./kg dry sediment based on mean measured concentrations. The NOEC was 2.740 mg a.s./kg dry sediment.

I. MATERIAL AND METHODS

Test item:	Mixture of non-radiolabeled pyraclostrobin (BAS 500 F; Reg. no.: 304 428, batch no. COD-001236; purity: 99.02%) and ^{14}C-radiolabeled pyraclostrobin (batch no. 579-6009; specific activity: 64.4 MBq/g; radiochemical purity: 99.4%; chemical purity: 100%).
Test species:	Marine amphipod (*Leptocheirus plumulosus*), 2 - 4 mm length; source: "Chesapeake Cultures", Hayes, Virginia, USA.
Test design:	Static system (10 days); 5 test concentrations plus a control and a solvent control, 8 replicates per test item concentration and per control group, 20 amphipods per replicate; assessment of survival after 10 days.
Endpoints:	NOEC and LC_{50}.
Test concentrations:	Control (dilution water), solvent control, 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./kg dry sediment (nominal); corresponding to geometric mean measured TRR concentrations (^{14}C-labeled pyraclostrobin equivalents) of 0.771, 1.51, 2.74, 5.05 and 11.3 mg a.s./kg dry sediment.
Test conditions:	1 L glass jars (17 cm height x 8.5 cm diameter) filled with 251.8 g natural marine sediment (27% sand, 55% silt, 18% clay, 6.4% organic matter), 600 mL dilution water (prepared by mixing a commercial sea salt mix to laboratory freshwater); salinity: 19.0 - 21.0‰; pH 7.6 - 8.5; oxygen content: 4.3 mg/L - 7.6 mg/L; water temperature: 24.3°C - 25.3°C; light intensity: 501 - 601 lux; photoperiod: 24 h light; continuous aeration.
Analytics:	Overlaying water, interstitial (pore) water and sediment were analyzed for total radioactive residues (TRR) using a liquid scintillation counting (LSC) method. The concentration of pyraclostrobin at the lowest treatment level was also measured in the overlying and interstitial water using an HPLC-method with UV-detection.
Statistics:	Descriptive statistics, Fisher's one-tailed exact test for determination of the NOEC value and for comparison of the dilution water control data and the solvent control data ($p = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of ^{14}C-labeled pyraclostrobin concentrations in the sediment, the overlying water and the interstitial water was conducted in the controls and in each concentration at the beginning and the end of the test via LSC analysis. Mean measured concentrations of ^{14}C-labeled pyraclostrobin TRR in the sediment were in a range between 117 and 125% of nominal concentrations at test initiation and between 89 and 120% of nominal at test termination. The geometric mean measured concentrations of ^{14}C-labeled pyraclostrobin in samples taken from the nominal concentrations of 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./kg dry sediment were determined to be 0.771, 1.51, 2.74, 5.05 and 11.3 mg a.s./kg dry sediment, respectively, which represent 101 to 122% of nominal concentrations. The geometric mean measured concentrations of ^{14}C-labeled pyraclostrobin in the overlaying water samples were 0.000526, 0.00136, 0.00282, 0.00541 and 0.0115 mg TRR/L for the 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./kg dry sediment samples, respectively. The respective geometric mean measured concentrations of ^{14}C-labeled pyraclostrobin in the interstitial water samples were 0.00169, 0.00338, 0.00625, 0.0115 and 0.0229 mg TRR/L.
Additionally, the concentration of pyraclostrobin in the controls and at the lowest treatment level was measured in overlying and interstitial water at test initiation and test termination via HPLC/UV analysis. Measured concentrations in overlaying water samples of two replicates of the 0.63 mg a.s./kg dry sediment treatment were 0.000128 and 0.000705 mg a.s./L at test initiation and 0.000342 and 0.0000492 mg a.s./L at test termination. In general, no residues of pyraclostrobin were detected in the control samples above the respective minimum quantifiable limits (MQL) in all measurements.
The following biological results are based on the geometric mean measured ^{14}C-labeled pyraclostrobin sediment concentrations. The TRR concentrations were corrected for dry weight of sediment.

Biological results: No statistically significant differences between the survival data in the dilution water control and the solvent control were detected (Fisher`s exact test; $p = 0.05$). The dilution control data were used for statistical evaluation of treatment related effects. After 10 days of exposure, the mean survival was 99 and 98% in the dilution water control and the solvent control, respectively. Survival rates at the test item concentrations of 0.771, 1.51, 2.74, 5.05 and 11.3 mg a.s./kg dry sediment were 98, 100, 97, 25 and 0.6%, respectively. Statistically significant differences compared to the dilution water control were observed at the two highest test concentrations (Fisher`s exact test; $p = 0.05$). The results are summarized in Table 8.2.5.4-1.

Table 8.2.5.4-1: Effect of pyraclostrobin on survival of *Leptocheirus plumulosus*

Concentration [mg a.s./kg dry sediment] (nominal)	**Control**	**Solvent control**	**0.63**	**1.3**	**2.5**	**5.0**	**10**
Concentration [mg a.s./kg dry sediment] (geometric mean measured)	**--**	**--**	**0.771**	**1.51**	**2.74**	**5.05**	**11.3**
Survival (10 d) [%]	99	98	98	100	97	25 *	0.6 *
	Endpoints [mg a.s./kg dry sediment] (geometric mean measured)						
LC_{50} (10 d)	4.412 (95% confidence limits: 4.192 - 4.643)						
NOEC (10 d)	2.740						

* Statistically significantly difference compared to the dilution water control (Fisher`s exact test; p = 0.05).

III. CONCLUSION

In a 10-day static acute sediment test with *Leptocheirus plumulosus*, the LC_{50} of pyraclostrobin was determined to be 4.412 mg a.s./kg dry sediment based on mean measured concentrations. The NOEC was 2.740 mg a.s./kg dry sediment.

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

The following 96-hour algae study on the freshwater green algae *Pseudokirchneriella subcapitata* has already been submitted and accepted during the previous Annex I inclusion process. Meanwhile the 72 h endpoints have been calculated from the original data for use in the aquatic risk assessment. The calculations and for completeness also the already submitted study are summarised below.

Report:	CA 8.2.6.1/1 Dohmen G.P., 1999a Effect of BAS 500 F on the growth of the green alga Pseudokirchneriella subcapitata 1999/11020
Guidelines:	OECD 201
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 8.2.6.1/2 Hoffmann F., 2009a Effect of BAS 500 F on the growth of the green alga Pseudokirchneriella subcapitata - Additional calculation of the inhibition values for growth rate and yield data after a test period of 72 h 2009/1037148
Guidelines:	OECD 201
GLP:	no

Executive Summary (originally submitted study, BASF DocID 1999/11020)

In a 96-hour static toxicity laboratory study, the effect of pyraclostrobin on the growth of the freshwater green algae *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 0.008, 0.016, 0.031, 0.063, 0.125, 0.250, 0.5, 1.0 mg a.s./L (corresponding to mean measured concentrations of 0 (control), 0.007, 0.014, 0.027, 0.057, 0.113, 0.213, 0.448 and 0.843 mg a.s./L). Assessment of growth was conducted 48 h, 72 h and 96 h after test initiation. Mean measured concentrations of the test item were 90.4% of nominal at the beginning and 86.0% at the end of the test. The biological results are based on mean measured concentrations of the test item. No morphological effects on algae were observed in the control group and any test item concentration tested throughout the test.

The effects on algal growth are summarized in Table 8.2.6.1-1

Table 8.2.6.1-1: Effect (96 h) of pyraclostrobin on the growth of the freshwater green alga *Pseudokirchneriella subcapitata*

Concentration [mg a.s./L] (nominal)	**0.008**	**0.016**	**0.031**	**0.063**	**0.125**	**0.250**	**0.500**	**1.0**
Concentration [mg a.s./L] (mean measured)	**0.007**	**0.014**	**0.027**	**0.057**	**0.113**	**0.213**	**0.448**	**0.843**
Inhibition in 96 h [%] (based on growth rate)	-1.1	3.3	6.8	6.5	12.2	15.6	27.6	49.5
Inhibition in 96 h [%] (based on biomass)	0.2	12.5	28.4	26.1	42.6	51.9	68.2	87.2
Endpoints [mg pyraclostrobin/L] (mean measured)								
E_rC_{50} (0 - 96 h)	> 0.843 extrapolated: 1.282 (95% confidence limits: 1.110 - 1.482)							
E_rC_{10} (0 - 96 h)	0.078 (95% confidence limits: 0.071 - 0.087)							
E_bC_{50} (0 - 96 h)	0.152 (95% confidence limits: 0.143 - 0.162)							
E_bC_{10} (0 - 96 h)	0.014 (95% confidence limits: 0.013 - 0.015)							

In a 96-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} of pyraclostrobin was determined to be > 0.843 mg a.s./L and the E_bC_{50} was 0.152 mg a.s./L (mean measured).

Executive Summary (recalculations; BASF DocID 2009/1037148)

The study BASF DocID 1999/11020 with the unicellular fresh water green alga *P. subcapitata* was conducted according to Good Laboratory Practice (GLP) following OECD Guideline 201, however, over a longer test duration than recommended (*i.e.* 96 h instead of 72 h). In the original study report only the 96 hour endpoints related to growth rate and biomass are reported.
Additional endpoints related to growth rate (r) and yield (y) after 72 hours of exposure were recalculated according to current recommendations (OECD 201, March, 2011). The following 72-hour endpoints were obtained based on mean measured concentrations of the test item:

E_rC_{50} (72 h) > 0.843 mg/L
E_rC_{10} (72 h) = 0.071 mg/L (95% confidence limits: 0.064 - 0.080 mg/L)
E_yC_{50} (72 h) = 0.148 mg/L (95% confidence limits: 0.140 - 0.158 mg/L)
E_yC_{10} (72 h) = 0.013 mg/L (95% confidence limits: 0.012 - 0.015 mg/L)

The following toxicity study on the freshwater green algae *Pseudokirchneriella subcapitata* performed with the metabolite BF 500-3 is provided in support of the aquatic risk assessment. The study was requested by Canadian authorities and has not been evaluated previously at the EU level.

Report:	CA 8.2.6.1/3 Hoffmann F., 2006a Effect of BF 500-3 (Reg.No. 340 266, metabolite of BAS 500 F) on the growth of the green alga Pseudokirchneriella subcapitata 2006/1038445
Guidelines:	OECD 201
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)

Executive Summary

In a 96-hour static toxicity laboratory study, the effect of BF 500-3 (metabolite of pyraclostrobin) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0.03, 0.075, 0.188, 0.469 and 1.172 mg BF 500-3/L. Additionally, a solvent (acetone) control and a dilution water control were set up. Assessment of growth was conducted 24, 48, 72 and 96 h after test initiation.

The biological results are based on nominal concentrations of the test item. No morphological effects on algae were observed in the control groups and at up to and including the highest test item concentration tested.

In a 96-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} (96 h) of BF 500-3 (metabolite of pyraclostrobin) was determined to be > 1.172 mg/L based on nominal concentrations. After 72 hours of exposure, the respective E_rC_{50} was equally determined to be > 1.172 mg/L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-3 (Reg. No. 340 266, synonym: 500M07; metabolite of pyraclostrobin), batch no. L74-118, purity: 99.9%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz); specification: SAG 61.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (96 hours); 5 test concentrations with 5 replicates for each plus a control with 10 replicates and a solvent control with 5 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 and 96 hours.

Test concentrations: Control, solvent control (0.1 mL acetone/L), 0.03, 0.075, 0.188, 0.469 and 1.172 mg BF 500-3/L (nominal).

Test conditions: 100 mL Erlenmeyer flasks, test volume: 60 mL; nutrient solution according to OECD 201; pH 8.1 at test initiation and pH 7.80 - 7.98 at test termination; temperature: 22 ± 1°C; initial cell densities: 3 x 10^3 cells/mL; continuous light at about 8000 lux, continuous shaking at about 135 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with MS detection.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values for growth rate and yield.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for BF 500-3 ranged from 74.0 to 98.8% of nominal concentrations at test initiation and from 20.6 to 56.5% of nominal at test termination. As the initially measured concentrations largely confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control groups and at up to and including the highest test item concentration tested. The effects on algal growth are summarized in Table 8.2.6.1-2.

Table 8.2.6.1-2: Effect of BF 500-3 (metabolite of pyraclostrobin) on the growth of the green algae *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**0.030**	**0.075**	**0.188**	**0.469**	**1.172**
Inhibition in 72 h (growth rate) [%]	0.9	6.2	5.8	9.0	12.2
Inhibition in 72 h (yield) [%]	3.5	22.6	20.8	31.4	40.0
Inhibition in 96 h (growth rate) [%] #	-0.5	1.2	4.3	11.9	11.9
Inhibition in 96 h (yield) [%] #	-2.5	5.9	19.7	45.9	45.9
Endpoints [mg BF 500-3/L] (nominal)					
E_rC_{50} (72 h)	> 1.172				
E_rC_{10} (72 h)	0.617 (95% confidence limits: 0.467 - 0.816)				
E_yC_{50} (72 h)	> 1.172				
E_yC_{10} (72 h)	0.038 (95% confidence limits: 0.030 - 0.047)				
E_rC_{50} (96 h)	> 1.172				
E_rC_{10} (96 h)	0.627 (95% confidence limits: 0.536 - 0.733)				
E_yC_{50} (96 h)	0.922 (95% confidence limits: 0.836 - 1.016)				
E_yC_{10} (96 h)	0.106 (95% confidence limits: 0.095 - 0.118)				

Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 96-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} (96 h) of BF 500-3 (metabolite of pyraclostrobin) was determined to be > 1.172 mg/L based on nominal concentrations After 72 hours of exposure, the respective E_rC_{50} value was equally determined to be > 1.172 mg/L.

The following study on the freshwater green algae *Pseudokirchneriella subcapitata* performed with the metabolite BF 500-5 has not been evaluated previously and was conducted to address a new metabolite found in a newly required e-fate study.

Report:	CA 8.2.6.1/4 Kuhl R., Frank C., 2014c Toxicity of Reg.No. 298327 (BF 500-5, metabolite of Pyraclostrobin) to Pseudokirchneriella subcapitata in an algal growth inhibition test 2013/1349202
Guidelines:	OECD 201 (2011), (EC) No 761/2009 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH 2009 - Part C.3: Algal Inhibition Test, OECD Series on Testing and Assessment No. 23 (2000) - Aquatic Toxicity Testing of Difficult Substances and Mixtures
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of BF 500-5 (metabolite of pyraclostrobin) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 0.10, 0.32, 1.0, 3.2 and 10.0 mg BF 500-5/L. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations of the test item. No morphological effects on algae were observed in the control group and at up to and including the highest test item concentration tested. Statistically significant differences for growth rate and yield compared to the control were observed at the two highest test item concentrations of 3.2 and 10.0 mg/L.

In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} of BF 500-5 (metabolite of pyraclostrobin) was determined to be 5.33 mg/L based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BF 500-5 (Reg. No. 298 327, synonym: 500M04; metabolite of pyraclostrobin), batch no. L84-174, purity: 99.6% ± 1%.

B. STUDY DESIGN

Test species:	Unicellular fresh water green alga, *Pseudokirchneriella subcapitata*; SAG 61.81; stock obtained from "Sammlung von Algenkulturen, Universität Göttingen", Göttingen, Germany.
Test design:	Static system (72 hours); 5 test concentrations with 3 replicates for each plus a control with 6 replicates; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72 hours.
Test concentrations:	Control, 0.10, 0.32, 1.0, 3.2 and 10.0 mg BF 500-5/L (nominal).
Test conditions:	50 mL Erlenmeyer flasks, test volume: 50 mL; nutrient solution according to OECD 201; pH 7.9 - 8.1 at test initiation and pH 8.0 - 9.7 at test termination; temperature: 22.1 - 22.7°C; initial cell densities: 5 x 10^3 cells/mL; continuous light at 4810 - 5870 lux.
Analytics:	Analytical verification of test item concentrations was conducted using an LC-method with MS/MS detection.
Statistics:	Descriptive statistics, probit analysis using linear max. likelihood regression for determination of EC_x values for growth rate and yield, Dunnett´s t-test for determination of the NOEC values ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for BF 500-5 ranged from 93 to 102% of nominal concentrations at test initiation. At test termination, the test item contents in the samples of all test item concentrations were below the LOQ (limit of quantification = 0.070 mg test item/L). As the initially measured concentrations confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control group and at up to and including the highest test item concentration tested. Statistically significant differences for growth rate and yield compared to the control were observed at the two highest test item concentrations of 3.2 and 10.0 mg/L (Dunnett´s t-test, $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.1-2.

Table 8.2.6.1-3: Effect of BF 500-5 (metabolite of pyraclostrobin) on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**0.10**	**0.32**	**1.0**	**3.2**	**10.0**
Inhibition in 72 h (growth rate) [%] #	-0.4	0.9	2.2	25.1 *	79.6 *
Inhibition in 72 h (yield) [%] #	-1.9	4.6	12.4	76.8 *	99.2 *
Endpoints [mg BF 500-5/L] (nominal)					
E_rC_{50} (72 h)	5.33 (95% confidence limits: 4.72 - 5.73)				
E_rC_{10} (72 h)	2.00 (95% confidence limits: 1.71 - 2.27)				
E_yC_{50} (72 h)	2.03 (95% confidence limits: 1.73 - 2.35)				
E_yC_{10} (72 h)	0.910 (95% confidence limits: 0.633 - 1.14)				

Negative values indicate stimulated growth compared to the control.
* Statistically significantly different compared to the control (Dunnett´s t-test, $\alpha = 0.05$)

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata,* the E_rC_{50} of BF 500-5 (metabolite of pyraclostrobin) was determined to be 5.33 mg/L based on nominal concentrations.

CA 8.2.6.2 Effects on growth of an additional algal species

The following alga study on the freshwater diatom *Navicula pelliculosa* performed with the active substance pyraclostrobin is not required for registration in the EU. The study was conducted due to U.S. data requirements and has not been evaluated previously on EU level. It is submitted for completeness.

Report:	CA 8.2.6.2/1 Boeri R.L. et al., 2000f Growth and Reproduction Toxicity Test with BAS 500F and the Freshwater Alga, Navicula pelliculosa 2000/5046
Guidelines:	EPA 123-2, EPA 850.5400, EPA Subdivision J of the Pesticide Assessment Guidelines
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The effect of pyraclostrobin on the growth of the freshwater diatom *Navicula pelliculosa* was investigated in a 120-hour static laboratory study. The following nominal concentrations were applied: 0.0013, 0.0025, 0.0050, 0.010 and 0.020 mg pyraclostrobin/L (corresponding to initial measured concentrations of 0.00118, 0.00240, 0.00486, 0.00908 and 0.0184 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted 24, 48, 72, 96 and 120 h after test initiation.

The biological results are based on initial measured concentrations of the test item. Statistically significant differences compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours.

In a 120 hour algae toxicity test with *Navicula pelliculosa,* the E_rC_{50} (120 h) for pyraclostrobin was determined to be > 0.0184 mg a.s./L. After 72 hours of exposure, the respective E_rC_{50} was determined to be 0.0158 mg a.s./.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C; purity: 97.09%.

B. STUDY DESIGN

Test species:	Freshwater diatom, *Navicula pelliculosa*, strain UTEX 664, stock originally obtained from the "Culture Collection of Algae", University of Texas, Austin, USA.
Test design:	Static system; test duration 120 hours; 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.
Endpoints:	EC_{50} with respect to biomass and growth rate after exposure over 72 and 120 hours.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.0013, 0.0025, 0.0050, 0.010 and 0.020 mg pyraclostrobin/L (nominal), corresponding to initial measured concentrations of 0.00118, 0.00240, 0.00486, 0.00908 and 0.0184 mg a.s./L.
Test conditions:	250 mL glass flasks; test volume 50 mL; sterile enriched medium supplemented with 0.2 g/L Na_2SiO_3 x 9 H_2O; pH 7.4 at test initiation and pH 7.3 - 7.6 at test termination; temperature: 23.2°C - 23.7°C; initial cell densities 3 x 10^3 cells/mL; continuous light at 4100 - 4500 lux; constant shaking at 100 rpm.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics; t-test (α = 0.05) for comparison of cell densities and growth rate data in the control and the solvent control; weighted least squares non-linear regression analysis for determination of EC_x values

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of pyraclostrobin ranged from 91 to 97% of nominal concentrations at test initiation and from 50 to 52% of nominal at test termination. The following biological results are based on initial measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Statistically significant differences of the cell numbers and average specific growth rates compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours (Bonferroni's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.2-1.

Table 8.2.6.2-1: Effect of pyraclostrobin on biomass development and growth of the freshwater diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (nominal)	**Water control**	**Solvent control**	**0.0013**	**0.0025**	**0.0050**	**0.010**	**0.020**
Concentration [mg a.s./L] (initial measured)	**--**	**--**	**0.00118**	**0.00240**	**0.00486**	**0.00908**	**0.0184**
Mean cell density (72 h) [% of water control]	--	108	78	33	26	19	19
Growth rate (72 h) [% of water control]	--	102	92	70	62	54	54
Mean cell density (120 h) [% of water control]	--	80	82	16	12	13	12
Growth rate (120 h) [% of water control]	--	96	96	72	68	70	68
Endpoints [mg pyraclostrobin/L] (initial measured)							
E_rC_{50} (72 h)	0.0158 (95% confidence limits: 0.00908 - > 0.0184)						
E_bC_{50} (72 h)	0.00165 (95% confidence limits: < 0.00118 - 0.00314)						
E_rC_{50} (120 h)	> 0.0184						
E_bC_{50} (120 h)	0.00150 (95% confidence limits: < 0.00118 - 0.00383)						
NOE_rC/NOE_bC (120 h)	0.00118						

III. CONCLUSION

In a 120 hour algae toxicity test with *Navicula pelliculosa,* the E_rC_{50} (120 h) for pyraclostrobin was determined to be > 0.0184 mg a.s./L. After 72 hours of exposure, the respective E_rC_{50} was determined to be 0.0158 mg a.s./L.

The following toxicity studies on the blue-green alga *Anabaena flos-aquae* and marine diatom *Skeletonema costatum* performed with the active substance pyraclostrobin are not required for registration in the EU. The studies were conducted due to U.S. data requirements and have not been evaluated previously on EU level.
Following the recommendations and validity criteria defined in the current OECD guideline 201 for alga testing (OECD, 2011) the 120 h studies on the algae *A. flos-aquae* and *S. costatum* are considered to be not valid. In both studies, at least one validity criterion is not met:

- the mean coefficients of variation (CV) for section by section specific growth rate in both the dilution water control and the solvent control were significantly > 35% after 0 - 72 hours, 0 - 96 hours and 0 - 120 hours in both studies;

- the CV of the average specific growth rate in the dilution water control was > 7% after 0 - 72 hours of exposure in the study on *A. flos-aquae* (i.e. 20%);

- the biomass increase was < 16 fold over the first 72 h in the dilution water control (*i.e.* 4-fold after 72 h) and over the first 96 h in the solvent control (*i.e.* 3-fold after 72 h and 13-fold after 96 h) in the study on *A. flos-aquae*; thus, no sufficient growth was demonstrated over the first 72 - 96 h of the test.

Nevertheless, summaries of the studies are provided for completeness.

Report:	CA 8.2.6.2/2 Boeri R.L. et al., 2000g Growth and reproduction toxicity test with BAS 500 F and the freshwater alga, Anabaena flos-aquae 2000/5036
Guidelines:	EPA 850.5400, FIFRA Subdivision J Series 123-2
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 120-hour static toxicity laboratory study, the effect of pyraclostrobin on growth of the blue-green alga *Anabaena flos-aquae* was investigated. The following nominal concentrations were applied: 0.13, 0.25, 0.50, 1.0 and 2.0 mg pyraclostrobin/L (corresponding to mean measured concentrations of 0.118, 0.219, 0.466, 0.911 and 1.78 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted 24 h, 48 h, 72 h, 96 h and 120 h after test initiation.

The biological results are based on mean measured concentrations of the test item. No statistically significant differences compared to the pooled control were observed at any test item concentrations after exposure over 120 hours.

In a 120-hour algae test with *Anabaena flos-aquae*, the E_rC_{50} (120 h) for pyraclostrobin was determined to be > 1.78 mg a.s./L, based on mean measured concentrations. After 96 hours of exposure, the respective E_rC_{50} value was determined to be 1.41 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C; purity: 97.09%.

B. STUDY DESIGN

Test species:	Freshwater blue-green alga, *Anabaena flos-aquae*; stock originally obtained from the University of Texas, Austin, USA; stock was maintained at test conditions for more than 14 days before the test.
Test design:	Static system (120 hours); 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.
Endpoints:	EC_{50} with respect to biomass and growth rate after exposure over 96 hours and 120 hours (72 h EC_{50} values could not be calculated because sufficient growth had not yet occurred during the first 72 hours in any of the test groups to allow the calculation to be made).
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.13, 0.25, 0.50, 1.0 and 2.0 mg pyraclostrobin/L (nominal), corresponding to mean measured concentrations of 0.118, 0.219, 0.466, 0.911 and 1.78 mg a.s./L.
Test conditions:	250 mL glass flasks; test volume: 50 mL; sterile enriched medium; pH 7.4 - 7.6 at test initiation and pH 7.8 - 8.0 at test termination; temperature: 23.7°C - 24.5°C; initial cell densities: 3 x 10^3 cells/mL; continuous light at about 2000 lux, continuous shaking at 100 rpm.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics, t-test ($\alpha = 0.05$) for comparison of cell densities and growth rate in the control and solvent control; Bonferroni's test for determination of the NOEC values ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured concentrations of pyraclostrobin ranged from 96.4 to 102.8% of nominal concentrations at test initiation and from 78.4 to 83.6% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. No statistically significant differences compared to the pooled control were observed at any test item concentrations after exposure over 120 hours (Bonferroni's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.2-2.

Table 8.2.6.2-2: Effect of pyraclostrobin on biomass development and growth of the blue-green alga *Anabaena flos-aquae*

Concentration [mg a.s./L] (nominal)	**Water control**	**Solvent control**	**0.13**	**0.25**	**0.50**	**1.0**	**2.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.118**	**0.219**	**0.466**	**0.911**	**1.78**
Mean cell density (96 h) [% of water control]	--	53	58	38	42	24	<14
Growth rate (96 h) [% of water control]	--	82	85	70	73	58	39
Mean cell density (120 h) [% of water control]	--	93	91	104	99	86	101
Growth rate (120 h) [% of water control]	--	100	98	102	100	98	100
Endpoints [mg pyraclostrobin/L] (mean measured)							
E_rC_{50} (96 h)	1.41 (95% confidence limits: 1.04 - > 1.78)						
E_bC_{50} (96 h)	0.367 (95% confidence limits: 0.184 - 0.734)						
E_rC_{50} (120 h)	> 1.78						
E_bC_{50} (120 h)	> 1.78						
NOE_rC/NOE_bC (120 h)	≥ 1.78						

III. CONCLUSION

In a 120-hour algae toxicity test with *Anabaena flos-aquae*, the E_rC_{50} (120 h) for pyraclostrobin was determined to be > 1.78 mg a.s./L based on mean measured concentrations. After 96 hours of exposure, the respective E_rC_{50} value was determined to be 1.41 mg a.s./L.

Report: CA 8.2.6.2/3
Boeri R.L.et al., 2000k
Growth and reproduction toxicity test with BAS 500F and the marine alga, Skeletonema costatum
2000/5035
Guidelines: EPA 123-2
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 120-h static toxicity laboratory study, the effect of pyraclostrobin on growth of the marine diatom *Skeletonema costatum* was investigated. The following nominal concentrations were applied: 0.013, 0.025, 0.050, 0.10 and 0.20 mg pyraclostrobin/L, corresponding to initial measured concentrations of 0.00973, 0.0194, 0.0381, 0.0816 and 0.159 mg a.s./L. Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted 24, 48, 72, 96 and 120 h after test initiation.

The biological results are based on initial measured concentrations of the test item. After 120 hours statistically significant differences compared to the pooled control were observed at the three and four highest test item concentrations for growth rate and mean cell density, respectively.

In a 120-h algae test with *Skeletonema costatum*, the E_rC_{50} (120 h) of pyraclostrobin was determined to be > 0.159 mg a.s./L based on initial measured concentrations. After 72 hours of exposure, the respective E_rC_{50} value was determined to be 0.0962 mg a.s./L.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C; purity: 97.09%.

B. STUDY DESIGN

Test species:	Marine diatom, *Skeletonema costatum*, strain UTEX LB 2308, in-house culture; stock originally obtained from the "Culture Collection of Algae", University of Texas, Austin, USA.
Test design:	Static system (120 hours); 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.
Endpoints:	EC_{50} with respect to biomass and growth rate after exposure over 72 hours and 120 hours.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.013, 0.025, 0.050, 0.10 and 0.20 mg pyraclostrobin/L (nominal), corresponding to initial measured concentrations of 0.00973, 0.0194, 0.0381, 0.0816 and 0.159 mg a.s./L.
Test conditions:	250 mL glass flasks; test volume: 50 mL; enriched marine media; pH 8.0 at test initiation and pH 8.3 - 9.6 at test termination; temperature: 19.2°C - 21.1°C; initial cell densities: 1 x 10^3 cells/mL; photoperiod: 16 hours light : 8 hours dark, light intensity: 3900 - 4000 lux, continuous shaking at 100 rpm.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC method with UV detection.
Statistics:	Descriptive statistics, t-test (α = 0.05) for comparison of cell densities and growth rate in the control and the solvent control; weighted least squares non-linear regression estimation procedure for determination of EC_x values, Bonferroni's test (α = 0.05) for determination of the NOEC (120 h) values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. At test initiation, mean measured concentrations of pyraclostrobin ranged from 75 to 82% of nominal concentrations. At test termination mean measured concentrations of pyraclostrobin were between 15 and 66% of nominal. The following biological results are based on initial measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; α = 0.05). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. After 120 hours statistically significant differences compared to the pooled control were observed at the three and four highest test item concentrations for growth rate and mean cell density, respectively (Bonferroni's t-test; α = 0.05). The effects on algal growth are summarized in Table 8.2.6.2-3.

Table 8.2.6.2-3: Effect of pyraclostrobin on biomass development and growth of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./L] (nominal)	**Water Control**	**Solvent control**	**0.013**	**0.025**	**0.050**	**0.10**	**0.20**
Concentration [mg a.s./L] (initial measured)	**--**	**--**	**0.00973**	**0.0194**	**0.0381**	**0.0816**	**0.159**
Mean cell density (72 h) [% of water control]	--	104	51	25	16	13	6
Growth rate (72 h) [% of water control]	--	100	85	71	62	59	41
Mean cell density (120 h) [% of water control]	--	98	99	86	59	51	15
Growth rate (120 h) [% of water control]	--	98	98	96	89	87	65
	Endpoints [mg pyraclostrobin/L] (initial measured)						
E_rC_{50} (72 h)	0.0962 (95% confidence limits: 0.0716 - 0.129)						
E_bC_{50} (72 h)	< 0.00973						
E_rC_{50} (120 h)	> 0.159						
E_bC_{50} (120 h)	0.0647 (95% confidence limits: 0.0498 - 0.0840)						
NOE_rC (120 h)	0.0194						
NOE_bC (120 h)	0.00973						

III. CONCLUSION

In a 120-h algae test with *Skeletonema costatum*, the E_rC_{50} (120 h) of pyraclostrobin was determined to be > 0.159 mg a.s./L based on initial measured concentrations. After 72 hours of exposure, the respective E_rC_{50} value was determined to be 0.0962 mg a.s./L.

CA 8.2.7 Effects on aquatic macrophytes

The following toxicity study on the aquatic plant *Lemna gibba* performed with the active substance pyraclostrobin is not required for registration in the EU. The study was conducted due to U.S. data requirements and has not been evaluated previously on EU level. It is submitted for completeness.

Report:	CA 8.2.7/1 Boeri R.L. et al., 2000h Growth and reproduction toxicity test with BAS 500F and the duckweed, Lemna gibba G3 2000/5037
Guidelines:	EPA 123-2, EPA 850.4400
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 14-day static toxicity laboratory study, the effect of pyraclostrobin on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0.13, 0.25, 0.50, 1.0 and 2.0 mg pyraclostrobin/L (corresponding to initial measured concentrations of 0.120, 0.202, 0.422, 0.896 and 1.72 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of plant growth and other effects was conducted 1, 4, 6, 8, 11 and 14 days after test initiation. Percent growth inhibition relative to the control was calculated for each test concentration based upon biomass for the parameters frond number and dry weight.

The biological results are based on initial measured concentrations of the test item. The duckweed population in the control vessels showed sufficient growth. At the end of the test, chlorotic fronds were observed in the control, the solvent control and in all test item concentrations tested. Statistically significant effects on the number of normal, non-chlorotic fronds and the plant biomass compared to the pooled controls were observed at the highest tested concentration of 1.72 mg a.s./L.

In a 14-day aquatic-plant test with *Lemna gibba,* the E_bC_{50} values of pyraclostrobin based on frond number and dry weight were determined to be > 1.72 mg a.s./L and 1.72 mg a.s./L, respectively, based on initial measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F; Reg. no.: 304 428), batch no. 27882/191/C; purity: 97.09%.

B. STUDY DESIGN

Test species:	Duckweed (*Lemna gibba* G3); inocula: 13 days old cultures; cultures maintained in-house; stock obtained from "Climate Stress Laboratory", USDA, Beltsville, Maryland, USA.
Test design:	Static system; test duration 14 days; 5 test item concentrations plus a control and a solvent control, 3 replicates for each test item concentration, the control and the solvent control; 3 plants with 3 - 4 fronds, total number of fronds at test initiation: 9 - 11 per replicate; assessment of growth and other effects on days 1, 4, 6, 8, 11 and 14.
Endpoints:	EC_{50} and NOEC with respect to biomass development after exposure over 14 days.
Test concentrations:	Control, solvent control (0.1 mL dimethylformamide/L), 0.13, 0.25, 0.50, 1.0 and 2.0 mg pyraclostrobin/L (nominal), corresponding to initial measured concentrations of 0.120, 0.202, 0.422, 0.896 and 1.72 mg a.s./L.
Test conditions:	500 mL glass flasks, test volume: 200 mL, M-Hoagland's media without sucrose or EDTA, pH 4.9 - 5.1 at test initiation and pH 5.5 - 5.7 at test termination; temperature: 24.7°C - 25.5°C, continuous light, light intensity: about 490 foot candles.
Analytics:	Analytical verification of the test item was conducted using an HPLC-method with UV-detection.
Statistics:	Descriptive statistics, t-test ($\alpha = 0.05$) for comparison of frond no. and dry weight in the control and solvent control, weighted least squares non-linear regression for determination of EC_x values based on frond no. and dry weight, Bonferroni's test for determination of the NOEC value ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of pyraclostrobin ranged from 81 to 92% of nominal at test initiation and from 17% to 34% of nominal at test termination. The following biological results are based on initial measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. The duckweed population in the control vessels showed sufficient growth, increasing from an average of 11 fronds per vessel to an average of 198 fronds per vessel, corresponding to an 18 x multiplication. At the end of the test, chlorotic fronds were observed in the control, the solvent control and in all test item concentrations tested. Statistically significant effects on the number of normal, non-chlorotic fronds and the plant biomass compared to the pooled controls were observed at the highest tested concentration of 1.72 mg a.s./L (Bonferroni's test; $\alpha = 0.05$). Effects on biomass development are summarized in Table 8.2.7-1.

Table 8.2.7-1: Effects of pyraclostrobin on the biomass development of duckweed *Lemna gibba*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.13**	**0.25**	**0.50**	**1.0**	**2.0**
Concentration [mg a.s./L] (initial measured)	**--**	**--**	**0.120**	**0.202**	**0.422**	**0.896**	**1.72**
Number of non-chlorotic fronds (14 d) [% of pooled control]	--	--	100	84	83	91	55
Mean dry weight of fronds [mg]	25.1	21.9	24.9	22.1	21.2	23.1	15.4
Endpoints [mg pyraclostrobin/L] (initial measured)							
E_bC_{50} (14 d) based on frond no	> 1.72						
E_bC_{50} (14 d) based on dry weight	1.72 (95% confidence limits: n.d.)						
NOEC (14 d)	0.896						

n.d. = not determined

III. CONCLUSION

In a 14-day aquatic-plant test with *Lemna gibba,* the E_bC_{50} values of pyraclostrobin based on frond number and dry weight were determined to be > 1.72 mg a.s./L and 1.72 mg a.s./L, respectively, based on initial measured concentrations.

CA 8.2.8 Further testing on aquatic organisms

The mesocosm study summarized below (BASF DocID 2000/1000011) was performed with the solo-formulation BAS 500 00 F (containing 250 g pyraclostrobin/L) and has already been submitted and accepted during the previous Annex I inclusion process for pyraclostrobin.

However, in recent years there was an increasing awareness of inconsistencies in both the way the same mesocosm data are interpreted and the AF applied by regulatory experts in different EU Member States. The Dutch Platform for Assessment of Higher-Tier Studies has produced a Guidance Document on how micro-/mesocosm data should be presented and evaluated in a uniform and transparent manner (de Jong et al., 2008; RIVM Guidance Document). The EFSA Aquatic Guidance Document (EFSA, 2013) proposes to largely use this document to present and evaluate micro-/mesocosm studies for regulatory purposes when placing plant protection products on the European market.
Therefore, the mesocosm study has been re-evaluated following the above mentioned RIVM Guidance Document.

This mesocosm study had been conducted under very conservative worst-case conditions with multiple test substance applications. Though conducted before certain additional guidances were published, which aid the assessment and evaluation of mesocosm studies, it covers all relevant requirements.
In order to further confirm this, an MDD analysis was conducted which is able to show that in this study far more than the required number of potentially sensitive species could be addressed with sufficient statistical power.

Executive summaries of the originally submitted study, the evaluation according to RIVM and the MDD analysis are provided below (for the detailed summary of the mesocosm study reference is made to the documents from the previous Annex I inclusion process for pyraclostrobin).

Report:	CA 8.2.8/1 Dohmen G.-P., 2000a The effect of BAS 500 00 F on aquatic ecosystems - An outdoor mesocosm investigation 2000/1000011
Guidelines:	EWOFFT, CLASSIC, HARAP, SETAC Europe, SETAC RESOLVE
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

A complex outdoor mesocosm study had been performed using multiple spray applications of the EC-formulated solo product BAS 500 00 F with eight applications of the test item in 14 day intervals and with rates increasing from 60 to 160 g a.s./ha during the season. A mixed, replicated ANOVA- regression approach was used with four treatment levels with concentrations reaching 0.9 to 24 µg a.s./L at the two last applications plus control, each with three replicate ponds. The mesocosm site consisted of 15 single ponds, each with a water volume of ca. 6.3 m^3. Water and sediment had been derived from a natural lake, which can be described as a holo/dimictic, meso- to slightly eutrophic, betameso-saprobic system. Parts of it are reserved as nature conservation area, and it is very rich in species. All relevant aquatic groups of phytoplankton, zooplankton, periphyton and benthic organisms were present in high diversity.

The results of this mesocosm study show, that most taxa were not affected even at the highest treatment level. Effects on a few species were observed at a concentration of 24 µg a.s./L. For all planktonic species these effects were found to be reversible. However, also fish and molluscs may be affected at this concentration. No clear effects were observed at 8 µg a.s./L. The multitude of endpoints and species and environmental conditions in this mesocosm study show clearly that at this (and lower) concentrations no adverse effects on aquatic communities can be expected even after multiple applications.

Parallel to the mesocosm study, the effects on fish under outdoor conditions were assessed using the same application scheme (omitting the first low application) in separate small ponds. Five ponds, each containing ca. 500 L water and sediment, were used for the control and the four pyraclostrobin treatments. Seven juvenile fish (*Cyprinus carpio*) were introduced initially into each of the ponds. Effects were only observed at the highest treatment level at the last application (during a period of high temperatures and low oxygen concentrations) causing fish mortality. No sublethal effects (behaviour, growth, gross pathological findings) were observed in any of the other treatments. Thus the outdoor fish study resulted in a NOEC = 8 µg a.s./L.

Based on the results of the mesocosm study, the NOEAEC for pyraclostrobin was determined to be between 8 µg a.s./L and 24 µg a.s./L; the NOEC of pyraclostrobin for aquatic ecosystems was 8 µg a.s./L.

Report: CA 8.2.8/2
Dohmen G.P., 2013a
Mesocosm study evaluation (BAS 500 00 F) - The effect of BAS 500 00 F on aquatic ecosystems - An outdoor mesocosm investigation
2012/1357084
Guidelines: none
GLP: no

Executive Summary

The mesocosm study was re-evaluated and presented according to the RIVM Guidance Document of the Dutch Platform for the assessment of higher tier studies (de Jong et al., 2008). The re-evaluation showed that the mesocosm test system is generally considered to allow very sensitive assessments of pesticide impact on aquatic ecosystems. Furthermore, the number of test substance applications is very much worst-case.
All relevant data are available and the study is considered acceptable and adequate for risk assessment. Given the data provided within the study report, a Reliability Index of 1 can be assigned.
The endpoints defined in the original study report were confirmed, i.e. the NOEAEC is between 8 µg a.s./L and 24 µg a.s./L; the NOEC of pyraclostrobin for aquatic ecosystems is 8 µg a.s./L.

Considering the multitude of endpoints covered, the inclusion of sensitive and relevant species, the worst case character of the study related to exposure and the realistic worst case test system applied, it can be concluded that this mesocosm study provides a sensitive, worst-case assessment of potential pyraclostrobin impacts on aquatic ecosystems.

Report: CA 8.2.8/3
Hommen U., 2016 a
MDD calculation for data sets of the BASF pond study on Pyraclostrobin (BASF DocID 2000/1000011)
2016/1345507
Guidelines: <none>
GLP: no

Executive Summary

- In 1999 BASF conducted an outdoor mesocosm study with the fungicide BAS 500 00 F (a.s.: pyraclostrobin). The test item was applied eight times every 14 days at four different treatment levels. Zooplankton, macroinvertebrates, emergence of insects and phytoplankton were monitored over up to 178 days after the first application.
- In the current 'Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters' (EFSA, 2013) it is required to report minimum detectable differences (MDD) for taxa assessed in a micro- or mesocosm study. To derive a regulatory acceptable concentration (RAC) it is recommended that for at least eight populations of the sensitive group the MDDs should be sufficiently low to allow an evaluation of direct effects.
- MDDs for the taxa considered for the evaluation were calculated using the Williams-test for each combination of taxon and sampling date following the proposal outlined in Brock et al. (2015).
- Considering the fungicide mode of action of the test item and the available laboratory toxicity data, invertebrates (mainly zooplankton, partly macroinvertebrates) and to a lower extent algae (see EFSA pesticide dossiers, EFSA 2016) can be considered as sensitive groups. Therefore, the objective of this report was to provide MDDs for the data sets including these groups and to determine for how many potentially sensitive populations a reliable evaluation of direct effects is possible in this study.
- From each of the data sets with frequent samplings, i.e. zooplankton and phytoplankton, at least eight taxa fulfil the MDD criterion proposed by Brock et al. (2015). These taxa cover different taxonomic groups including Cladocera, Copepoda, Ostracoda, Rotifera, and different classes of algae.
- Also in the data sets with only three or four samplings (MASS and insect emergence), the MDDs indicate that for several taxa a reliable statistical analysis was possible (e.g. for different midges, mayflies, snails and mussels).
- Thus, the study meets the requirement by EFSA (2013) that for at least eight populations of the sensitive group(s) a statistical evaluation of direct effects should be possible.

Summary of Relevant Literature Data

The following literature data has not been used or evaluated during the previous Annex I inclusion process.
From the literature search the following peer-reviewed scientific study on *Chironomus dilutus* and *Hyalella azteca* was considered relevant and reliable (with restrictions, RI 2) for the aquatic risk assessment of pyraclostrobin. In this study, *C. dilutus* larvae and juveniles of *H. azteca* were exposed to nine different pesticides. In the following summary only the experimental data and results for pyraclostrobin are presented.

Report: CA 8.2.8/4
Ding Y. et al., 2010a
Toxicity of sediment associated pesticides to *Chironomus dilutus* and *Hyalella azteca*
2014/1143797
Guidelines: none
GLP: no

Executive Summary

Chironomus dilutus larvae and juveniles of *Hyalella azteca* were exposed to pyraclostrobin in a series of 10-day flow-through spiked sediment studies. In the tests with *C. dilutus* sediments were spiked with test item concentrations as high as necessary to obtain LC_{50} values. In the tests with *H. azteca*, nominal test item concentrations up to and including 20 mg/kg dry sediment were used. Additionally, a solvent (acetone) control and a water control were set up for all tests. The *C. dilutus* test was conducted with 5 replicates per test item concentration and the *H. azteca* tests were conducted with 3 replicates per test item concentration. 10 larvae were added to each test vessel in all tests. Three series of tests with *H. azteca* using 3 different sediments with different organic carbon (OC) contents (0.5, 2 and 2.8%) were conducted, while *C dilutus* was only tested with 1 type of sediment (2% organic carbon). Assessment of survival was performed at test end. In addition, a sublethal growth endpoint using average individual ash-free dry mass was measured for *C. dilutus*.

The biological results are based on mean measured sediment concentrations. Survival of *C. dilutus* in the control groups was higher than 88% and average ash-free dry weight (AFDM) values ranged from 0.58 to 0.67 mg for control organisms after the 10-day bioassays. Mean survival in the control groups of the *H. azteca* tests was 95%. *H. azteca* mortality did not reach 50% even at the highest test item concentration (20 mg/kg dry sediment). The apparent differences in endpoints between different OC contents are merely due to different analyzed concentrations (lower recovery at higher OC content).

In a 10-day flow-through spiked sediment test with *Chironomus dilutus* the LC_{50} of pyraclostrobin was determined to be 6.913 mg a.s./kg dry sediment (mean measured). In respective tests with *Hyalella azteca* the LC_{50} values for pyraclostrobin were > 21.6, > 19.3 and > 13.2 mg a.s./kg dry sediment (mean measured) using sediment with an organic carbon content of 0.5, 2.0 and 2.8%, respectively. The NOEC for growth in the test with *C. dilutus* was determined to be 3.2 mg a.s./kg dry sediment based on ash-free dry weight (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F), CAS no.: 175013-18-0; supplier ChemService (West Chester, PA, USA), purity > 95%, log K_{ow}: 4.0, log K_{oc}: 3.8 - 4.2.

B. STUDY DESIGN

Test species: *Chironomus dilutus*, third instar larvae, source: in-house culture maintained at the Fisheries and Illinois Aquaculture Center, Southern Illinois University at Carbondale, originally obtained from "USEPA Environmental Research Laboratory", Duluth, MN, USA.
Hyalella azteca, juveniles, 7 - 14 days old; source: University of California, Berkeley, USA.

Test design: Flow-through system; exposure duration: 10 days; test substance was spiked into sediment; several test item treatments plus a solvent (acetone) control and a water control for both the *C. dilutus* and *H. azteca* tests. After a 12-day aging period, wet sediment was distributed into each test beaker and the sediment settled overnight. Ten organisms were randomly placed into each beaker.
C. dilutus: tests were performed with 4 - 6 test item concentrations; 5 replicates for all test item concentrations and controls, each replicate contained 10 animals; overlying water was renewed by addition of moderately hard water three times per day by an automatic water delivery system (total additions were approximately 180 ml/d), with the excess water overflowing of the beaker through a screened hole; *C. dilutus* was tested with 1 type of sediment only (2.0% organic carbon content).
H. azteca: tests were performed with 5 - 7 test item concentrations; 3 replicates for all test item concentrations and controls; each replicate contained 10 animals; overlying water was replaced by the addition of 500 ml water/d (each renewal was 100 ml, and five renewals were used daily); 3 series of tests were set up with *H. azteca* using 3 different sediments with different organic carbon content (0.5, 2.0 and 2.8%).

Assessment of survival and ash-free dry weight (AFDM) for *Chironomus dilutus* and assessment of survival for *H. azteca* was performed at test end.

Endpoints: NOEC and LC_{50} (regarding survival and growth) for *C. dilutus* and LC_{50} (regarding survival) for *H. azteca.*

Test concentrations:	Solvent control (< 700 µL acetone/kg wet sediment), water control for both test species; *C. dilutes*: 4 - 6 test item concentrations (as high as necessary to obtain LC_{50} values); *H. azteca:* 4 - 7 test item concentrations; nominal test item concentrations were not higher than 20 mg/kg dry sediment on the assumption that higher concentrations would be environmentally unrealistic.
Test conditions:	350 mL beakers with 60 g spiked wet sediment and approx. 300 ml overlying water; sediments were collected from drinking water reservoirs and a lake near Berkeley, California, USA with organic carbon contents of 0.5, 2.0 and 2.8%; temperature: 23°C; photoperiod: 16 h light : 8 h dark; food (daily): 1.0 mL of TetraFin suspension (6 g/L) for *C. dilutus* and yeast, cerophyll, and trout food mixture for *H. azteca*; some relevant information (pH, oxygen content, hardness and conductivity of dilution water) is missing in the study report.
Analytics:	Analytical verification of test item concentrations was conducted using a GC-method with micro-electron capture detection.
Statistics:	Descriptive statistics, probit analysis or trimmed Spearman–Karber analysis (Abbott's correction was applied in cases where the data were non-monotonic) for calculation of the LC_{50} values; ANOVA followed by Dunnett's test for determination of the NOEC values ($p < 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: The actual concentrations of the test items in the sediment were determined for each sediment type at the beginning and the end of the test. In the tests with *H. azteca* 95, 93 and 83% of nominal pyraclostrobin concentrations were found in sediments containing 0.5, 2 and 2.8% organic carbon, respectively. The measured pyraclostrobin content in the 2% organic carbon sediment used in the test with *C. dilutus* was 65% of nominal concentrations. The following biological results are based on mean measured sediment concentrations.

Biological results: Survival of *C. dilutus* in the control groups was higher than 88% and average ash-free dry weight values ranged from 0.58 to 0.67 mg for control organisms after the 10-day bioassays. There were no significant differences (Dunnett's test; $p < 0.05$) in the measurements between the negative and solvent controls. Mean survival in the control groups of the *Hyalella azteca* tests was 95% and no significant differences between control and solvent control were found. *Hyalella azteca* mortality did not reach 50% even at the highest test item concentration (20 mg/kg dry sediment). The apparent differences in endpoints between different OC contents are merely due to different analysed concentrations (lower recovery at higher OC content).
The results are summarized in Table 8.2.8-1.

Table 8.2.8-1: Effects of pyraclostrobin on survival and growth of *Chironomus dilutus* and survival of *Hyalella azteca*

Test organism	Organic carbon content of sediment [%]	Endpoint *	Value in mg a.s./kg dry sediment (mean measured)	Value in mg a.s./kg organic carbon content [1)] (mean measured)
Chironomus dilutus	2.0	10 d LC_{50}	6.913 (95% confidence limits: 4.958 - 8.443)	346 (95% confidence limits: 248 - 422)
		10 d NOEC [#]	3.2	160
Hyalella azteca	0.5	10 d LC_{50}	> 21.6	> 4330
	2.0	10 d LC_{50}	> 19.3	> 963
	2.8	10 d LC_{50}	> 13.2	> 470

* LC_{50} and NOEC values are based on mean measured test item concentrations.
[#] NOEC values for growth based on ash-free dry weight
[1)] Normalized to sediment organic carbon content

III. CONCLUSION

In a 10-day flow-through sediment test with *Chironomus dilutus* the LC_{50} of pyraclostrobin was determined to be 6.913 mg a.s./kg dry sediment (mean measured). In respective tests with *Hyalella azteca* the LC_{50} values for pyraclostrobin were > 21.6, > 19.3 and > 13.2 mg a.s./kg dry sediment (mean measured) using sediment with an organic carbon content of 0.5, 2.0 and 2.8%, respectively. The NOEC for growth in the test with *C. dilutus* was determined to be 3.2 mg a.s./kg dry sediment based on ash-free dry weight (mean measured).

From the literature search the following peer-reviewed scientific study on *Hyalella azteca* was considered relevant and reliable (with restrictions; RI 2). In this study, *H. azteca* amphipods were exposed to two different strobilurin fungicide formulations and their active substances. In the following summary only the experimental data and results for pyraclostrobin and the pyraclostrobin containing formulation 'Headline' are presented.

Report: CA 8.2.8/5
Morrison S.A. et al., 2013a
Acute toxicity of Pyraclostrobin and Trifloxystrobin to Hyalella azteca
2014/1143857
Guidelines: none
GLP: no

Executive Summary

Hyalella azteca amphipods were exposed to the fungicide formulation Headline and its active substance pyraclostrobin solved in acetone in a 7-day static study. *Hyalella azteca* was exposed in a water-only exposure scenario and in microcosm tests including sediment. In the first microcosm exposure scenario the test item was added directly to the overlying water and in the second scenario the sediment was over-sprayed with test item 24 h prior to the addition of water. An additional microcosm test was conducted to determine the degree of pyraclostrobin partitioning to the sediment (fate test). These microcosm treatments were set up using the same methods as microcosm tests outlined above to include both sediment- and water-treated pathways. 3 replicates were used. Nominal test concentrations were 0.005, 0.012, 0.030, 0.070 and 0.150 mg a.s./L for the water-only tests and 0.005, 0.014, 0.039, 0.107 and 0.284 mg a.s./L for the microcosm tests. Additionally, a water control was set up for all tests. In the water-only tests all test item concentrations and the water control had 4 replicates, whereas in the microcosm tests 6 replicates were tested for each treatment. 10 amphipods were added to each test vessel. The assessment of mortality was performed after 7 days.

The biological results are based on nominal concentrations. Additionally, biological results are presented based on average measured water concentrations over 96 h.

Water-only tests: Control mortality was < 5% for all experiments. Most of the observed mortality (> 80%) occurred by 96 h for all treatments. After 96 h of exposure, statistically significant differences compared to the control were observed at the three highest and the four highest test item concentrations in the pyraclostrobin treatment and the Headline treatment, respectively. There was a significant difference, albeit slight, in mortalities between Headline and pyraclostrobin treatments. However, the LC_{50} and LC_{10} values were similar and 95% confidence intervals overlapped.

Microcosm tests: Control mortality was < 10% across all experiments. Observed toxicity was lower when Headline was applied to the overlying water in the microcosm, compared with the water-only tests. Sediment-water microcosm systems reduced the toxicity by a factor of 5 compared to the observed toxicity in water only tests when the overlying water was treated, based on the total amount of fungicide applied to the system (LC_{50} value of 0.017 mg/L compared to 0.0855 mg/L). No significant differences were observed in mortality between the controls and the sediment treated microcosms up to and including the highest tested concentration. Thus, water versus sediment application of Headline resulted in significantly different mortalities at the 2 highest test item concentrations and non-overlapping LC_{50} and LC_{10} values between exposure scenarios.

In a 7-day spiked water test (water-only exposure) with *Hyalella azteca* the LC_{50} values of pyraclostrobin were determined to be 0.022 and 0.017 mg a.s./L (nominal) for the pyraclostrobin solved in acetone and the pyraclostrobin formulation treatment, respectively (0.0251 and 0.021 mg a.s./L based on 96 h measured average water concentrations, respectively).
In a 7-day spiked water test including sediment (Headline water overspray) with *Hyalella azteca* the LC_{50} value of pyraclostrobin was 0.0855 mg a.s./L based on nominal concentrations (0.0183 mg a.s./L based on 96 h measured average water concentrations). In a 7-day sediment test (Headline sediment overspray) with *Hyalella azteca* the LC_{50} value of pyraclostrobin was > 0.284 mg a.s./L based on nominal concentrations (> 0.0087 mg a.s./L based on 96 h measured average water concentrations).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F), purchased from Sigma- Aldrich, purity: 99.9%.
Pyraclostrobin formulation Headline (US-EPA Reg. No. 7969-186);
some relevant information (batch no., density of pyraclostrobin formulation, content of a.s. in pyraclostrobin formulation) is missing in the study report.

B. STUDY DESIGN

Test species: *Hyalella azteca*, juveniles, size: 250 - 500 µm, source: in-house culture.

Test design: Static system (7 days); pyraclostrobin formulation Headline was suspended in dechlorinated water and added to experimental units; solutions containing the active substance were dissolved in acetone to achieve the same concentration of active substance as in formulation concentrations, based on percentages of active substance in the formulation; 10 amphipods per replicate.
Water-only tests: exposure to pyraclostrobin and pyraclostrobin formulation; 5 test item concentrations plus a water control, 4 replicates for each treatment; amphipods were introduced to the experimental units prior to the addition of fungicides, assessment of survival after 6 h and then every 12 h after the start of the exposure until test end.
Microcosm tests: exposure to pyraclostrobin formulation only; 6 replicates for each treatment; experimental units were treated with pyraclostrobin formulation in 2 ways: 1) pyraclostrobin formulation diluted in water was added directly to the overlying water and gently stirred into solution, 2) sediment was sprayed across the sediment surface 24 h prior to the addition of overlying water.
Amphipods were added to water-treated microcosms prior to treating the overlying water and were added to sediment-treated microcosms immediately following the addition of water to the system; assessment of mortality at 7 d.
Fate test: An additional test was conducted to determine the degree of pyraclostrobin partitioning to the sediment. Microcosm treatments were set up using the same methods as microcosm toxicity tests outlined above to include both sediment- and water-treated pathways except only 3 replicates were used. The amount of pyraclostrobin applied to the sediment and water system corresponded to a water concentration of 0.300 mg/L (assuming full water incorporation) or a sediment concentration of 0.230 mg/kg (assuming complete adsorption to the sediment). Three replicates and a control were destructively taken down for each treatment at 3 h, 12 h, 96 h and 168 h.

Endpoints: NOEC (regarding survival); LC_{10} and LC_{50}.

Test concentrations: Water-only tests: water control, 0.005, 0.012, 0.030, 0.070 and 0.150 mg pyraclostrobin/L.
Microcosm tests: water control, 0.005, 0.014, 0.039, 0.107 and 0.284 mg a.s./L, the given concentrations represent the concentration based on full water incorporation into 800 mL of overlying water.

Test conditions:	Water-only tests: 600 mL glass beakers containing 500 mL dechlorinated water. Microcosm tests: glass jars containing 800 mL dechlorinated water and 100 g of sediment. All tests: pH 7.2 - 7.5; oxygen content: 3.2 mg/L - 9.0 mg/L; temperature: 23 ± 1°C; photoperiod: 16 h light : 8 h dark; food: 1.0 mL of TetraMin fish food solution (1800 mg/L) per replicate daily. Dilution water: dechlorinated water obtained by carbon filtration of tap water (pH: 7.5 - 7.7; hardness: 80 - 100 mg $CaCO_3$/L; dissolved oxygen: 6.7 - 9.0 mg/L. Sediment: collected from a wetland from the Rainwater Basin of Central Nebraska, soil type: silty clay loam (16.2% sand, 50% silt and 33.8% clay), overall organic matter content: 6.78%; no detectable pesticides were found in sediment (<10 mg/kg). Some relevant information (light intensity, aeration) is missing in the study report.
Analytics:	Analytical verification of test item concentrations was conducted using a GC-method with selected ion monitoring detection.
Statistics:	Descriptive statistics, log probit analysis for calculation of LC_x values; ANOVA followed by Tukey's honestly significant difference test for determination of NOEC values ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in the water-only tests and the microcosm tests at multiple time points throughout the tests.
Water-only tests: measured water concentrations for pyraclostrobin in the water-only toxicity tests were 122 ± 28% of targeted concentrations over the entire test. On average, measured water concentrations of pyraclostrobin declined 13 ± 9% from initial concentrations over 168 h.
Microcosm - water overspray: At 4 h after test initiation, pyraclostrobin water concentrations in the spiked water microcosms across all treatment concentrations were 28 ± 2% of that expected based on full water incorporation. Water concentrations of pyraclostrobin continued to decline throughout the test, with 13 ± 8% remaining at 96 h and 9 ± 7% after 168 h. Based on the parallel fate experiment, pyraclostrobin water concentrations declined by 69%, whereas sediment concentrations increased by 56% from initial measurements by 168 h. However, no differences were observed in the total mass balance of pyraclostrobin in system from beginning (59 ± 6%) to the end (62 ± 4%) due to the dissipation from the water column. Thus, partitioning of pyraclostrobin into the sediment accounts for the majority of the declining water concentrations with other dissipation processes (e.g., biotic- or abiotic-transformations, hydrolysis, etc.) contributing to the 30 to 40% being lost from the system.

Microcosm - sediment overspray: Application of Headline to the sediment 24 h prior to adding water resulted in minimal partitioning of pyraclostrobin from the sediment into the water column. Highest pyraclostrobin water concentrations across all treatments (2 ± 3%) were observed at the first measurement (4 h), with concentrations decreasing to < 1% of the total concentration expected from full water incorporation for the remainder of the microcosm toxicity tests. Measured water and sediment concentrations declined to 65 and 45% of initial measured concentrations, respectively, by 168 h based on the parallel fate experiment. There was a pronounced loss of pyraclostrobin from the system when Headline was applied to the sediment with total mass balance recoveries declining from 81 ± 10% at 4 h to 43 ± 3% at 168 h.
The following biological results are based on nominal concentrations. Additionally, biological results are presented based on average measured water concentrations over 96 h.

Biological results:
Water-only tests: Control mortality was < 5% for all experiments. Most of the observed mortality (> 80%) occurred by 96 h for all treatments. After 96 h of exposure, statistically significant differences compared to the control were observed at the three highest and the four highest test item concentrations in the pyraclostrobin treatment and the Headline treatment, respectively (ANOVA followed by Tukey's honestly significant difference test; $p < 0.05$). There was a significant difference, albeit slight, in mortalities between Headline and pyraclostrobin treatments ($p = 0.035$). However, the LC_{50} and LC_{10} values were similar and 95% confidence intervals overlapped.
Microcosm tests: Control mortality was < 10% across all experiments. Observed toxicity was lower when Headline was applied to the overlying water in the microcosm, compared with the water-only toxicity tests. Sediment-water microcosm systems reduced the toxicity by a factor of 5 compared to the observed toxicity in water only tests when the overlying water was treated, based on the total amount of fungicide applied to the system (LC_{50} value of 0.017 mg/L compared to 0.0855 mg/L). No significant differences were observed in mortality between the controls and the sediment treated microcosms up to and including the highest tested concentration (ANOVA followed by Tukey's honestly significant difference test; $p < 0.05$). Thus, water versus sediment application of Headline resulted in significantly different mortalities at the 2 highest test item concentrations and non-overlapping LC_{50} and LC_{10} values between exposure scenarios.

The results are summarized in Table 8.2.8-2.

Table 8.2.8-2: Effects of pyraclostrobin and Headline on survival of *Hyalella azteca*

Exposure scenario	Fungicide treatment	Endpoint	Endpoints [mg a.s./L]	
			Based on nominal	Based on 96 h average water concentrations (measured)
water-only exposure *	pyraclostrobin	7 d LC_{10}	0.0096 (95% confidence limits: 0.0061 - 0.0125)	0.0121 (95% confidence limits: 0.0094 - 0.00147)
		7 d LC_{50}	0.022 (95% confidence limits: 0.0172 - 0.0282)	0.0251 (95% confidence limits: 0.0213 - 0.0297)
		96 h NOEC	0.012	--
	pyraclostrobin formulation (Headline)	7 d LC_{10}	0.0074 (95% confidence limits: 0.0052 - 0.00094)	0.0098 (95% confidence limits: 0.0074 - 0.012)
		7 d LC_{50}	0.017 (95% confidence limits: 0.0142 - 0.0204)	0.021 (95% confidence limits: 0.018 - 0.0248)
		96 h NOEC	0.005	--
microcosm test - water overspray #	pyraclostrobin formulation (Headline)	7 d LC_{10}	0.020 (95% confidence limits: 0.0095 - 0.0313)	0.0039 (95% confidence limits: 0.0012 - 0.0070)
		7 d h LC_{50}	0.0855 (95% confidence limits: 0.0587 - 0.1341)	0.0183 (95% confidence limits: 0.0110 - 0.0354)
		7 d NOEC	0.039	--
microcosm test - sediment overspray #	pyraclostrobin formulation (Headline)	7 d LC_{10} 7 d LC_{50}	> 0.284	> 0.0087
		7 d NOEC	≥ 0.284	--

* 96-h average concentrations were calculated by averaging measurements from 6 h to 96 h.
# As pyraclostrobin formulation concentrations in the microcosm test declined throughout the exposures, 96 h weighted water concentration averages over time were calculated using an first-order exponential decay function.

III. CONCLUSION

In a 7-day spiked water test (water-only exposure) with *Hyalella azteca* the LC_{50} values of pyraclostrobin were determined to be 0.022 and 0.017 mg a.s./L (nominal) for the pyraclostrobin and the pyraclostrobin formulation treatment, respectively (0.0251 and 0.021 mg a.s./L based on 96 h measured average water concentrations, respectively).
In a 7-day spiked water test including sediment (Headline water overspray) with *Hyalella azteca* the LC_{50} value of pyraclostrobin was 0.0855 mg a.s./L based on nominal concentrations (0.0183 mg a.s./L based on 96 h measured average water concentrations). In a 7-day sediment test (Headline sediment overspray) with *Hyalella azteca* the LC_{50} value of pyraclostrobin was > 0.284 mg a.s./L based on nominal concentrations (> 0.0087 mg a.s./L based on 96 h measured average water concentrations).

From the literature search the following peer-reviewed scientific study on glochidia and juvenile life stages of freshwater mussels was considered relevant and reliable (with restrictions; RI 2). In this study, glochidia and juvenile life stages of freshwater mussels were exposed to different technical-grade current-use pesticides. However, in the following summary only the experimental data and results for pyraclostrobin are presented.

Report: CA 8.2.8/6
Bringolf R.B. et al., 2007a
Contaminant sensitivity of freshwater mussels - Acute and chronic toxicity of technial-grade pesticides to glochidia and juveniles of freshwater mussels (unionidae)
2014/1143854
Guidelines: none
GLP: no

Executive Summary

In an acute toxicity laboratory study, glochidia of the freshwater mussel *Lampsilis siliquoidea* were exposed over 48 hours under static conditions. Similarly, juvenile of *L siliquoidea* were exposed over 96 hours in a semi-static test system. Animals in both tests were exposed to a dilution water control, a solvent control and to up to 6 test item concentrations. Glochidia were exposed in groups of 100 - 200 individuals while juvenile test groups consisted of 7 mussels per replicate. Viability of glochidia was observed 24 and 48 hours after start of exposure. Survival of juveniles was assessed 48 and 96 hours after test initiation.

The biological results are based on mean measured concentrations of the test item. After 48 hours of exposure control viability was > 90% in the test with glochidia. Regarding the acute test with juvenile mussels control survival exceeded 93% after 96 hours of exposure. Juvenile mussels were more sensitive to pyraclostrobin than glochidia, at the end of the respective exposure times of 96 and 48 hours.

In an acute toxicity study with early life stages of the freshwater mussel *Lampsilis siliquoidea* the EC_{50} values of pyraclostrobin were 0.08 mg a.s./L (48 h) and 0.03 mg a.s./L (96 h) for glochidia and juveniles, respectively, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Pyraclostrobin (BAS 500 F) purchased from Chem Service (West Chester, PA, USA), purity: 99%. Some relevant information (batch no.) is missing in the study report.

B. STUDY DESIGN

Test species:	Freshwater mussel (*Lampsilis siliquoidea)*, glochidia and juveniles; juveniles 8 weeks post-transformation and less than two months old; source: in-house culture, mussels originally collected from Silver Fork of Perche Creek, Boone County, Missouri, USA.
Test design:	Glochidia acute toxicity tests: Static system (48 h); 5 - 6 test item concentrations plus a dilution water control and a solvent control, 3 replicates per treatment; 100 - 200 glochidia in each replicate; assessment of viability 24 and 48 hours after start of exposure. Juvenile acute toxicity tests: Semi-static system (96 h); renewal of test solutions 48 hours after test initiation; 5 - 6 test item concentrations plus a dilution water control and a solvent control, 3 replicates per treatment; 7 mussels in each replicate; assessment of survival 48 and 96 hours after start of exposure.
Endpoints:	EC_{50} based on viability (glochidia) or survival (juveniles).
Test concentrations:	Control (dilution water), solvent control (acetone), 5 - 6 test item concentrations approaching water solubility of the test item.
Test conditions:	Test chambers: 90 x 50 mm glass crystallizing dishes, test volume: 100 mL; dilution water: reconstituted hard water; hardness: 170 - 192 mg $CaCO_3$/L; temperature: 20.1 - 21.9°C; pH 8.32 - 8.61; oxygen content: > 80% saturation; conductivity: 523 - 625 µS/cm; some relevant information (aeration; feeding) is missing in the study report.
Analytics:	Analytical verification of test item concentrations was conducted using a liquid chromatography-tandem mass spectrometry method.
Statistics:	Descriptive statistics; Trimmed Spearman-Karber method for calculation of EC_{50} values.

II. RESULTS AND DISCUSSION

Analytical measurements: Pesticide exposure concentrations were determined for at least two treatment concentrations in each toxicity test. The mean measured content of pyraclostrobin was 122.6 ± 5.7% of nominal concentrations for toxicity tests with glochidia and juvenile mussels. The following biological results are based on mean measured concentrations of the test item.

Biological results: After 48 hours of exposure control viability was > 90% in the test with glochidia. Regarding the acute test with juvenile mussels control survival exceeded 93% after 96 hours of exposure. Juvenile mussels were more sensitive to pyraclostrobin than glochidia, at the end of the respective exposure times of 96 and 48 hours. The results are summarized in Table 8.2.8-3.

Table 8.2.8-3: Acute toxicity of pyraclostrobin to early life stages of the freshwater mussel *Lampsilis siliquoidea*

Life stage	Endpoint	Value in mg a.s./L (mean measured)
Glochidia	EC_{50} (24 h)	0.48 (95% confidence limits: 0.19 - 1.21)
	EC_{50} (48 h)	0.08 (95% confidence limits: 0.04 - 0.19)
Juvenile mussels	EC_{50} (96 h)	0.03 (95% confidence limits: 0.03 - 0.04)

III. CONCLUSION

In an acute toxicity study with early life stages of the freshwater mussel *Lampsilis siliquoidea* the EC_{50} values of pyraclostrobin were 0.08 mg a.s./L (48 h) and 0.03 mg a.s./L (96 h) for glochidia and juveniles, respectively, based on mean measured concentrations.

In the following summary of a study with several substances only the results for pyraclostrobin and its formulation Headline® are presented.

Report: CA 8.2.8/7
Hooser E.A. et al., 2012a
Acute toxicity of three Strobilurin fungicide formulations and their active ingredients to tadpoles
2014/1143796
Guidelines: <none>
GLP: no

Executive Summary

In a 96-hour static acute toxicity laboratory study, tadpoles of Great Plains toad (*Bufo cognatus*) were exposed to pyraclostrobin and the formulation Headline® at nominal concentrations of 0 (solvent control), 0.0005, 0.0017, 0.0050 and 0.015 mg/L in 3 replicates per treatment each with 7 tadpoles per aquarium. Tadpoles were observed for survival every 2 hours for the first 12 hours and then every 12 hours through 96 hours after start of exposure.

The following biological results are based on nominal concentrations. Mortalities in the solvent control were < 2% for the entire experiment. Pyraclostrobin and the formulation caused 100% mortality at the highest concentration. Toxicity was comparable between pyraclostrobin and the formulation for all concentrations, with the exception of 0.005 mg/L, where formulation exposure resulted in 79% mortality versus no mortality from exposure to pyraclostrobin. The authors conclude that pyraclostrobin is toxic to tadpoles and that adjuvant(s) in the Headline® formulation also contribute to mortality. However, it has to be considered that spacing of concentrations was rather wide and that the dose response is rather steep.

In a static acute toxicity study with tadpoles of *Bufo cognatus*, the LC_{50} (72 h) of pyraclostrobin was determined to be 0.010 mg a.s./L based on nominal concentrations, while the LC_{50} (72 h) for the formulation Headline® was 0.0037 mg/L (nominal). The NOEC values were 0.005 mg a.s./L and 0.0017 mg/L (nominal) for pyraclostrobin and the formulation Headline®, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Headline®: EPA Reg. No. 7969-186; purchased from a local distributor. Pyraclostrobin: purchased from Sigma-Aldrich (St. Louis, MO, USA); analytical standard grade (Pestanal®); dissolved using HPLC grade acetone to produce the same concentrations as the formulations.

B. STUDY DESIGN

Test species: Great Plains toad (*Bufo cognatus*); exposure of 6 days old tadpoles, obtained from in-house culture; adults were captured in central Oklahoma, USA.

Test design: Static system; exposure duration: 96 hours; 4 test item concentrations plus solvent control; 7 tadpoles per aquarium; 3 aquaria per tank (3 replicates; 21 tadpoles per tank), 1 tank per concentration and control; assessment of mortality every 2 hours for the first 12 hours and then every 12 hours through 96 hours after start of exposure. Stock solution of the a.s. pyraclostrobin was prepared by dissolving in acetone; the stock solutions of the formulations was prepared with water.

Endpoints: LC_{50}, NOEC, based on mortality after 72 h.

Test concentrations: Solvent control (deionized water and 0.5 mL acetone per aquarium),and 0.0005, 0.0017, 0.005 and 0.015 mg a.s./L for both the active substance and the formulation, respectively.

Test conditions: 9.5 L glass aquaria, test volume: 6 L, dilution water: dechlorinated water; temperature: 25 ±°2°C; dissolved oxygen: > 5.4°mg/L; photoperiod 13 h light : 11 h dark.

Analytics: Analytical verification of test item concentrations was conducted using a GC-method with MS detection.

Statistics: Descriptive statistics, one-way ANOVA, Tukey´s multiple range test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in the lowest treatment groups for both the formulation treatment and the active substance treatment after 3 and 96 hours and also in the highest treatment group for the formulation after 3 hours (value for active substance is missing). For the highest treatment group no analysis was done after 96 hours, because all individuals were dead within 24 hours for pyraclostrobin and Headline®. After 3 hours the mean measured values for pyraclostrobin and Headline® in the lowest treatment group were 84% and 126% of nominal values, respectively. After 96 hours the mean measured values for pyraclostrobin and Headline® were 22% and 91% of nominal, respectively. In the highest treatment group, the mean measured value of Headline® was 98% of nominal after 3 hours.
The following biological results are based on nominal concentrations.

Biological results: Mortalities in the solvent control were < 2%. 100% mortality was observed at the highest tested concentration for the a.s. and the formulation. Statistically significant differences in toxicity between pyraclostrobin and formulation exposure occurred at 0.005 mg/L only. The results are summarized in Table 8.2.8-4.

The authors speculate that constituents of the formulation may be responsible for the observed difference in toxicity at 0.005 mg/L. However, it has to be considered that spacing of concentrations was rather wide and that the dose response is rather steep.

Table 8.2.8-4: Acute toxicity of the active substance pyraclostrobin and the formulation Headline® to tadpoles of Bufo cognatus

Test substance	Endpoint	Value in mg a.s./L (nominal)
Pyraclostrobin	LC_{50} (72 h)	0.010
	NOEC (72 h)	0.005
Headline®	LC_{50} (72 h)	0.0037
	NOEC (72 h)	0.0017

III. CONCLUSION

In a static acute toxicity study with tadpoles of *Bufo cognatus*, the LC_{50} (72 h) of pyraclostrobin was determined to be 0.010 mg a.s./L based on nominal concentrations, while the LC_{50} (72 h) for the formulation Headline® was 0.0037 mg/L (nominal). The NOEC values were 0.005 mg a.s./L and 0.0017 mg/L (nominal) for pyraclostrobin and the formulation Headline®, respectively.

The following study investigated sublethal and chronic effects of three different strobilurin-containing fungicide formulations to *Bufo cognatus* tadpoles. In the following summary only the results for pyraclostrobin and the pyraclostrobin containing solo formulation Headline® are presented.

Report: CA 8.2.8/8
Hartman E.A.H. et al., 2014a
Chronic effects of Strobilurin fungicides on development, growth and mortality of larval great plains toads (Bufo cognatus)
2014/1143856
Guidelines: <none>
GLP: no

Executive Summary

In a 90-day semi-static chronic limit test, tadpoles of Great Plains toad (*Bufo cognatus*) were exposed throughout metamorphosis to the formulation Headline® at a single nominal concentration of 0.0017 mg pyraclostrobin/L and a dilution water control in 6 replicates per treatment each with 21 tadpoles per aquarium. Aquaria were checked daily for metamorphosed individuals and mortality. With the onset of metamorphosis the time to metamorphosis was recorded and weight and snout-vent length (SVL) were measured. At test termination surviving tadpole larvae were measured, staged and counted as incomplete metamorphosis.

The following biological results are based on nominal concentrations. Mortalities in the control were < 15% for the entire experiment. Mean body mass and snout-vent length of tadpoles in the control were 19.3 g and 11.4 mm. The average time to metamorphosis was 68.8 days for animals in the control, which is significantly longer than observed normally in the field (18-49 days). The time to metamorphosis of tadpoles in the Headline® treatment was statistically significantly reduced (it was also reduced non significantly in another treatment group). Headline® exposure caused no mortality and no statistically significant effects on body mass and snout-vent length were observed. In conclusion, headline exposure did not result in any adverse effects, and it may be speculated whether the observed difference to the control might be due to a particular poor performance of the control group.

In a semi-static chronic test with tadpoles of *Bufo cognatus*, application of the formulation Headline®, corresponding to a nominal test concentration of 0.0017 mg pyraclostrobin/L, resulted in a shorter development time as compared to the control. There was no impact on mortality or on biomass development or snout-vent length and accordingly no adverse effect of the Headline® treatment.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Headline® (EPA Reg. No. 7969-186; purchased from a local distributor) containing the active substance pyraclostrobin.

B. STUDY DESIGN

Test species: Great Plains toad (*Bufo cognatus*); tadpoles were captive breed from three pairs of adults captured in central Oklahoma (USA) in spring 2012.

Test design: Semi-static system; renewal of test water every 96 hours with fresh spike solutions; limit test: 1 test item concentration plus a water control; 21 six day old tadpoles per aquarium; 6 aquaria per treatment (n = 6 replicates). Tadpoles were allowed to acclimate to the test chambers 24 hours prior to test initiation; exposure duration: up to 90 days; daily assessment of mortality (failure to move after gentle probing with a glass rod) and metamorphosed individuals. Individuals that reached Gosner stage 44 were removed and placed into a tank with dilution water and substrate and allowed to absorb their tail and complete metamorphosis (Gosner stage 46). Time to metamorphosis was recorded and mass and snout–vent length (SVL) were measured at this time. Animals that had not finished metamorphosis after 90 days were measured, staged and counted as incomplete metamorphosis.

Endpoints: Mortality, time to metamorphosis, body mass and snout–vent length (SVL)

Test concentrations: Water control and 0.0017 mg pyraclostrobin/L (nominal).

Test conditions: 9.5 L glass aquaria, test volume: 6 L of dechlorinated water with continuous aeration, dilution water: dechlorinated water; temperature: 25 ±°2°C; dissolved oxygen: > 5.4°mg/L; photoperiod 13 h light: 11 h dark; feeding: tadpoles were fed *ad libitum* a mixture of commercial rabbit food and TetraMin® fish flakes (Tetra).

Analytics: Analytical verification of test item concentrations was conducted using a GC-method with MS detection. Please note, due to volume limitations of collected test water, pyraclostrobin measurements of the test items could not be achieved

Statistics: Descriptive statistics, one-way ANOVA, Wilcoxon test ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentration was conducted in fresh and old solutions (i.e. 2 and 96 hours after spiking) at each test water renewal in two of the six replicates. Due to volume limitations of collected test water, pyraclostrobin measurements could not be achieved. In addition, spiking solutions were confirmed to be within 10% of expected, which is similar to the precision of the instrument. The following biological results are reported using nominal concentrations.

Biological results: Mortalities in the control were < 15% for the entire experiment. Mean body mass and snout-vent length of tadpoles in the control were 19.3 g and 11.4 mm. The average time to metamorphosis was 68.8 days for animals in the control, which is significantly longer than observed normally in the field (18-49 days). The time to metamorphosis of tadpoles in the Headline® treatment was statistically significantly reduced (it was also reduced non significantly in another treatment group). Headline® exposure caused no mortality and no statistically significant effects on body mass and snout-vent length were observed. In conclusion, headline exposure did not result in any adverse effects, and it may be speculated whether the observed difference to the control might be due to a particular poor performance of the control group.

III. CONCLUSION

In a semi-static chronic test with tadpoles of *Bufo cognatus*, application of the formulation Headline®, corresponding to a nominal test concentration of 0.0017 mg pyraclostrobin/L, resulted in a shorter development time as compared to the control. There was no impact on mortality or on biomass development or snout-vent length and accordingly no adverse effect of the Headline® treatment.

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CA 8.3 Effects on arthropods

CA 8.3.1 Effects on bees

Since Annex I inclusion of the active substance pyraclostrobin (BAS 500 F), new studies on honeybees and bumblebees have been performed with the active substance. As a result, there are new endpoints, which are considered in the honey bee risk assessment. Summaries of these new studies are provided below and an overview on studies and endpoints is given in Table 8.3.1-1.

Table 8.3.1-1: List of studies and endpoints with bees and the active substance pyraclostrobin (BAS 500 F)

Substance	Test species	Endpoint	Value	Reference (BASF DocID)	EU agreed
Pyraclostrobin	honeybee	48 h acute oral LD_{50}	> 73.1 µg a.s./bee	1999/11457	yes
		48 h acute contact LD_{50}	> 100.0 µg a.s./bee		
	honeybee	48 h acute oral LD_{50}	> 110 µg a.s./bee	2013/1003210	no, new study
		48 h acute contact LD_{50}	> 100 µg a.s./bee		
	bumblebee	96 h acute oral LD_{50}	> 97.2 µg a.s./bee	2016/1000530	no, new study
		96 h acute contact LD_{50}	> 100 µg a.s./bee		
Pyraclostrobin (tested as Pristine)[3)]	honeybee	honeybee development	No unacceptable effects in queen development, queen survival and adult worker bee health using 400 ppm Pristine treated (measured concentration: 22 ppm pyraclostrobin)	2013/1416303	no, new study
Pyraclostrobin[1)]	--	residues in honeybee feed items (sunflower)	Applied rate: 143 g pyraclostrobin/ha Highest residue: 16.6 mg pyraclostrobin/kg (90^{th} percentile, found in pollen)	2014/1000204	no, new study
Pyraclostrobin[1)]	--	residues in honeybee feed items (oilseed rape)	Applied rate: 143 g pyraclostrobin/ha Highest residue: 6.7 mg pyraclostrobin/kg (90^{th} percentile, found in pollen)	2014/1000182	no, new study
Pyraclostrobin[2)]	honeybee	semi-field tunnel test	No unacceptable lethal or sublethal effects on honeybee colonies exposed to 250 g a.s./ha	2011/1112669	no, new study

1) Study was carried out with BAS 556 03 F as a surrogate for pyraclostrobin. BAS 556 03 F contains 130 g pyraclostrobin/L and 80 g metconazole/L and is an EC formulation like BAS 500 06 F. The study is presented as additional information.
2) Study was carried out with BAS 500 06 F as a surrogate for pyraclostrobin because active substances can only be applied as formulated products.
3) Peer-reviewed scientific study was carried out with Pristine containing 25.2% boscalid and 12.8% pyraclostrobin (a formulation similar to BAS 516 07 F). The study summary is shown in M-CP 10.3.1.3 of the BAS 516 07 F dossier.

Report:	CA 8.3.1/1 Mack P., 2014a Determination of residues of BAS 556 03 F in nectar, pollen and flowers of sunflowers after one application in 2013 2014/1000204
Guidelines:	EEC 7029/VI/95 rev. 5, EU Regulation 1107/2009 with Regulation 283/2013, EU Regulation 1107/2009 with Regulation 284/2013
GLP:	yes (certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The purpose of the study was to collect samples for later determination of residues in nectar, pollen and flowers from sunflowers treated once with BAS 556 03 F under field conditions. During the 2013 growing season five separate field trials were conducted in different representative growing areas in Southern Germany, Italy and Greece. Actual applied rates of pyraclostrobin in the five trials were 145.9, 144.3, 146.0, 144.3, and 137.4 g a.s./ha. The results were compared to an untreated control.

In the control treatments no residues of pyraclostrobin were detected in pollen, nectar and flower matrices. The matrices with the highest residues analyzed over all trials were pollen samples. Residues ranged from 2.20 to 17.00 mg/kg at the first sampling (0-1 DAA) and from 0.05 to 1.3 mg/kg at the second sampling (6-7 DAA). In flower samples residues of all trials ranged from 0.68 mg/kg to 1.50 mg/kg at the first sampling (0-1 DAA). At the second sampling (6-7 DAA) residues were between 0.04 mg/kg and 0.32 mg/kg. In nectar samples lowest residues of all matrices were analysed and ranged from 0.04 mg/kg to 0.53 mg/kg at the first sampling (0-1 DAA). At the second sampling the residues were < 0.010 mg/kg (below LOQ).

Based on the application rate of 143 g pyraclostrobin/ha (applied as BAS 556 03 F) the highest residues (90th percentile), found in pollen, was determined to be 16.6 mg pyraclostrobin/kg at the first sampling with an average value of 9.76 mg pyraclostrobin/kg.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 556 03 F, batch no. 380014, content: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 130.0 g/L nominal (132.6 g/L analyzed); metconazole (BAS 555 F, Reg. No. 4 056 343): 80.0 g/L nominal (80.2 g/L analyzed); density: 1.077 g/cm^3.

B. STUDY DESIGN

Test design: Nectar, pollen and flower samples collected from sunflowers were analyzed for residues of the test item BAS 556 03 F. The study comprised five trials, three in Southern Germany (trials -*01* to -*03*), one in Italy (trial -*04*) and one in Greece (trial -*05*). For the trials -*01* and -*03* to -*05* two treatment groups were set up per trial, one test item treatment and one untreated control.

Field site: The three German fields were located in the region of Baden-Wuerttemberg; the two fields in Italy in the region of Emilia-Romagna. All fields were cropped with sunflowers.

Sampling: Two samplings were performed per trial, one within 24 hours after application of the treatment field and one six to seven days after application. For trial -*02* one field site was used, where one sampling was performed before application and used as control and two further samplings were performed one and seven days after application. For pollen and nectar samples, on each sampling day a pooled sample of approx. 0.4 g was collected and divided into two subsamples samples (A and R) of at least 0.2 g, if enough material was available. For flower samples on each sampling day 12 flower heads from at least 12 different locations across the plot were collected. Depending on the size of the flower head they were cut into pieces and at least 1/8 of the flower head was taken for a pooled sample. The samples were divided into two subsamples (A and R) of at least 12 flower heads, each.

Application rates: Actual applied rates of pyraclostrobin: trial -*01*: 145.9 g a.s./ha, trial -*02*: 144.3 g a.s./ha, trial -*03*: 146.0 g a.s./ha, trial -*04*: 144.3 g a.s./ha and trial -*05*: 137.4 g a.s./ha. All applications were carried out between BBCH 63 and 65 with 300 L water/ha.

II. RESULTS AND DISCUSSION

In the control treatments no residues of pyraclostrobin were detected in pollen, nectar and flower matrices. The matrices with the highest residues analyzed over all trials were pollen samples. Residues ranged from 2.20 to 17.00 mg/kg at the first sampling (0-1 DAA) and from 0.05 to 1.3 mg/kg at the second sampling (6-7 DAA). In flower samples residues of all trials ranged from 0.68 mg/kg to 1.50 mg/kg at the first sampling (0-1 DAA). At the second sampling (6-7 DAA) residues were between 0.04 mg/kg and 0.32 mg/kg. In nectar samples lowest residues of all matrices were analysed and ranged from 0.04 mg/kg to 0.53 mg/kg at the first sampling (0-1 DAA). At the second sampling the residues were < 0.010 mg/kg (below LOQ). The results are presented in Table 8.3.1-2.

Table 8.3.1-2: Analyzed residues of pyraclostrobin in nectar, pollen and flower samples

Matrix	Treatment	Sampling	Timing [DAA[1)]]	Range of residues over all trials [mg/kg]	90th percentile over all trials [mg/kg]
Nectar	Control	S1[2)]	-1 – 1	< LOQ	--
		S2[2)3)]	7		
	BAS 556 03 F	S1[2)]	0 – 1	0.04 – 0.53	0.42
		S2[2)]	7	< LOQ	--
Pollen	Control	S1	-3 – 1	< LOQ	--
		S2[3)]	0 – 7		
	BAS 556 03 F	S1	0 – 1	2.20 – 17.00	16.60
		S2	6 – 7	0.05 – 1 3	1.07
Flowers	Control	S1	-3 – 1	< LOQ	--
		S2[3)]	6 – 7		
	BAS 556 03 F	S1	0 – 1	0.68 – 1.50	1.46
		S2	6 – 7	0.04 – 0.32	0.31

< LOQ (below limit of quantification): < 0.010 mg/kg
1) Days after application
2) Nectar samples of four trials (no sample in trial *-04*)
3) No sample for trial *-02*

For all matrices a reduction between the first and second sampling is visible. Table 8.3.1-3 shows the residue reduction from the 1st to the 2nd sampling in percent, calculated from the average residue values from all 5 trials.

Table 8.3.1-3: Reduction of pyraclostrobin residues in nectar, pollen and flowers between samplings

Matrix	Sampling	Timing [DAA [1)]]	Average residue [mg/kg]	Reduction [%]
Nectar	S1[2)]	0 – 1	0.20	--
	S2[2)3)]	7	< 0.01	95.00
Pollen	S1	0 – 1	9.76	--
	S2	6 – 7	0.51	94.77
Flowers	S1	0 – 1	1.13	--
	S2	6 – 7	0.19	83.19

1) Days after application
2) Nectar samples of four trials (no sample in trial *-04*)
3) Values below the limit of quantification (0.010 mg/kg) were set to 0.01 mg/kg.

III. CONCLUSION

Based on the application rate of 143 g pyraclostrobin/ha (applied as BAS 556 03 F) the highest residues (90th percentile), found in pollen, was determined to be 16.6 mg pyraclostrobin/kg at the first sampling with an average value of 9.76 mg pyraclostrobin/kg.

Report:	CA 8.3.1/2 Barth M., 2014a Determination of residues of BAS 556 03 F in oilseed rape inflorescences and their respective honeybee food items 2014/1000182
Guidelines:	EEC 1607/VI/97 rev. 2 10.06.1999, EU Regulation 1107/2009 with Regulation 283/2013, EU Regulation 1107/2009 with Regulation 284/2013, SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The purpose of the study was to collect samples for later determination of residues in nectar, pollen and flowers from winter oilseed rape treated once with BAS 556 03 F under field conditions. During the 2013 growing season five separate field trials were conducted in different representative growing areas in Europe. Trial FR 13 044 01 was performed in East Germany, trials FR 13 044 02 and -03 were performed in South Germany and trial FR 13 044 04 was located in West Germany. Trial FR 13 044 05 was performed in Poland. Actual applied rates of pyraclostrobin in the five trials were 134.7, 138.6, 139.5, 142.4 and 149.3 g a.s./ha. The results were compared to an untreated control.

In the control treatments no residues at or above the limit of quantification of pyraclostrobin were detected in pollen, nectar and flower matrices. The matrices with the highest residues analyzed over all trials were flower samples. Residues ranged from 8.557 to 20.994 mg/kg at the first sampling (0 DAA) and from 0.361 to 2.915 mg/kg at the second sampling (7 DAA). In pollen samples residues of all trials ranged from 1.075 mg/kg to 7.787 mg/kg at the first sampling (0 DAA). At the second sampling (7 DAA) residues were between 0.010 mg/kg and 0.222 mg/kg. In nectar samples lowest residues of all matrices were analyzed and ranged from 0.057 mg/kg to 0.231 mg/kg at the first sampling (0 DAA). At the second sampling the residues were < LOD to 0.023 mg/kg.

Based on the application rate of 143 g pyraclostrobin/ha (applied as BAS 556 03 F) the highest residues (90th percentile), found in pollen, was determined to be 6.7 mg pyraclostrobin/kg at the first sampling with an average value of 3.3 mg pyraclostrobin/kg.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 556 03 F, batch no. 380014, content: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 130.0 g/L nominal (132.6 g/L analyzed); metconazole (BAS 555 F, Reg. No. 4 056 343): 80.0 g/L nominal (80.2 g/L analyzed); density: 1.077 g/cm^3.

B. STUDY DESIGN

Test design: Nectar, pollen and flower samples collected from winter oilseed rape were analyzed for residues of the test item BAS 556 03 F. The study comprised five trials, which covered different geographical regions in Europe. Each trial comprised a test item treatment and an untreated control. The minimum distance between test item treatment and control was at least 10 m. Two samplings were performed per trial, one on day of application and one seven days after application.

Field site: The four German fields were located in East Germany near Motterwitz (Saxony), in South Germany (Kleinrheinfeld and Traustadt, Bavaria) and in West Germany near Goch-Nierswalde (North Rhine Westfalia); the field in Poland was located near Urbanowice. All fields were cropped with winter oilseed rape.

Sampling: For determination of residues in *Brassica napus* L. the flower samples and the respective bee food items (pollen and nectar) were sampled in the test item treatment and the respective control of each trial. For pollen and nectar samples, on each sampling day a pooled sample of approx. 2 g was collected and divided into two subsamples samples (A and R) of at least 1 g for analysis. For flower samples on each sampling day a pooled sample of at least 10 g of winter oil seed rape inflorescences were collected divided into two subsamples samples (A and R) of at least 5 g, each. The sampling on DAA 0 was performed within 24 hours after application of the test item. The second sampling took place on DAA 7.

Application rates: Actual applied rates of pyraclostrobin: trial -*01*: 134.7 g a.s./ha, trial -*02*: 138.6 g a.s./ha, trial -*03*: 139.5 g a.s./ha, trial -*04*: 142.4 g a.s./ha and trial -*05*: 149.3 g a.s./ha. All applications were carried out at BBCH 65 with 300 L water/ha.

II. RESULTS AND DISCUSSION

In the control treatments no residues at or above the limit of quantification of pyraclostrobin were detected in pollen, nectar and flower matrices. The matrices with the highest residues analyzed over all trials were flower samples. Residues ranged from 8.557 to 20.994 mg/kg at the first sampling (0 DAA) and from 0.361 to 2.915 mg/kg at the second sampling (7 DAA). In pollen samples residues of all trials ranged from 1.075 mg/kg to 7.787 mg/kg at the first sampling (0 DAA). At the second sampling (7 DAA) residues were between 0.010 mg/kg and 0.222 mg/kg. In nectar samples lowest residues of all matrices were analyzed and ranged from 0.057 mg/kg to 0.231 mg/kg at the first sampling (0 DAA). At the second sampling the residues were < LOD to 0.023 mg/kg. The results are presented in Table 8.3.1-4.

Table 8.3.1-4: Analyzed residues of pyraclostrobin in nectar, pollen and flower samples

Matrix	Treatment	Sampling	Timing [DAA[1)]]	Range of residues [mg/kg]	90th percentile [mg/kg][2)]
Nectar	Control	S1	0	< LOQ	--
		S2	7		
	BAS 556 03 F	S1	0	0.057 - 0.231	0.211
		S2	7	< LOQ - 0.023	0.021
Pollen	Control	S1	0	< LOQ	--
		S2	7		
	BAS 556 03 F	S1	0	1.075 – 7.787	6.727
		S2	7	0.010 - 0.222	0.186
Flowers	Control	S1	0	< LOQ	--
		S2	7		
	BAS 556 03 F	S1	0	8.557 – 20.994	29.602
		S2	7	0.361 - 2.915	2.012

< LOQ (below limit of quantification): < 0.01 mg/kg.
1) Days after application.
2) 90th percentile over all trails.

For all matrices a reduction between the first and second sampling is visible. Table **8.3.1-3** shows the residue reduction from the 1st to the 2nd sampling in percent, calculated from the average residue values from all 5 trials:

Table 8.3.1-5: Reduction of pyraclostrobin residues in nectar, pollen and flowers between samplings

Matrix	Sampling	Timing [DAA[1)]]	Average residue [mg/kg]	Reduction [%]
Nectar	S1	0	0.14	--
	S2[2)]	7	0.01	91.4
Pollen	S1	0	3.30	--
	S2	7	0.11	96.6
Flowers	S1	0	19.26	--
	S2	7	1.00	94.8

1) Days after application.
2) Values below the limit of quantification (0.010 mg/kg) were set to 0.01 mg/kg.

III. CONCLUSION

Based on the application rate of 143 g pyraclostrobin/ha (applied as BAS 556 03 F) the highest residues (90th percentile), found in pollen, was determined to be 6.7 mg pyraclostrobin/kg at the first sampling with an average value of 3.3 mg pyraclostrobin/kg.

CA 8.3.1.1 Acute toxicity to bees

In the original dossier the study BASF DocID 1999/11457 had been included. However, since the effect of the toxic reference item was slightly out of range, the study was repeated for the renewal of approval. The results of the new study confirm the outcome of the original study. For risk assessment purposes the values of the new study are used.

CA 8.3.1.1.1 Acute oral toxicity

Report:	CA 8.3.1.1.1/1 Sekine T., 2013a Effects of BAS 500 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory 2013/1003210
Guidelines:	OECD 213 (1998), OECD 214 (1998)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In a limit test, young adult worker bees (*Apis mellifera carnica*) were exposed orally to pyraclostrobin (BAS 500 F). Therefore, a nominal dose of 100.0 µg a.s./bee was tested, resulting in an actual uptake of 110.0 µg a.s./bee. Additionally, honeybees were treated with dimethoate as reference item at 0.05 to 0.32 µg/bee (nominal) or with water and solvent as control treatments. The test was conducted with 5 replicates per treatment group; each replicate contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

No mortality was observed after 48 hours in the control and the test item treatments. The LD_{50} was determined to be > 110.0 µg a.s./bee. No behavioral abnormalities of the bees could be observed.

The oral LD_{50} value (48 h) for pyraclostrobin was > 110.0 µg a.s./bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 500 F, batch no. 10-510009, content: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 100% (± 1.0%).
Test species:	Honeybee *Apis mellifera carnica*; young adult worker bees; derived from a healthy and queen-right colony, source: in-house culture; collected on the morning of use.

B. STUDY DESIGN

Test design: Limit test for oral toxicity; duration 48 h; 3 treatment groups (water control, solvent control, test item and 4 concentrations of the reference item) with 5 replicates per treatment group, each replicate consisting of 10 bees per cage; assessment of mortality after 4, 24 and 48 hours.

Endpoints: Mortality, resulting in an LD_{50} value; behavioral abnormalities.

Reference item: Dimethoate (nominal 400 g/L).

Test doses: Water control, solvent control and 100.0 µg a.s./bee (nominal) resulting in an actual uptake of 110.0 µg a.s./bee.

Test conditions: Temperature: 24 – 25 °C; relative humidity: 56% – 81%; photoperiod: 24 h darkness.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No mortality was observed after 48 hours in the control and the test item treatments. The LD_{50} was determined to be > 110.0 µg a.s./bee. No behavioral abnormalities of the bees could be observed. The results are summarized in Table 8.3.1.1.1-1.

Table 8.3.1.1.1-1: Toxicity of pyraclostrobin to honeybees (Apis mellifera) in an oral toxicity test

Consumed dosage [µg a.s./bee]	**Mortality [%]**		
	4 h	**24 h**	**48 h**
Water control	0.0	0.0	0.0
Solvent control	0.0	0.0	0.0
110.0	0.0	0.0	0.0
Endpoint [µg a.s./bee]			
LD_{50} (48 h)	> 110.0		

The LD_{50} value (24 h and 48 h) for the reference item in the oral toxicity test was determined to be 0.23 µg a.s./bee.

III. CONCLUSION

The oral LD_{50} value (48 h) for pyraclostrobin was > 110.0 µg a.s./bee.

Report:	CA 8.3.1.1.1/2 Amsel K., 2016 a Acute toxicity of BAS 500 F to the bumblebee Bombus terrestris L. under laboratory conditions 2016/1000530
Guidelines:	Van der Steen (1996), Van der Steen (2001), OECD 213 (1998), OECD 214 (1998), Hanewald et al. (2013)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In an acute oral limit test, young adult worker bumblebees (*Bombus terrestris*) were exposed to pyraclostrobin (BAS 500 F). The toxicity of the test item was determined at a dose rate of 100.0 µg a.s./bumblebee; the resulting oral uptake was 97.2 µg a.s./bumblebee (based on analyzed purity). Additionally, bumblebees were treated with Dimethoate EC 400 (a.s. dimethoate) as a reference item at dose rates ranging from 0.25 to 1.47 µg consumed dimethoate/bumblebee (analyzed), and furthermore with a 50% (w/v) sucrose solution and 50% (w/v) sucrose solution including 5% acetone as controls.

After 96 hours of oral exposure, no mortality occurred in the control group fed with 50% (w/v) sucrose solution or with the sucrose solution including 5% acetone. In the test item treatment, slight mortality of 1.7 % was observed after oral consumption of 97.2 µg a.s./bumblebee after 96 hours.

No behavioral effects of bumblebees were observed at all tested dose rates in the oral toxicity test.

In the acute oral toxicity test with pyraclostrobin (BAS 500 F), the resulting LD_{50} after 96 hours was estimated to be > 97.2 µg consumed a.s./bumblebee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 500 F, batch no. COD-001236, content: pyraclostrobin (BAS 500 F, Reg.No. 304428): purity: 99.02% analyzed.
Test species:	*Bombus terrestris* L. (bumblebee), young adult worker bumblebees derived from healthy and queen-right hives; source: Biobest Belgium N.V., Westerlo, Belgium; collected on the morning prior to use.

B. STUDY DESIGN

Test design:	In a 96-hour test, adults of *Bombus terrestris* were exposed to 1 dose of BAS 500 F in treated food (50% (w/v) sucrose solution including 5% acetone). In total, the test design consisted of 1 dose rate of the test item, 2 control groups (1 dose rate each) and 4 dose rates of the reference item. 60 replicates were used for the test item and sucrose control including 5% acetone. 30 replicates were used for the sucrose control and the reference item. 1 bumblebee was used per replicate. Assessments of bumblebee mortality and behavioral effects were done after 4, 24, 48, 72 and 96 hours.
Endpoints:	Mortality, behavioral impairments
Reference item:	Dimethoate EC 400 (dimethoate, 400 g/L nominal)
Test doses:	Control: 50% (w/v) sucrose solution; acetone control: 50% (w/v) sucrose solution including 5% acetone; BAS 500 F: 100.0 µg/bumblebee (resulting in an actual uptake of 97.2 µg BAS 500 F/bumblebee); reference item: 0.25, 0.45, 0.80 and 1.47 µg dimethoate/bumblebee
Test conditions:	Temperature: 24.9 – 25.1 °C, relative humidity: 59.6 – 60.5%, photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution
Statistics:	Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$)

II. RESULTS AND DISCUSSION

After 96 hours of oral exposure, no mortality occurred in the control group fed with 50% (w/v) sucrose solution or with the sucrose solution including 5% acetone. In the test item treatment, slight mortality of 1.7% was observed after oral consumption of 97.2 µg a.s./bumblebee after 96 hours. The results are summarized below.

Table 8.3.1.1.1-2: Toxicity of BAS 500 F to *Bombus terrestris* (bumblebee) in an oral toxicity test

Treatment	Dosage	Mortality [%]			
		24 h	48 h	72 h	96 h
Control	Sucrose	0.0	0.0	0.0	0.0
	Sucrose + 5% acetone	0.0	0.0	0.0	0.0
BAS 500 F [µg consumed a.s./bumblebee]	97.2	1.7	1.7	1.7	1.7
Endpoint [µg consumed a.s./bumblebee]					
LD_{50} (96 h)	> 97.2				

III. CONCLUSION

In the acute oral toxicity test with pyraclostrobin (BAS 500 F), the resulting LD_{50} after 96 hours was estimated to be > 97.2 µg consumed a.s./bumblebee.

CA 8.3.1.1.2 Acute contact toxicity

Report:	CA 8.3.1.1.2/1 Sekine T., 2013a Effects of BAS 500 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory 2013/1003210
Guidelines:	OECD 213 (1998), OECD 214 (1998)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

In a limit test, young adult worker bees (*Apis mellifera carnica*) were exposed to pyraclostrobin (BAS 500 F). The toxicity was determined in a contact limit test at a nominal dose of 100.0 µg a.s./bee. Additionally, honeybees were treated with dimethoate as reference item at 0.10 to 0.30 µg/bee or with water and solvent as a control groups. The test was conducted with 5 replicates per treatment group, each replicate contained 10 bees. Assessment of mortality was done after 4, 24 and 48 hours.

No mortality was observed in the control and test item treatments. The LD_{50} was determined to be >100.0 µg a.s./bee. One single bee showed a moving coordination problem during the 48 hours assessment. This was the only occurrence of test item related behavioral abnormalities during the entire time of the contact test.

The contact LD_{50} value (48 h) for pyraclostrobin was > 100.0 µg a.s./bee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 F, batch no. 10-510009, content: pyraclostrobin (BAS 500 F, Reg. No. 304 428): 100% (± 1.0%).

Test species: Honeybees *Apis mellifera carnica*; young adult worker bees; derived from a healthy and queen-right colony, source: in-house culture; collected on the morning of use.

B. STUDY DESIGN

Test design: Limit test for contact toxicity; duration 48 h; 4 treatment groups (water control, solvent control, test item and 4 concentrations of the reference item) with 5 replicates per treatment group, each replicate consisting of 10 bees per cage; assessment of mortality after 4, 24 and 48 hours.

Endpoints: Mortality, resulting in a LD_{50} value; behavioral abnormalities.

Reference item: Dimethoate (nominal 400 g/L).

Test doses: Water control, solvent control and 100.0 µg a.s./bee.

Test conditions: Temperature: 24 – 25 °C; relative humidity: 56% – 81%; photoperiod: 24 h darkness.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No mortality was observed in the control and test item treatments. The LD_{50} was determined to be >100.0 µg a.s./bee. One single bee showed a moving coordination problem during the 48 hours assessment. This was the only occurrence of test item related behavioral abnormalities during the entire time of the contact test. The results are summarized in Table 8.3.1.1.2-1.

Table 8.3.1.1.2-1: Toxicity of pyraclostrobin to honeybees (Apis mellifera) in a contact toxicity test

Treatment [µg a.s./bee]	Mortality [%]		
	4 h	24 h	48 h
Water control	0.0	0.0	0.0
Solvent control	0.0	0.0	0.0
100.0	0.0	0.0	0.0
	Endpoint [µg a.s./bee]		
LD_{50} (48 h)	> 100.0		

The LD_{50} value (24 h) for the reference item in the contact toxicity test was determined to be 0.27 µg a.s./bee.

III. CONCLUSION

The contact LD_{50} value (48 h) for pyraclostrobin was > 100.0 µg a.s./bee.

Report:	CA 8.3.1.1.2/2 Amsel K., 2016 a Acute toxicity of BAS 500 F to the bumblebee Bombus terrestris L. under laboratory conditions 2016/1000530
Guidelines:	Van der Steen (1996), Van der Steen (2001), OECD 213 (1998), OECD 214 (1998), Hanewald et al. (2013)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In an acute contact limit test, young adult worker bumblebees (*Bombus terrestris*) were exposed to pyraclostrobin (BAS 500 F). The toxicity of the test item was determined at a dose rate of 100.0 µg a.s./bumblebee (based on analyzed purity). Additionally, bumblebees were treated with Dimethoate EC 400 (a.s. dimethoate) as a reference item at dose rates ranging from 2.5 to 10.1 µg dimethoate/bumblebee (analyzed), and furthermore with deionized water, TritonX solution and acetone as controls.

After 96 hours, 3.3% mortality occurred in the control group treated with deionized water and no mortality occurred in the control groups treated with TritonX solution and acetone. In the test item treatment, no mortality occurred after thoracic application of 100.0 µg a.s./bumblebee after 96 hours.

No behavioral effects of bumblebees were observed at all tested dose rates in the contact toxicity test.

In the acute contact toxicity test with pyraclostrobin (BAS 500 F), the resulting LD_{50} after 96 hours was estimated to be > 100.0 µg a.s./bumblebee.

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 500 F, batch no. COD-001236, content: pyraclostrobin (BAS 500 F, Reg. No. 304 428): purity: 99.02% analyzed

B. STUDY DESIGN

Test species:	*Bombus terrestris* L. (bumblebee), young adult worker bumblebees derived from healthy and queen-right hives; source: Biobest Belgium N.V., Westerlo, Belgium; collected on the morning prior to use.
Test design:	In a 96 hour test, adults of *Bombus terrestris* were exposed to 1 dose rate of BAS 500 F in an appropriate carrier (acetone) placed on the dorsal bumblebee thorax. In total, the test design consisted of 1 dose rate of the test item, 3 control groups (1 dose rate each) and 4 dose rates of the reference item. 60 replicates were used for the test item and the acetone control and 30 replicates for the reference item, water and TritonX control (1 bumblebee per replicate). Assessments of bumblebee mortality and behavioral effects were done after 4, 24, 48, 72 and 96 hours.
Endpoints:	Mortality, behavioral impairments
Reference item:	Dimethoate EC 400 (dimethoate, 400 g/L nominal)
Test doses:	Control: deionized water; acetone control: pure acetone; TritonX control: 0.5% (v/v) TritonX solution; BAS 500 F: 100.0 µg/bumblebee (the test item was solved in acetone); reference item: 2.5, 4.0, 6.4 and 10.1 µg dimethoate/bumblebee
Test conditions:	Temperature: 24.9 – 25.1 °C, relative humidity: 59.6 – 60.5%, photoperiod: 24 h darkness; food: 50% (w/v) sucrose solution
Statistics:	Descriptive statistics; Fisher's Exact Binomial Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$)

II. RESULTS AND DISCUSSION

After 96 hours, 3.3% mortality occurred in the control group treated with deionized water and no mortality occurred in the control groups treated with TritonX solution and acetone. In the test item treatment, no mortality occurred after thoracic application of 100.0 µg a.s./bumblebee after 96 hours. The results are summarized below.

Table 8.3.1.1.2-2 Toxicity of BAS 500 F to *Bombus terrestris* (bumblebee) in a contact toxicity test

Treatment	Dosage	Mortality [%]			
		24 h	48 h	72 h	96 h
Control	deionized water	0.0	0.0	3.3	3.3
	0.5% TritonX	0.0	0.0	0.0	0.0
	acetone	0.0	0.0	0.0	0.0
BAS 500 F [µg a.s./ bumblebee]	100	0.0	0.0	0.0	0.0
Endpoint [µg a.s./bumblebee]					
LD_{50} (96 h)	> 100.0				

III. CONCLUSION

In the acute contact toxicity test with pyraclostrobin (BAS 500 F), the resulting LD_{50} after 96 hours was estimated to be > 100.0 µg a.s./bumblebee.

CA 8.3.1.2 Chronic toxicity to bees

Based on uncertainties with regard to the implementation process of the EFSA Guidance Document on Honey Bee Risk Assessment, the applicant had decided to conduct a study on the chronic toxicity of pyraclostrobin to honeybees. Due to technical problems of keeping the test item sufficiently stable in the feed solution, results are currently not available. Investigations on chronic toxicity of pyraclostrobin to bees will be continued.
Due to the situation described above, the study is not part of the supplemental dossier, although it was included in the study list of the application for renewal of approval as data point KCA 8.3.1.2/1 (BASF DocID 2013/1235056).
It should be considered that a study on chronic toxicity to honeybees is not required for AIR 3 substances submitted before January 1st, 2015 according to SANCO/10606/2014 (May 16th, 2014). Furthermore, a bee brood tunnel study conducted with BAS 500 06 F (CP 10.3.1.5/1, BASF DocID 2011/1112669) is available, which is a higher tier option to address this data point.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Since this study type requires exposure of bees to sprayed flowering plants, the test item used needs to be formulated in a way suitable for spray applications. Therefore, the study was conducted with the representative solo-formulation BAS 500 06 F. The study is intended to address the active substance, but is summarized in the dossier of BAS 500 06 F (CP 10.3.1.5/1, BASF DocID 2011/1112669).

CA 8.3.1.4 Sub-lethal effects

A tunnel study is available, which covers sublethal effects to honeybees (see CP 10.3.1.5/1).
All standard and higher tier studies demonstrate that pyraclostrobin does not pose an unacceptable risk to honeybees. Consequently, further tests are not necessary.

CA 8.3.2 Effects on non-target arthropods other than bees

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

No new studies available with the active substance, because studies have been carried out with the formulated products (please refer to M-CP 10.3.2.1).

CA 8.3.2.2 Effects on *Typhlodromus pyri*

No new studies available with the active substance, because studies have been carried out with the formulated products (please refer to M-CP 10.3.2.1).

CA 8.4 Effects on non-target soil meso- and macrofauna

Since Annex I inclusion of the active substance pyraclostrobin (BAS 500 F), new studies on soil macro organisms have been performed with the active substance and its soil metabolites. As a result, there are new endpoints, which are considered in the respective risk assessment. Summaries of these new studies are provided below and an overview on studies and endpoints is given in Table 8.4-1.

Table 8.4-1 Toxicity to non-target soil meso- and macrofauna of pyraclostrobin and relevant metabolites

Substance	Species	Endpoint	Value [mg/kg dry soil]	Reference (BASF Doc ID)	EU agreed
Pyraclostrobin	*Eisenia fetida*	NOEC	11.6 *	2014/1000461	no, new study
BF 500-6	*Eisenia fetida*	NOEC	≥ 160 *	2013/1003174	no, new study
BF 500-7	*Eisenia fetida*	NOEC	≥ 160 *	2013/1224029	no, new study
BF 500-6	*Folsomia candida*	NOEC	≥ 1000	2013/1068054	no, new study
BF 500-7	*Folsomia candida*	NOEC	≥ 800	2013/1224030	no, new study
Pyraclostrobin[1)]	*Folsomia candida*	NOEC	23.9	2008/1037495	no, new study
	Hypoaspis aculeifer	NOEC	≥ 17.3	2012/1129444	no, new study

* Toxicity endpoint is re-adjusted using a soil factor of 2 to address the organic content of the soil (peat 10%), and the log P_{ow} of the substance is > 2.

1) Study was carried out with BAS 500 06 F as a surrogate for pyraclostrobin. For a study summary please refer to the formulation dossier of BAS 500 06 F, M-CP 10.4.2.1.

CA 8.4.1 Earthworms – sub-lethal effects

Report: CA 8.4.1/1
Friedrich S., 2014a
Sublethal toxicity of BAS 500 F (Pyraclostrobin) to the earthworm Eisenia fetida in artificial soil
2014/1000461
Guidelines: OECD 222 (2004)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of BAS 500 F on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations of the test item (8.0, 13.6, 23.1, 39.3 and 66.8 mg a.s./kg dry soil) were incorporated into the soil (10% peat) with 4 replicates per treatment and 8 replicates for the solvent control (each containing 10 worms). Assessment of adult worm mortality, biomass development and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

The mortality of adult worms was about 0 – 5.0% in the five test item treatments and 1.3% in the solvent control group. No statistically significant differences compared to the solvent control were observed at any test item concentration. The weight change of adult worms was about -6.8 – 36.4% in the test item treatments and 35.2% in the solvent control group. Statistically significant differences on worm weight of *Eisenia fetida* were determined at concentrations of 39.3 and 66.8 mg a.s./kg dry soil. The reproduction rate was statistically significantly different compared to the solvent control at concentrations of 39.3 and 66.8 mg a.s./kg dry soil. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity of adult worms was reduced at the concentrations of 39.3 and 66.8 mg a.s./kg dry soil.

In a 56-day earthworm reproduction study with BAS 500 F on earthworms (*Eisenia fetida*), the NOEC for mortality was determined to be 66.8 mg a.s./kg soil dry weight. The NOEC for biomass and reproduction was determined to be 23.1 mg test item/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 500 F, Reg. No. 304 428, batch No. COD-001236; purity: 99.2% (tolerance ± 1.0 %).

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 301 mg – 498 mg), age: approximately 3 months old; source: "W. Neudorff GmbH KG" followed by in-house culture.

B. STUDY DESIGN

Test design:	56-day test in treated artificial soil according to OECD 222 (10 % peat); 5 different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, solvent control); 4 replicates for the test item treatments, 8 replicates for the solvent control, 10 worms each. Assessment of adult worm mortality, behavioral effects and biomass development was done after 28 days. Reproduction rate was determined after additional 28 days (assessed 56 days after application).
Endpoints:	NOEC; effects on mortality, weight change, reproduction rate, feeding activity.
Reference item:	Nutdazim 50 FLOW (carbendazim, SC 500). The effects of the reference item were investigated in a separate study.
Test concentrations:	Control, 8.0, 13.6, 23.1, 39.3 and 66.8 mg a.s./kg dry soil.
Test conditions:	Artificial soil according to OECD 222 (with 10% peat); pH 5.99 - 6.06 at test initiation, pH 5.70 – 5.76 at test termination; water content: 56.4% - 56.5% of maximum water holding capacity (WHC) at test initiation, 55.6% - 56.2% of WHC at test termination; temperature: 18.6 °C – 21.8 °C; photoperiod: 16 h light : 8 h dark, light intensity: 580 lux; food: horse manure.
Statistics:	Descriptive statistics, Fisher's Exact Binomial Test for mortality ($\alpha = 0.05$), Williams-t-test for weight change and reproduction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

The mortality of adult worms was about 0 – 5.0% in the five test item treatments and 1.3% in the solvent control group. No statistically significant differences compared to the solvent control were observed at any test item concentration (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). The weight change of adult worms was about -6.8 – 36.4% in the test item treatments and 35.2% in the solvent control group. Statistically significant differences on worm weight of *Eisenia fetida* were determined at concentrations of 39.3 and 66.8 mg a.s./kg dry soil (Williams-t-test, $\alpha = 0.05$, one-sided smaller). The reproduction rate was statistically significantly different compared to the solvent control at concentrations of 39.3 and 66.8 mg a.s./kg dry soil (Williams-test, $\alpha = 0.05$, one-sided smaller). No behavioral abnormalities were observed in any of the treatment groups. The feeding activity of adult worms was reduced at the concentrations of 39.3 and 66.8 mg a.s./kg dry soil. The results are summarized below (see Table 8.4.1-1).

Table 8.4.1-1: Effects of BAS 500 F on earthworms (*Eisenia fetida)* in a 56-day reproduction study

BAS 500 F [mg/kg dry soil]	Solvent control	8.0	13.6	23.1	39.3	66.8
Mortality (day 28) [%]	1.3	0.0	0.0	2.5	5.0	2.5
Weight change (day 28) [%]	35.2	36.4	32.7	28.0	9.4 *	-6.8 *
Mean no. of juveniles (day 56)	118.8	127.8	123.8	107.5	36.8 *	14.8 *
Reproduction (day 56) [% of control]	--	107.6	104.2	90.5	30.9 *	12.4 *
Endpoints [mg BAS 500 F/kg dry soil]						
$NOEC_{mortality}$ (day 28)	66.8					
$NOEC_{weight}$ (day 28)	23.1					
$NOEC_{reproduction}$ (day 56)	23.1					

* Statistically significantly different from solvent control (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

III. CONCLUSION

In a 56-day earthworm reproduction study with BAS 500 F on earthworms (*Eisenia fetida*), the NOEC for mortality was determined to be 66.8 mg a.s./kg soil dry weight. The NOEC for biomass and reproduction was determined to be 23.1 mg test item/kg dry soil.

Report:	CA 8.4.1/2 Ganssmann M., 2013a Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms Eisenia fetida in artificial soil 2013/1003174
Guidelines:	OECD 222 - Earthworm Reproduction Test (2004), ISO 11268-2 (1998), EC 1107/2009 (14 June 2011)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The effects of BF 500-6 (Reg. No. 364 380, synonym: 500M01), a metabolite of pyraclostrobin, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations (20, 40, 80, 160 and 320 mg BF 500-6/kg dry soil) were incorporated into the soil (10% peat) with 4 replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of adult worm mortality, biomass development and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure a slight mortality of 2.5% was observed at the test item treatment concentration of 80 mg BF 500-6/kg dry soil, which was not statistically significantly different compared to the control. The body weight changes and reproduction rates were also not statistically significantly different compared to the control up to and including the highest concentration tested. Neither behavioral abnormalities nor effects on feeding activity were observed in any of the treatment groups.

In a 56-day earthworm reproduction study with BF 500-6 (Reg. No. 364 380, a metabolite of pyraclostrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, feeding activity and reproduction was determined to be ≥ 320 mg BF 500-6/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 364 380 (metabolite of pyraclostrobin, BF 500-6, synonym: 500M01) batch no. 01311-142; purity: 99.2% (tolerance ± 1.0 %).

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 300 mg – 592 mg), age: approximately 10 to 11 months old; source: in-house culture.

B. STUDY DESIGN

Test design:	56-day test in treated artificial soil according to OECD 222 (10% peat); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. The artificial soil was treated and filled into test vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioral effects and biomass development after 28 days; assessment of reproduction rate (number of offspring) after another 28 days (56 days after application).
Endpoints:	NOEC; effects on mortality, weight change, reproduction rate, feeding activity.
Reference item:	Luxan Carbendazim 500 FC (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.
Test concentrations:	Control, 20, 40, 80, 160 and 320 mg Reg. No. 364 380/kg dry soil.
Test conditions:	Artificial soil according to OECD 222 (with 10% peat); pH 6.4 - 6.5 at test initiation, pH 6.0 - 6.1 at test termination; water content: 54.3% - 54.9% of maximum water holding capacity (WHC) at test initiation, 52.5% - 58.5% of WHC at test termination; temperature: 18 °C - 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: 400 lux to 800 lux; food: cattle manure.
Statistics:	Descriptive statistics, Fisher's Exact Test for mortality ($\alpha = 0.05$), Williams t-test for weight change and reproduction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure a slight mortality of 2.5% was observed at the test item treatment concentration of 80 mg BF 500-6 /kg dry soil, which was not statistically significantly different compared to the control (Fisher`s Exact Test, $\alpha = 0.05$). The body weight changes and reproduction rates were also not statistically significantly different compared to the control (Williams t-test, $\alpha = 0.05$) up to and including the highest concentration tested. Neither behavioral abnormalities nor effects on feeding activity were observed in any of the treatment groups. The results are summarized below (see Table 8.4.1-2).

Table 8.4.1-2: Effects of BF 500-6 (Reg. No. 364 380), a metabolite of pyraclostrobin, on earthworms (Eisenia fetida) in a 56-day reproduction study

BF 500-6 [mg/kg dry soil]	Control	20	40	80	160	320
Mortality (day 28) [%]	0.0	0.0	0.0	2.5	0.0	0.0
Weight change (day 28) [%]	51.7	52.7	52.4	55.9	56.7	50.1
Mean no. of juveniles (day 56)	276	324	330	308	293	303
Reproduction (day 56) [% of control]	--	117.4	119.5	111.5	106.2	109.7
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg BF 500-6/kg dry soil]						
$NOEC_{mortality, weight}$ (day 28)	≥ 320					
$NOEC_{reproduction}$ (day 56)	≥ 320					

III. CONCLUSION

In a 56-day earthworm reproduction study with BF 500-6 (Reg. No. 364 380, a metabolite of pyraclostrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, feeding activity and reproduction was determined to be ≥ 320 mg BF 500-6/kg dry soil.

Report:	CA 8.4.1/3 Ganssmann M., 2013b Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction and growth of earthworms Eisenia fetida in artificial soil with 10% peat 2013/1224029
Guidelines:	OECD 222 - Earthworm Reproduction Test (2004), ISO 11268-2 (2012)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The effects of BF 500-7 (Reg. No. 369 315, synonym: 500M02), a metabolite of pyraclostrobin, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations (20, 40, 80, 160 and 320 mg BF 500-7/kg dry soil) were incorporated into the soil (10% peat) with 4 replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of adult worm mortality, biomass development and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure a slight mortality of 2.5% was observed at the test item treatment concentrations of 20, 40 and 320 mg BF 500-7/kg dry soil, respectively, which was not statistically significantly different compared to the control, where 1.3% of the worms died. The body weight changes and reproduction rates were also not statistically significantly different compared to the control up to and including the highest concentration tested. Neither behavioral abnormalities nor effects on feeding activity were observed in any of the treatment groups.

In a 56-day earthworm reproduction study with BF 500-7 (Reg. No. 369 315, a metabolite of pyraclostrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, feeding activity and reproduction was determined to be ≥ 320 mg BF 500-7 /kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 369 315 (metabolite of pyraclostrobin, BF 500-7, synonym: 500M02) batch no. L83-168; purity: 96.2%.

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 301 mg – 598 mg), age: approximately 7 months old; source: in-house culture.

B. STUDY DESIGN

Test design:	56-day test in treated artificial soil according to OECD 222 (10% peat); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. The artificial soil was treated and filled into test vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioral effects and biomass development after 28 days; assessment of reproduction rate (number of offspring) after another 28 days (56 days after application).
Endpoints:	NOEC; effects on mortality, weight change, reproduction rate, feeding activity.
Reference item:	Luxan Carbendazim 500 FC (carbendazim, 500 g/L nominal). The effects of the reference item were investigated in a separate study.
Test concentrations:	Control, 20, 40, 80, 160 and 320 mg Reg. No. 369 315/kg dry soil.
Test conditions:	Artificial soil according to OECD 222 (with 10% peat); pH 6.0 at test initiation and termination; water content: 51.2% - 53.7% of maximum water holding capacity (WHC) at test initiation, 55.4% - 61.5% of WHC at test termination; temperature: 18 °C - 22 °C; photoperiod: 16 h light : 8 h dark, light intensity: 400 lux to 800 lux; food: cattle manure.
Statistics:	Descriptive statistics, Fisher's Exact Test for mortality ($\alpha = 0.05$), Williams t-test for weight change and reproduction ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure a slight mortality of 2.5% was observed at the test item treatment concentrations of 20, 40 and 320 mg BF 500-7/kg dry soil, respectively, which was not statistically significantly different compared to the control, where 1.3% of the worms died (Fisher`s Exact Test, $\alpha = 0.05$). The body weight changes and reproduction rates were also not statistically significantly different compared to the control (Williams t-test, $\alpha = 0.05$) up to and including the highest concentration tested. Neither behavioral abnormalities nor effects on feeding activity were observed in any of the treatment groups. The results are summarized below (see Table 8.4.1-3).

Table 8.4.1-3: Effects of BF 500-7 (Reg. No. 369 315), a metabolite of pyraclostrobin, on earthworms (Eisenia fetida) in a 56-day reproduction study

BF 500-7 [mg/kg dry soil]	**Control**	**20**	**40**	**80**	**160**	**320**
Mortality (day 28) [%]	1.3	2.5	2.5	0.0	0.0	2.5
Weight change (day 28) [%]	21.5	26.6	22.4	20.5	26.7	13.6
Mean no. of juveniles (day 56)	239	233	301	312	293	253
Reproduction (day 56) [% of control]	--	97.6	125.9	130.5	122.8	105.8
Food consumption [g]	25.0	25.0	25.0	25.0	25.0	25.0
Endpoints [mg BF 500-7/kg dry soil]						
$NOEC_{mortality, weight}$ (day 28)	≥ 320					
$NOEC_{reproduction}$ (day 56)	≥ 320					

III. CONCLUSION

In a 56-day earthworm reproduction study with BF 500-7 (Reg. No. 369 315, a metabolite of pyraclostrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass, feeding activity and reproduction was determined to be ≥ 320 mg BF 500-7 /kg dry soil.

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Report:	CA 8.4.2/1 Ganssmann M., 2013c Effects of Reg.No. 364380 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction of the collembola Folsomia candida in artificial soil with 5% peat 2013/1068054
Guidelines:	OECD 232 (2009), ISO 11267 (1999)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The effects of BF 500-6 (Reg. No. 364 380, synonym: 500M01), a metabolite of pyraclostrobin, on mortality and reproduction of Collembola *(Folsomia candida)* were investigated in a laboratory study over 28 days. Five concentrations (62.5, 125, 250, 500 and 1000 mg BF 500-6 /kg dry soil) were incorporated into the soil with 4 replicates per test item treatment. An untreated control with 8 replicates was included. All replicates contained 10 collembolans. Assessment of mortality, reproduction rate (number of juveniles) and behavior was carried out after 28 days.

After 28 days of exposure a slight mortality of up to 15% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 8% of the Collembola died. Reproduction of the collembolans exposed to BF 500-6 was not statistically significantly different compared to the control up to and including the highest test concentration of 1000 mg/kg dry soil. No behavioral abnormalities were observed in any of the treatment groups.

In a 28-day Collembola reproduction study with BF 500-6 (Reg. No. 364 380, a metabolite of pyraclostrobin) the NOEC based on mortality and reproduction was determined to be ≥ 1000 mg BF 500-6/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 364 380 (metabolite of pyraclostrobin, BF 500-6, synonym: 500M01) batch no. 01311-142; purity: 99.2% (tolerance ± 1.0 %).

Test species: Collembola (*Folsomia candida)*, juveniles (10 - 12 days old); source: in-house culture.

B. STUDY DESIGN

Test design: 28-day chronic laboratory test in treated artificial soil (5% peat) according to OECD 232 and ISO 11267; different concentrations of the test item were mixed homogenously into artificial soil and filled into glass vessels after which collembolans were introduced on top of the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for each test item treatment and 8 replicates for the control, each containing 10 collembolans. Assessment of adult mortality, reproduction (number of juveniles) and behavioral effects after 28 days.

Endpoints: Mortality, reproduction rate after 28 days.

Reference item: Boric acid (100.3 % analyzed). The effects of the reference item were investigated in a separate study.

Test rates: Control, 62.5, 125, 250, 500 and 1000 mg Reg. No. 364 380/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (peat: 5 %); pH 6.1 at test initiation, pH 5.9 - 6.0 at test termination; water content at study initiation 52.2 % - 54.6 % of maximum water holding capacity and 46.6 % - 52.7 % of maximum WHC at test termination; temperature: 18 C – 22 C; photoperiod: 16 h light : 8 h dark, light intensity: 400 lux - 800 lux; food: 2 mg granulated dry yeast at the start of the test and after 14 days.

Statistics: Descriptive statistics; Fisher's Exact Test for mortality ($\alpha = 0.05$), Williams t-test for reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure a slight mortality of up to 15% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 8% of the Collembola died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).
Reproduction of the collembolans exposed to BF 500-6 was not statistically significantly different compared to the control up to and including the highest test concentration of 1000 mg/kg dry soil (Williams t-test, $\alpha = 0.05$, one-sided smaller).
No behavioral abnormalities were observed in any of the treatment groups. The results are summarized in Table 8.4.2-1.

Table 8.4.2-1: Effect of BF 500-6 (Reg. No. 364 380) on Collembola (Folsomia candida) in a 28-day reproduction study

BF 500-6 [mg/kg dry soil]	**Control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality (day 28) [%]	8	8	5	10	15	10
No. of juveniles (day 28)	437	471	467	527	451	419
Reproduction (day 28) [% of control]	--	108	107	121	103	96
Endpoints [mg BF 500-6/kg dry soil]						
$NOEC_{mortality,\ weight}$ (day 28)	≥ 1000					
$NOEC_{reproduction}$ (day 28)	≥ 1000					

III. CONCLUSION

In a 28-day Collembola reproduction study with BF 500-6 (Reg. No. 364 380, a metabolite of pyraclostrobin) the NOEC based on mortality and reproduction was determined to be ≥ 1000 mg BF 500-6/kg dry soil, the highest concentration tested.

Report:	CA 8.4.2/2 Ganssmann M., 2013d Effects of Reg.No. 369315 (metabolite of BAS 500 F, Pyraclostrobin) on reproduction of the collembola Folsomia candida in artificial soil with 5% peat 2013/1224030
Guidelines:	OECD 232 (2009)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The effects of BF 500-7 (Reg. No. 369 315, synonym: 500M02, a metabolite of pyraclostrobin, on mortality and reproduction of Collembola *(Folsomia candida)* were investigated in a laboratory study over 28 days. Five concentrations (50, 100, 200, 400 and 800 mg BF 500-7/kg dry soil) were incorporated into the soil with 4 replicates per test item treatment. An untreated control with 8 replicates was included. All replicates contained 10 collembolans. Assessment of mortality, reproduction rate (number of juveniles) and behavior was carried out after 28 days.

After 28 days of exposure a slight mortality of up to 15% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 6% of the Collembola died. Reproduction of the collembolans exposed to BF 500-7 was not statistically significantly different compared to the control up to and including the highest test concentration of 800 mg/kg dry soil. No behavioral abnormalities were observed in any of the treatment groups.

In a 28-day Collembola reproduction study with BF 500-7 (Reg. No. 369 315, a metabolite of pyraclostrobin) the NOEC based on mortality and reproduction was determined to be ≥ 800 mg BF 500-7/kg dry soil, the highest concentration tested.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Reg. No. 369 315 (metabolite of pyraclostrobin, BF 500-7, synonym: 500M02) batch no. L83-168; purity: 96.2%.

Test species: Collembola (*Folsomia candida)*, juveniles (10 - 12 days old); source: in-house culture.

B. STUDY DESIGN

Test design:	28-day chronic laboratory test in treated artificial soil (5% peat) according to OECD 232 and ISO 11267; different concentrations of the test item were mixed homogenously into artificial soil and filled into glass vessels after which collembolans were introduced on top of the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for each test item treatment and 8 replicates for the control, each containing 10 collembolans. Assessment of adult mortality, reproduction (number of juveniles) and behavioral effects after 28 days.
Endpoints:	Mortality, reproduction rate after 28 days.
Reference item:	Boric acid (100.3 % analyzed). The effects of the reference item were investigated in a separate study.
Test rates:	Control, 50, 100, 200, 400 and 800 mg Reg. No. 369 315/kg dry soil.
Test conditions:	Artificial soil according to OECD 232 (peat: 5 %); pH 6.1 - 6.2 at test initiation, pH 6.0 - 6.1 at test termination; water content at study initiation 43.6 % - 45.7 % of maximum water holding capacity and 41.4 % - 44.3 % of maximum WHC at test termination; temperature: 18 C – 22 C; photoperiod: 16 h light : 8 h dark, light intensity: 400 lux - 800 lux; food: 2 mg granulated dry yeast at the start of the test and after 14 days.
Statistics:	Descriptive statistics; Fisher's Exact Test for mortality ($\alpha = 0.05$), Williams t-test for reproduction data ($\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 28 days of exposure a slight mortality of up to 15% was observed in the test item treated groups, which was not statistically significantly different compared to the control, where 6% of the Collembola died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).
Reproduction of the collembolans exposed to BF 500-7 was not statistically significantly different compared to the control up to and including the highest test concentration of 800 mg/kg dry soil (Williams t-test, $\alpha = 0.05$, one-sided smaller).
No behavioral abnormalities were observed in any of the treatment groups. The results are summarized in Table 8.4.2-2.

Table 8.4.2-2: Effect of BF 500-7 (Reg. No. 369 315) on Collembola (Folsomia candida) in a 28-day reproduction study

BF 500-7 [mg/kg dry soil]	**Control**	**50**	**100**	**200**	**400**	**800**
Mortality (day 28) [%]	6	8	10	10	13	15
No. of juveniles (day 28)	886	861	1041	921	873	860
Reproduction (day 28) [% of control]	--	97	117	104	98	97
Endpoints [mg BF 500-7/kg dry soil]						
$NOEC_{mortality,\ weight}$ (day 28)	≥ 800					
$NOEC_{reproduction}$ (day 28)	≥ 800					

III. CONCLUSION

In a 28-day Collembola reproduction study with BF 500-7 (Reg. No. 369 315, a metabolite of pyraclostrobin) the NOEC based on mortality and reproduction was determined to be ≥ 800 mg BF 500-7/kg dry soil, the highest concentration tested.

CA 8.4.2.1 Species level testing

Further studies are not triggered.

CA 8.5 Effects on nitrogen transformation

Since Annex I inclusion of the active substance pyraclostrobin (BAS 500 F) a new study on nitrogen transformation has been performed with the new representative formulation BAS 500 06 F. As a result, there is a new endpoint, which is considered in the risk assessment. A summary of this new study is provided in the formulation dossier of BAS 500 06 F (M-CP 10.5). An overview on studies and endpoints is given in Table 8.5-1.

Table 8.5-1 Toxicity to nitrogen transformation of pyraclostrobin and its metabolites

Substance	Endpoint	NOEC [mg/kg dry soil]	Reference (BASF DocID)	EU agreed
BF 500-6	Effects on nitrogen transformation	1.0	1999/11311	yes
BF 500-7	Effects on nitrogen transformation	0.5	1999/11311	yes
pyraclostrobin [1)]	Effects on nitrogen transformation	3.33	2012/1129443	no, new study

[1)] Study was carried out with BAS 500 06 F as a surrogate for pyraclostrobin. For the study summary please refer to the formulation dossier of BAS 500 06 F, M-CP 10.5.

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

Non-target plant GLP studies with the representative formulations are available. Further screening data are not required.

CA 8.6.2 Testing on non-target plants

Studies have been conducted with the representative formulations and are described in M-CP 10.6.2.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

Studies with the active substance are not required. Studies conducted with the representative formulations are described in M-CP 10.7 (including studies which are not required under 1107/2009).

CA 8.8 Effects on biological methods for sewage treatment

The results of the already peer-reviewed and accepted study are still valid and they are summarized in Table 8.8-1. No new study has been performed.

Table 8.8-1: Effects on biological methods for sewage treatment

Test item	Study type	Endpoint [mg a.s./L]	Reference (BASF DocID)	EU agreed
BAS 500 F (pyraclostrobin)	Respiration inhibition test (inhibition of oxygen consumption activated sludge from wastewater plant)	EC_{20} (0.5 h) > 1000	1999/10656	yes

CA 8.9 Monitoring data

According to the knowledge of the applicant, there are currently no published ecotoxicological monitoring data available for pyraclostrobin or its metabolites, which would provide additional knowledge on the ecotoxicological assessment not covered by this dossier.

During literature search a few publications were found dealing with environmental monitoring of various pesticides in surface water, groundwater and/or sediment outside EU (e.g. US, Australia, Costa Rica) mentioning also pyraclostrobin. The results showed that pyraclostrobin could be detected in local areas characterized by intensive fungicide use during the spraying season, however, frequency of detection as well as measured concentrations were usually very low.

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT M-CA, Section 9

LITERATURE DATA

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Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-July-2014		BASF DocID 2014/1162250 (version 1)
09-June-2015	The document was amended in order to make it more transparent how the literature search report fits to the documents listing the literature references. New or changed text is marked in yellow.	BASF DocID 2015/1117502 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 9 LITERATURE DATA

A literature search on pyraclostrobin and the common product trade names was performed by the BASF Group Information Center. The Literature Search Report on pyraclostrobin describes the general search and evaluation process as well as details on search profiles, search histories and summary tables.

The complete search report is provided in K-CA 9 (BASF DocID 2014/1172994).

The first step of the search result processing based on summary records was done by the Information Center and involved the separation into "hits" and "ballast" (obviously irrelevant records). The "ballast" was not further processed.

The "hits" were further evaluated by the scientific experts and categorized into "not relevant", "relevant, but not reliable", and "used for dossier". The result of this evaluation is documented in different EXCEL files which are contained on the CADDY CD as additional attachments to the search report in K-CA 9 with the file names as listed below (alphabetical order):

Analytics:	Pyraclostrobin Literature Analytics
Ecotoxicology:	Pyraclostrobin Literature Ecotox aquatic
	Pyraclostrobin Literature Ecotox general
	Pyraclostrobin Literature Ecotox terrestric
	Pyraclostrobin Literature Ecotox wildlife
E-fate:	Pyraclostrobin Literature E-fate
Consumer Safety:	Pyraclostrobin Literature Metabolism and Residues in Animals
	Pyraclostrobin Literature Metabolism and Residues in Plants
Product Chemistry:	Pyraclostrobin Literature Product Chemistry
Toxicology:	Pyraclostrobin Literature Toxicology (including the search topics operator exposure, toxicology in animals and humans, mode of action and QSAR as shown in the Literature Search Report)

All literature seen as both, relevant and reliable, has been discussed in Doc M. The respective articles are part of the reference lists of the different sections as contained in Doc L.

The hits in Ecotox general, Ecotox wildlife (not including amphibians), E-Fate, Metabolism and Residues in Animals as well as Product Chemistry did not contribute to the risk assessment and were therefore not further discussed in the dossier.

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT M-CA, Section 10

CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

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[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 10 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

The following harmonized classification and labelling was adopted for pyraclostrobin:

Legislation	**Classification**	**Labelling**	**Concentration limits**
Directive 67/548/EEC	T, N R: 23-38-50-53	T, N R: 23-38-50/53 S: 1/2-45-60-61-63	None
Regulation (EC) No 1272/2008	Hazard class and category code: Acute Tox. 3 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1 Hazard statement code: H331, H315, H400, H410	Pictogram signal word code: GHS06 GHS09 Danger Hazard statement code: H331, H315, H410	M-factor = 100

Single accidental exposure of humans to spray mist of pyraclostrobin containing formulations resulted in symptoms of respiratory irritation (see M-CA 5.9.3). Based on this direct observation in humans and supportive evidence for respiratory irritation observed in subchronic inhalation studies in rats (see CA 5.3.3/1 and 5.3.3/2), BASF self-classified pyraclostrobin with STOT SE Category 3 (H335, May cause respiratory irritation). Consequently BASF proposes for pyraclostrobin the classification and labelling as shown below.

Legislation	**Classification**	**Labelling**	**Concentration limits**
Directive 67/548/EEC	T, N R: 23-37-38-50-53	T, N R: 23-37/38-50/53 S: 1/2-45-60-61-63	None
Regulation (EC) No 1272/2008	Hazard class and category code: Acute Tox. 3 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1 STOT SE 3 Hazard statement code: H331, H315, H400, H410, H335	Pictogram signal word code: GHS06 GHS09 Danger Hazard statement code: H331, H315, H410, H335	M-factor = 100

BASF
The Chemical Company

Pyraclostrobin

Document N1

OVERALL CONCLUSIONS

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18-Jul-2014		BASF DocID 2014/1165141 (version 1)
27-Feb-2017	Chapter 5.1.3: Update of methods for risk assessment Chapter 5.2: Addition of methods supporting toxicological studies Chapter 6.1.1: Inclusion of information from new metabolism studies Chapter 6.1.2: Additional data on acute oral toxicity in mice have been considered. Chapter 6.1.4: A new kinetic study in mice has been considered. Chapter 6.1.9: Additional toxicological studies on two new metabolites are now reflected. Chapter 7.4.1: Consideration of a new residue study in potatoes Chapter 7.9: Update of the dietary risk assessment for metabolites Chapter 9.1: Updated/revised risk assessment for mammals Chapter 9.3: Consideration of a new bumblebee study Changed and new text is marked in yellow.	BASF DocID 2017/1032927 (version 2)

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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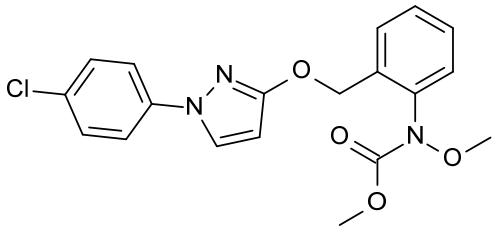
1 IDENTITY

1.1 Summary of identity

Pyraclostrobin, chemical name (IUPAC) methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl] oxymethyl}phenyl)-(N-methoxy)carbamate, is a fungicide and belongs to the chemical class of strobilurines.



BASF Number:	BAS 500 F
BASF Registry Number:	Reg.No. 304428
Molecular formula:	$C_{19}H_{18}ClN_3O_4$
Molecular mass:	387.8 g/mol
CAS No.:	175013-18-0
CIPAC No.:	657
EC No.:	not assigned

BAS 500 06 F is an emulsifiable concentrate (EC) containing 200 g/L of the active ingredient pyraclostrobin.

BAS 516 07 F is a water dispersible granule (WG) containing 6.7% w/w pyraclostrobin and 26.7% w/w boscalid.

2 PHYSICAL AND CHEMICAL PROPERTIES

2.1 Summary of physical and chemical properties of the active substance

Purified pyraclostrobin is a white to light beige crystalline solid, while the commercially produced technical material is an amber-coloured glass-like solid.
Pyraclostrobin is neither flammable, nor explosive, nor corrosive, nor oxidising. The pure active ingredient (PAI) has a vapour pressure of 2.6 x 10^{-8} Pa at 20°C. It is slightly soluble in pure water (1.9 mg/L at 20°C and pH 5.8). Its n-octanol/water partition coefficient (log Pow) is 3.99. Due to the lack of acidic protons, pyraclostrobin does not dissociate. The active substance absorbs light in the UV area (absorption bands around 205 and 275 nm), showing sensitivity to photolysis (measured with PAI).

2.2 Summary of physical and chemical properties of the plant protection product

The product BAS 500 06 F is an EC formulation. It is a dark yellow clear liquid with a moderate naphthalene-like odour. It is not explosive, has no oxidising properties and is not considered to be a self-heating substance. It has a self-ignition temperature of 450°C. The product is not considered to be highly flammable. In aqueous solution it has a pH value around 6-7. The stability data indicate a shelf life of at least 2 years at ambient temperature. Its technical characteristics are acceptable for an EC formulation.

The product BAS 516 07 F is a WG formulation. It is a brown fine granule with a smoky odour. It is not explosive, has no oxidising properties and is not considered to be a self-heating substance. It has a self-ignition temperature of 246°C. The product is not considered to be highly flammable. In aqueous solution it has a pH value around 5. The stability data demonstrate a shelf life of at least 2 years at ambient temperature. Its technical characteristics are acceptable for a WG formulation.

3 DATA ON APPLICATION AND EFFICACY

3.1 Summary of effectiveness

Pyraclostrobin is a strobilurine fungicide which is used worldwide in many crops for the control of a broad range of important pathogens from the classes of ascomycetes, basidiomycetes, deuteromycetes and oomycetes. Pyraclostrobin is active against different fungal stages both on the plant surface and in the plant tissue. After application to the plant, the active ingredient is taken up via the leaf and then translocated at low rates via the transpiration flow. Due its relatively low mobility, it shows local systemic and translaminar activity. Because of its very high intrinsic activity, pyraclostrobin has been observed to have systemic effects in a number of authorized uses. By that, it can control fungal stages which have already become established in deeper tissue layers. Pyraclostrobin is thus suitable for preventative and curative treatments.

Pyraclostrobin belongs to the QoI group of fungicides. The mode of action is the inhibition of mitochondrial respiration resulting from a blockage of the mitochondrial electron transport chain, thus blocking phosphorylation further down in the respiratory chain. In consequence, this leads to a reduction of energy-rich ATP which is required to support a range of essential processes in the fungal cell. In the end, the various fungal development processes of spore germination, formation of infection structures, mycelium growth and sporulation are permanently disrupted.

In addition to its fungicidal effects, plant physiology is also affected by the application of pyraclostrobin. Among these effects higher yield and better product quality in absence of diseases as well as improvement of the assimilation rate and delayed senescence have been reported. Some studies showed better stress tolerance to abiotic stresses (e.g. drought or frost). Furthermore, a reduction of physiological leaf spots in cereals has been observed after treatment with pyraclostrobin-containing products.

3.2 Summary of information on the development of resistance

Pyraclostrobin belongs to the QoI group of fungicides. Most important resistance mechanisms to QoIs are target site mutations, in particular the G143A in the cytochrome *b*. G143A leads to strong resistance and reduced field efficacy. Two other mutations, F129L and G137R, have been described, which lead to lower resistance factors.

All QoI fungicides are in the same cross-resistance group and should be managed accordingly. QoI resistance has developed in some diseases (www.frac.info). However, several fungal species were not yet able to develop field resistance to QoI fungicides, which can be explained by the structure of the target gene (“intron pathogens”). In such pathogens, development of G143A resistance is unlikely and thus long-term efficacy of pyraclostrobin is expected.

3.3 Summary of adverse effects on treated crops

Pyraclostrobin has been applied in all EU member states since many years with several different formulations across a wide range of crops without reports of phytotoxic effects on target or succeeding crops. Consequently, no negative impact is expected on treated crops.

3.4 Summary of observations on other undesirable or unintended side-effects

There is no evidence of any undesirable or unintended side-effect.

4 FURTHER INFORMATION

4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Handle in accordance with good industrial hygiene and safety practice. Use appropriate personal protective equipment, e.g. particle filter with high efficiency for solid and liquid particles, suitable chemical resistant safety gloves, safety glasses with side-shields, protecting boots and chemical-protection suits. Store work clothes separately.

Ensure thorough ventilation of stores and work areas. Protect the product from direct sunlight, temperatures above 40 °C and moisture. Segregate the product from foods and animal feeds.

For transport consider hazard class 9, packing group III, ID number UN 3077 and hazard label 9, EHSM. As proper shipping name use “Environmentally hazardous substance, solid, N.O.S. (contains pyraclostrobin)”.

Prevent electrostatic charge and keep fire extinguishers handy.

In case of fire wear self-contained breathing apparatus and chemical-protective clothing. Do not breathe fumes. Keep containers cool by spraying with water if exposed to fire. Dispose of fire debris and contaminated extinguishing water in accordance with official regulations. Collect contaminated extinguishing water separately, do not allow to reach sewage or effluent systems.

4.2 Summary of procedures for destruction or decontamination

Clean contaminated floors and objects thoroughly with water and detergents, observing environmental regulations. Cleaning operations should be carried out only while wearing breathing apparatus.
Collect waste in suitable containers, which can be labeled and sealed. Waste must be sent to a suitable incineration plant, observing local regulations. Contaminated packaging should be emptied as far as possible and disposed of in the same manner as the product.

4.3 Summary of emergency measures in case of an accident

Use personal protective clothing and avoid contact of product with skin, eyes and clothing. Do not allow the product to reach drains or surface waters.

First aid personnel should pay attention to their own safety.

Treat patients according to symptoms (no specific antidote is known) and seek for medical attention. If the patient is likely to become unconscious, place and transport in stable sideways. Immediately remove contaminated clothing. After skin contact, wash thoroughly with soap and water. In case of contact with eyes, wash affected eyes for at least 15 minutes under running water with eyelids held open. If inhaled, keep patient calm and move to fresh air. If ingested, rinse mouth and then drink 200-300 ml of water.

5 METHODS OF ANALYSIS

5.1 Methods used for the generation of pre-authorisation data

5.1.1 Analysis of the active substance as manufactured

Pyraclostrobin
Pyraclostrobin is determined by reverse phase high performance liquid chromatography with UV detection and external calibration. The separation of the samples is achieved by using gradient elution and quantification of the active ingredient by UV-detection at 275 nm and comparison with reference material. This method was evaluated during the previous Annex I inclusion process.

Dimethyl sulfate (relevant impurity)
Dimethyl sulfate is determined by solid-phase microextraction (SPME) coupled to GC/MS and external calibration. This method was evaluated during the previous Annex I inclusion process.

5.1.2 Formulation analysis

Analytical methods for BAS 500 06 F

The method CF-A 669 is applicable for the determination of **pyraclostrobin** in the EC formulation BAS 500 06 F. A reversed phase HPLC method is used, utilizing a 4µm J'sphere ODS-H80 column. Detection is done by a UV detector at 230 nm and quantification is made by external standard.

Dimethyl sulfate: A method of analysis was developed in order to determine the relevant impurity dimethyl sulfate in BAS 500 06 F. The method applied is a Headspace GC method, MS detection with quantification by means of standard addition. The method was developed and validated in accordance with current GLP regulations.

Analytical methods for BAS 516 07 F

The active substances of BAS 516 07 F can be quantified using the analytical reversed phase-HPLC method CF-A 598. This method was developed and validated for quantifying **pyraclostrobin and boscalid** in BAS 516 00 F, but specific chromatograms prove the applicability for BAS 516 07 F as well. This method is a reversed phase-HPLC method with UV-detection and external calibration to determine pyraclostrobin and boscalid in water dispersible granules (WG).

Dimethyl sulfate: The GC method AM/01120/01e was developed to determine the relevant impurity dimethyl sulfate in BAS 516 07 F. Quantification is done by means of standard addition. This analytical method is a GC method equipped with headspace autosampler and a mass spectrometer detector.

5.1.3 Methods for Risk Assessment

Plants and plant products

The analytical methods for the determination of pyraclostrobin in foodstuffs of plant origin were evaluated in the previous Annex I inclusion process and during more recent evaluations performed by EFSA in the context of MRL applications. Pyraclostrobin residues in commodities of plant origin can be determined by LC/MS-MS with LOQs of 0.02 mg/kg or 0.01 mg/kg. The BASF methods 421/0, 445/0, 535/1 (synonym: L0076/01) allow the determination of pyraclostrobin and its metabolite 500M07 (synonym: BF 500-3) in multiple crops. The metabolite 500M07 is not part of the residue definition in Europe.

The data generation methods are fully validated in separate GLP studies. Furthermore, an extensive set of concurrent fortification experiments exist from supervised field trials or processing studies. They are also supported by extractability investigations performed in the context of metabolism studies. The general suitability of these methods has been already confirmed by EFSA in the recently published Reasoned Opinion on MRLs (Review of established MRLs according to Reg. 396/2005 (Art. 12); EFSA Journal 2011;9(8):2344).

Due to the broad use of pyraclostrobin containing formulations, the scope of the methods has been considerably expanded over the past years. In 2011, EFSA re-evaluated the safety of the established EU MRLs according to Reg. 396/2005, Art. 12. In the context of this evaluation a data gap for a validated method in coffee beans has been identified. The method 445/0 (SOP-PA.0243) has been used for the analysis of the samples generated during the supervised field trials in Brazil.

In addition, a method was developed for the analysis of representative samples generated during supervised field trials. It is intended to support the dietary risk assessment for metabolite 500M04 and its conjugates. For proving the suitability of the enzymatic cleavage step applied, the glycoside 500M79 was selected.

Summaries of the validation studies for the new methods are presented in M-CA 4.1.2 and a summary of the methods including the respective reference in the dossier is listed in the table below:

Method	LOQ (mg/kg)	Matrices	Analyte(s)	Detection
SOP-PA.0243 (CA 4.1.2/13)	0.02	Coffee (grain), soybean (grain) and wheat (grain)	Pyraclostrobin and its metabolite BF 500-3 (500M07)	LC-MS/MS
L0220/01 (CA 4.1.2/14) (CA 4.1.2/15)	0.01	Lettuce (head), white cabbage and leek	Metabolite 500M04 (BF 500-5) and its glycoside conjugate 500M79 (Reg.No. 597091)	LC-MS/MS
L0076/09 (CA 4.1.2/16)	0.01	Dry beans, soya bean (grain), citrus, wheat, tomato	Pyraclostrobin and its metabolite BF 500-3 (500M07)	LC-MS/MS

Food of animal origin

The analytical methods for the determination of pyraclostrobin in foodstuffs of animal origin were evaluated in the previous Annex I inclusion process.

Pyraclostrobin parent residues can be determined by LC-UV method with a validated LOQ of 0.01 mg/kg in milk and 0.05 mg/kg in muscle, liver, kidney, fat and eggs. In the context of this dossier, method L0151/01 is provided. In order to allow an efficient enforcement of pyraclostrobin parent residues using up-to-date technology (LC-MS/MS), it replaces the previously submitted LC-UV method 439/0. It was also validated in blood. As required in SANCO 825/00 and the relevant OECD guidance document, the validation data include a confirmatory technique (second transition).

For data generation purposes, a common moiety method was developed in the context of the previous Annex I inclusion process. It is based in the hydrolytic degradation of BAS 500 F and its metabolites to 500M04 and 500M85. For proving the suitability of the method, which has been used in the cow feeding study, the common moiety has been newly validated for key metabolites identified in the livestock metabolism studies (BASF Method no. 446/2, L0058/03). The principle of the common moiety approach from 2000 was kept; only the final quantitation technique (LC-MS/MS instead of GC-MS) was changed.

In order to support the poultry feeding study, the common residue analytical method (D9902) used for sample analysis is provided as supplemental information. The poultry feeding study was submitted to US EPA.

Summaries of the validation studies for the new methods are presented in M-CA 4.1.2 and a summary of the methods including the respective reference in the dossier is listed in the table below:

Method	LOQ (mg/kg)	Matrices	Analyte(s)	Detection
L0058/03 (CA 4.1.2/17) (CA 4.1.2/18)	0.01	Muscle, kidney, fat, liver, milk and egg	Pyraclostrobin and metabolites 500M04 (BF 500-5), 500M85 (BF 500-8) – common moiety approach	LC-MS/MS
L0151/01 (CA 4.1.2/19) (CA 4.1.2/20)	0.01	Cow: muscle, kidney, fat, liver, milk, skim milk, cream hen: egg swine: blood	Pyraclostrobin	LC-MS/MS
D9902 (CA 4.1.2/21)	0.05	Hen: egg, liver, muscle, fat	Pyraclostrobin and metabolite BF 500-16 [hydrolysed into BF 500-5 and BF 500-9] – common moiety approach	LC-MS/MS

Soil

BASF method L0166/01 fulfilling the European requirements for data generation was developed for the quantitation of pyraclostrobin and its metabolites (shown below) in soil. Additionally, another validated method for parent in soil is provided as supplemental information. This method has been used to analyse petri dish samples collected in a new field dissipation study.

A summary of the methods including the respective reference in the dossier is listed in the table below:

Method	LOQ (mg/Kg)	Matrices	Analyte(s)	Detection
L0166/01 (CA 4.1.2/1)	0.001	soil	Pyraclostrobin and its metabolites BF 500-6 (500M01), BF 500-7 (500M02) and BF 500-3 (500M07)	LC-MS/MS
L0161/01 (CA 4.1.2/2)	0.001	soil	Pyraclostrobin	LC-MS/MS

Water

Two methods fulfilling the European requirements for data generation were developed for the quantitation of pyraclostrobin and its metabolites (shown below) in water. Additionally, another validated method for parent in water is provided as supplemental information. This method has been used to analyse spray solution samples collected in a new field dissipation study.

A summary of the methods including the respective reference in the dossier is listed in the table below:

Method	LOQ (µg/L)	Matrices	Analyte(s)	Detection
L0182/02 (CA 4.1.2/4)	0.003 for BAS 500 F and 0.03 for metabolites	Water (ground/ surface and tap)	Pyraclostrobin (BAS 500 F) and its metabolites BF 500-11 (500M60), BF 500-12 (500M59), BF 500-13 (500M62), BF 500-14 (500M76) and BF 500-15 (500M78)	LC-MS/MS
L0182/02 (CA 4.1.2/5)	0.03	Water (ground/ surface)	BF 500-5 (500M04)	LC-MS/MS
APL0500/01 (CA 4.1.2/3)	1	Water	Pyraclostrobin	LC-MS

Air

The method L0197/01 fulfilling the European requirements for data generation was developed for the quantitation of pyraclostrobin in air. A summary of the method including the respective reference in the dossier is listed in the table below:

Method	**LOQ (ng/L)**	**Matrices**	**Analyte(s)**	**Detection**
L0197/01 (CA 4.1.2/6)	4.44	Air	Pyraclostrobin	LC-MS/MS

5.2 Methods for post-authorisation control and monitoring purposes

Plants and plant products

Based on the residue definition for MRL setting and enforcement, a residue analytical method is required for the parent molecule pyraclostrobin in food of plant origin. For this purpose, residue analytical method 421/0 was already provided in the previous Annex I inclusion process. The method was found to be suitable to quantify residues in different commodity groups, but also in crop matrices which are considered as "difficult" to analyse. Due to an extended use pattern of pyraclostrobin, an Independent Laboratory Validation study in coffee is provided in M-CA 4.2.

In addition, the QuEChERS method (determination using GC-MS and/or LC-MS/MS) is sufficiently validated at the LOQ of 0.01 mg/kg for the determination of pyraclostrobin in plant matrices. The use of the multi residue method for the determination of pyraclostrobin residues is widely published in the Internet.

A summary of the methods including the respective reference in the dossier is listed in the table below:

Method	LOQ (mg/kg)	Matrices	Analyte(s)	Detection
421/0 (CA 4.2/1)	0.02	Multiple commodities	Pyraclostrobin	LC-MS/MS
QuEChERS	0.01	Multiple commodities	Pyraclostrobin	LC-MS/MS

Food of animal origin

Based on the residue definition for MRL setting and enforcement, a residue analytical method is required for the parent molecule Pyraclostrobin in food of animal origin. In order to allow an efficient enforcement of Pyraclostrobin parent residues using up-to-date technology (LC-MS/MS) method L0151/01 replaces the previously submitted LC-UV method 439/0. It was also validated in blood. As required in SANCO 825/00 and the relevant OECD guidance document, the validation data include a confirmatory technique (second transition).

A summary of the methods including the respective reference in the dossier is listed in the table below:

Method	LOQ (mg/kg)	Matrices	Analyte(s)	Detection
L0151/01 (CA 4.2/3)	0.01	Cow: muscle, kidney, fat, liver, milk, skim milk, cream hen: egg swine: blood	Pyraclostrobin	LC-MS/MS

Soil

The soil methods developed for risk assessment as listed in chapter 5.1.3 can also be used for monitoring purposes.

Method	LOQ (mg/Kg)	Matrices	Analyte(s)	Detection
L0166/01 (CA 4.1.2/1)	0.001	soil	Pyraclostrobin and its metabolites BF 500-6 (500M01), BF 500-7 (500M02) and BF 500-3 (500M07)	LC-MS/MS
L0161/01 (CA 4.1.2/2)	0.001	soil	Pyraclostrobin	LC-MS/MS

Water

The water method developed for risk assessment as listed in chapter 5.1.3 can also be used for monitoring purposes. For confirmation, an independent laboratory validation was performed.

Method	LOQ (µg/L)	Matrices	Analyte(s)	Detection
L0182/02 (CA 4.2/5)	0.003 for parent 0.03 for metabolites	Water (surface/drinking)	Pyraclostrobin and its metabolites BF 500-5 (500M04), BF 500-11 (500 M60), BF 500-12 (500M59), BF 500-13 (500M62), BF 500-14 (500M76) and BF 500-15 (500M78)	LC-MS/MS

Air

The air method developed for risk assessment as listed in chapter 5.1.3 can also be used for monitoring purposes.

Method	LOQ (ng/L)	Matrices	Analyte(s)	Detection
L0197/01 (CA 4.1.2/6)	4.44	Air	Pyraclostrobin	LC-MS/MS

Methods used in support of toxicological studies

Methods developed for supporting toxicological studies as listed in M-CA 5.1.3.

Method	LOQ	Matrices	Analyte(s)	Detection
13/0173_04 (CA 4.1.2/7)	50 ng/mL	plasma	Reg.No. 5916421 (500M24)	LC-MS/MS
99/0251_01-0 (CA 4.1.2/8)	50 ng/mL	plasma	Reg.No. 411847 (500M60)	LC-MS/MS
99/0249_01 (CA 4.1.2/9)	50 ng/mL	plasma	Reg.No. 413038 (500M76)	LC-MS/MS
14/0701_04 (CA 4.1.2/10)	50 ng/mL	plasma	Reg.No. 399379 (500M106) Reg.No. 298327 (500M04)	LC-high resolution MS
14/0701_03-01 (CA 4.1.2/11)	0.1 mg/mL	CMC	Reg.No. 399379 (500M106)	LC-UV
14/0701_03-01 (CA 4.1.2/12)	0.5 mg/mL	CMC	Reg.No. 399379 (500M106)	LC-UV

6 IMPACT ON HUMAN AND ANIMAL HEALTH

In the EU Review Report for pyraclostrobin (8 September 2004; SANCO/1420/2001-final) the toxicological properties of pyraclostrobin were fixed. The endpoints are still valid. The database has been extended with studies fulfilling new data requirements, studies conducted to fulfil data requirements of other authorities, updated metabolism studies using state of the art metabolite identification, by inclusion of additional toxicological evaluation on significant metabolites and by assessment of impurities and theoretical impurities for potential toxicological relevance.

6.1 Effects Having Relevance to Human and Animal Health

6.1.1 Summary of adsorption, distribution, metabolism and excretion

Pyraclostrobin has been extensively studied for absorption, distribution, metabolism and excretion. Endpoints set during the previous Annex I inclusion process reflect the rapid absorption and excretion as well as the extensive metabolism in mammalian systems.

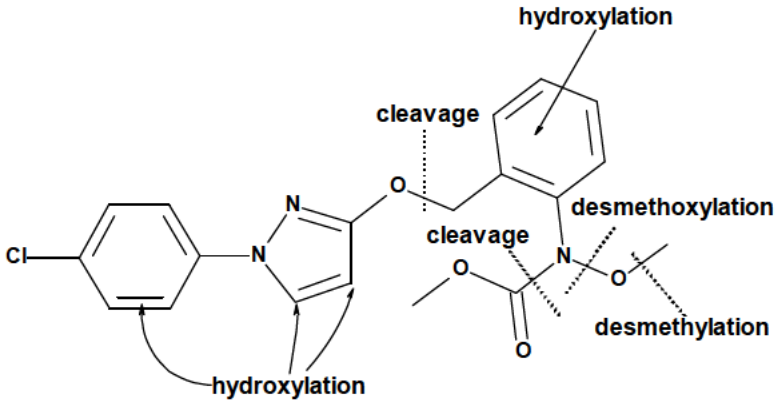
In addition, a new rat study focussing on detailed metabolism in plasma is now available, which extends the knowledge obtained during previous studies. Given the degree of metabolism of pyraclostrobin, it is not to be expected to match all metabolites or percentage between the old and new study. In the recently performed plasma study in rats, several metabolites have been reconfirmed and some new metabolites (500M104, 500M106, 500M107 and 500M108) have been identified. The relevant pathways are provided in Figure 6.1.1-1 and Figure 6.1.1-2.

Since the submission of the supplemental dossier in July 2014, further investigations have been performed. Main purpose of these studies was to get a better understanding of the formation, but also the metabolism of metabolites 500M106, 500M107 and 500M02 which were predominantly found in human and rabbit cell cultures, but only in minor amounts in rats.

When all studies (including the comparative *in-vitro* metabolism and the subsequent investigations on 500M106) are compared, the following general conclusions can be drawn:

- Pyraclostrobin is rapidly excreted via urine and feces.
- Excretion of radioactivity is similar for both sexes. The majority of the radioactivity was excreted via feces (>80% of the dose) and smaller amounts via urine <15% of the dose). Via bile, about 35% of dose is excreted resulting in a bioavailability of approximately 50%.
- There is no evidence of any cumulative potential of pyraclostrobin. Throughout the time course of the experiments, highest radioactivity concentrations were found in the GI tract. Most organs and tissues had values similar to the ones of the plasma. Liver (factor: 4 – 5) and to a smaller extent kidneys (factor: 2) were tissues with higher values than plasma. Radioactivity concentrations were lowest in bone and brain.
- The metabolite patterns in feces, urine, bile, liver, kidney and plasma were largely comparable for both sexes and for all dose groups investigated.

- In total six transformation steps were observed in rats; they are also depicted in the figure below:



  - Desmethoxylation of the side chain
  - Hydroxylation of the chlorophenyl pyrazole ring system
  - Hydroxylation of the tolyl ring system
  - Cleavage of the ether bond resulting in chlorophenyl pyrazole or anthranilic acid derivatives
  - Desmethylation of the side chain
  - Cleavage of the amide bond in the side chain

- The combination of these reactions followed by conjugation steps results in a huge number of metabolites.

In accordance with the new data requirements for active substances of Commission Regulation No 283/2013 of 1 March 2013, and in alignment with RMS Germany a comparative *in-vitro* metabolism study of pyraclostrobin was conducted. There is no guideline for this type of study available yet, therefore the study was conducted according to an internal protocol, which was aligned with RMS Germany.

- Pyraclostrobin was found to be extensively metabolized by liver hepatocytes or microsomes of humans, rabbits, rats and dogs (microsomes only).
- The study showed no human metabolites which were not also observed in at least one of the animal species.
- The same key metabolic degradation steps were observed as under *in-vivo* conditions. Metabolites as 500M04, 500M73, 500M108, 500M103, 500M104 and 500M88 are common to all test species. Some quantitative differences were noted between the different test species. In human and rabbit, the formation of metabolite 500M106 is the major degradation pathway. Subsequently, 500M106 is further metabolized by conjugation with glucuronic acid to 500M107. In human and rabbit, metabolite 500M02 (which is formed by dimerization) has been also identified.
- Based on these results, three metabolites require further consideration. For metabolites 500M106 and 500M02, further toxicological studies have been performed (see M-CA 5.8). The glucuronic acid conjugate 500M107 is covered by the investigations of 500M106. The relevant endpoints are considered for estimating the dietary exposure (see M-CA 6.9) as the conversion of parent into the three metabolites cannot be excluded under *in-vivo* conditions.
- For improving the understanding of the formation and metabolism of 500M106, additional investigations were performed.
- In a rat metabolism study where 500M106 was dosed to male rats, the same excretion pattern was found as for pyraclostrobin. The metabolic pathway is presented in Figure 6.1.1-3. The results of the metabolism study demonstrate that rats are an appropriate species for further toxicological testing, but they also show that metabolite 500M02 is covered by the investigations on 500M106 as it has been found in considerable amounts.
- By means of further *in-vitro* investigations, the enzyme resulting in the formation of 500M106 has been identified as human carboxylesterase 1. This type of enzymes is also present in rats.

Altogether and taking the results summarized in M-CA 5.8 into account, the new studies support the use of the previous studies for end point setting (ADI, ARfD) and also the resulting interpretation of consumer risk.

Figure 6.1.1-1: Proposed metabolic pathway of pyraclostrobin in rats

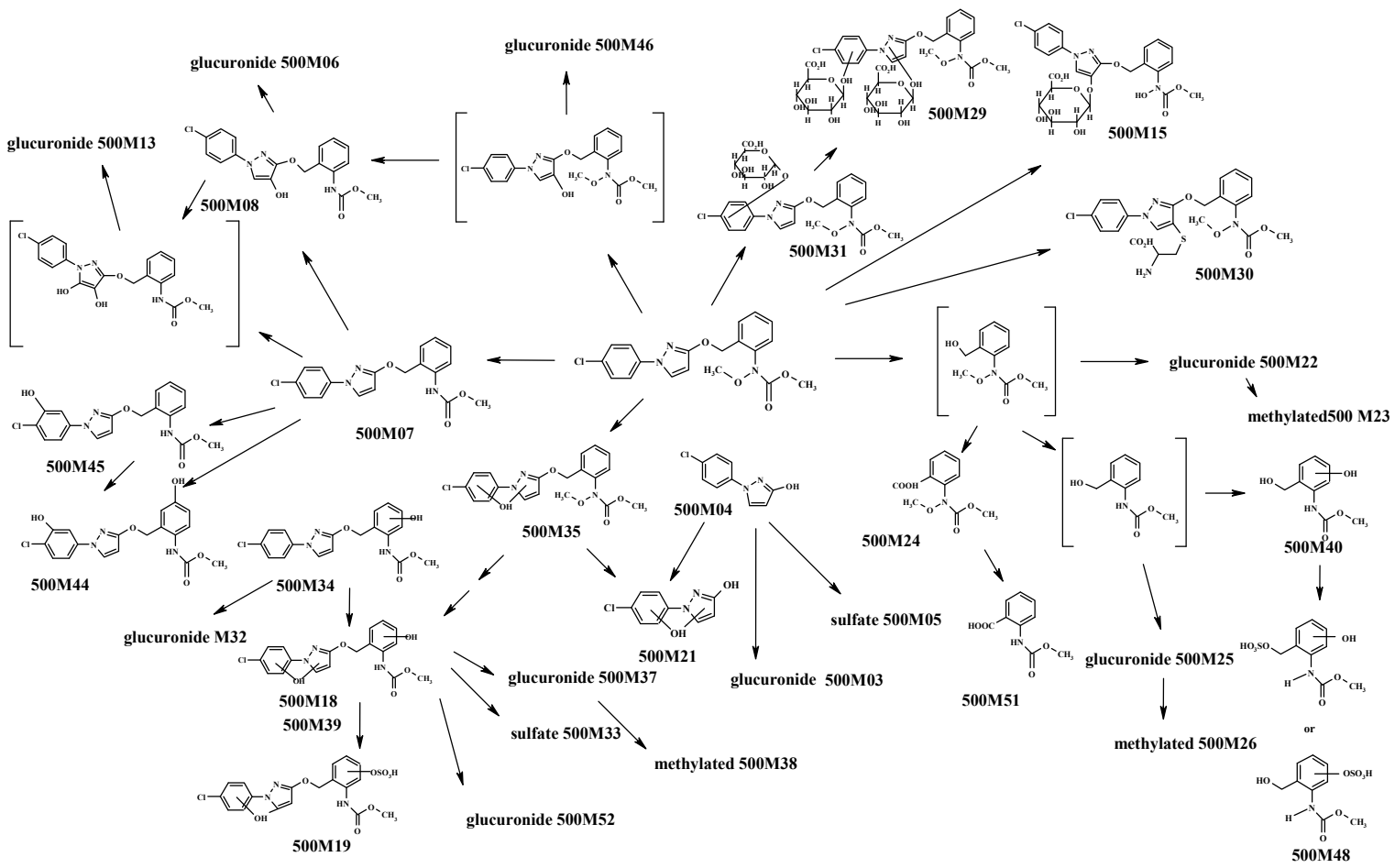


Figure 6.1.1-2: Proposed metabolic pathway of pyraclostrobin in rat plasma

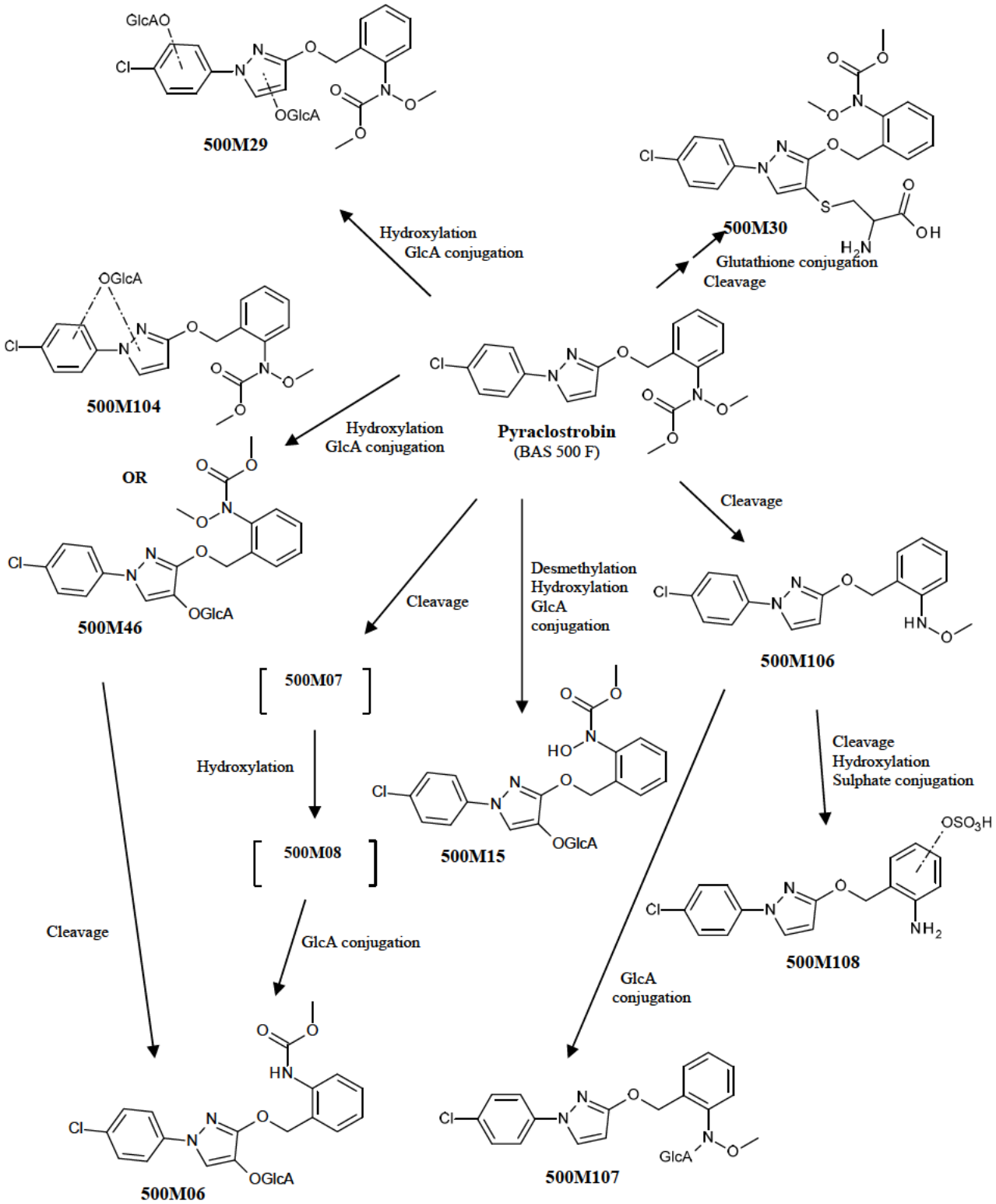
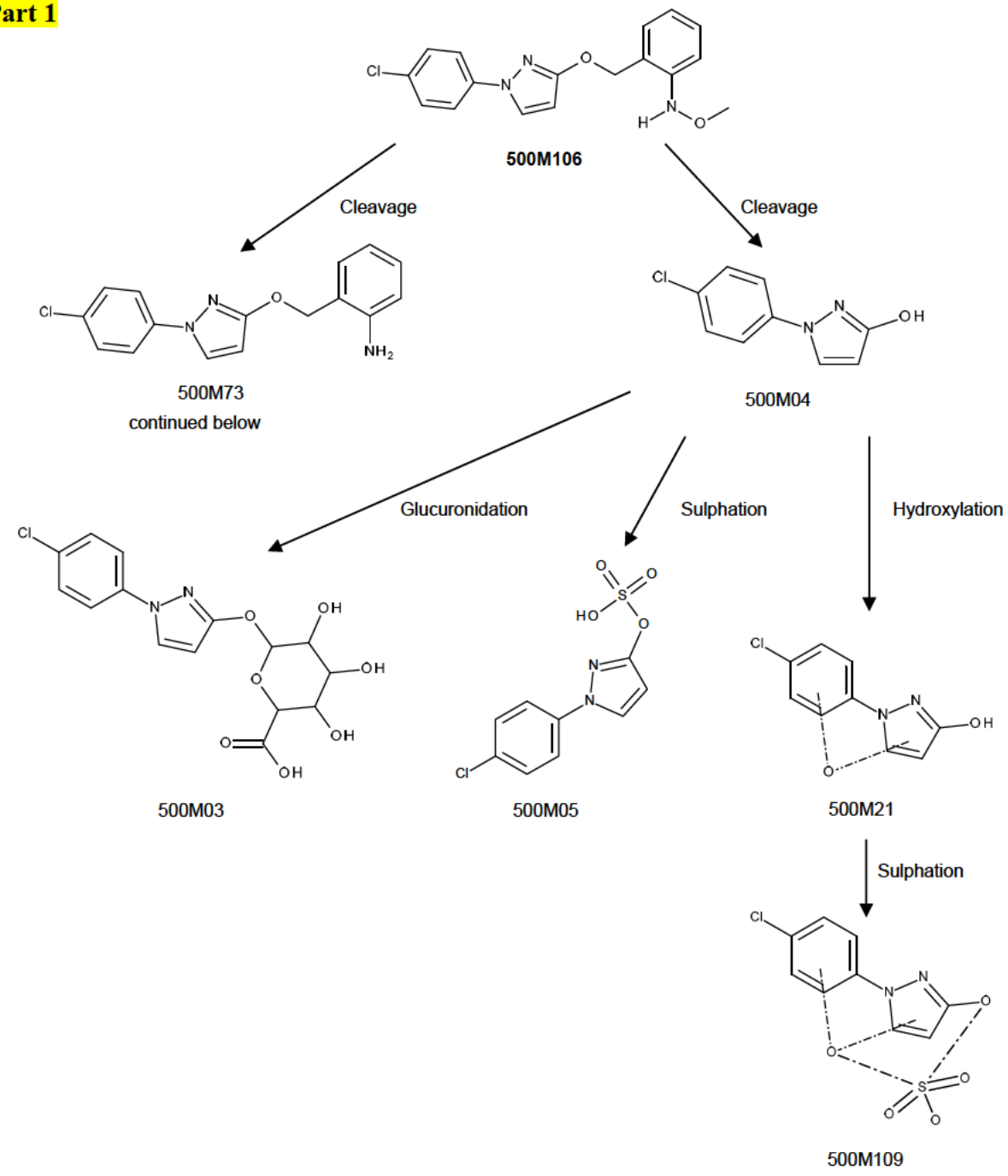
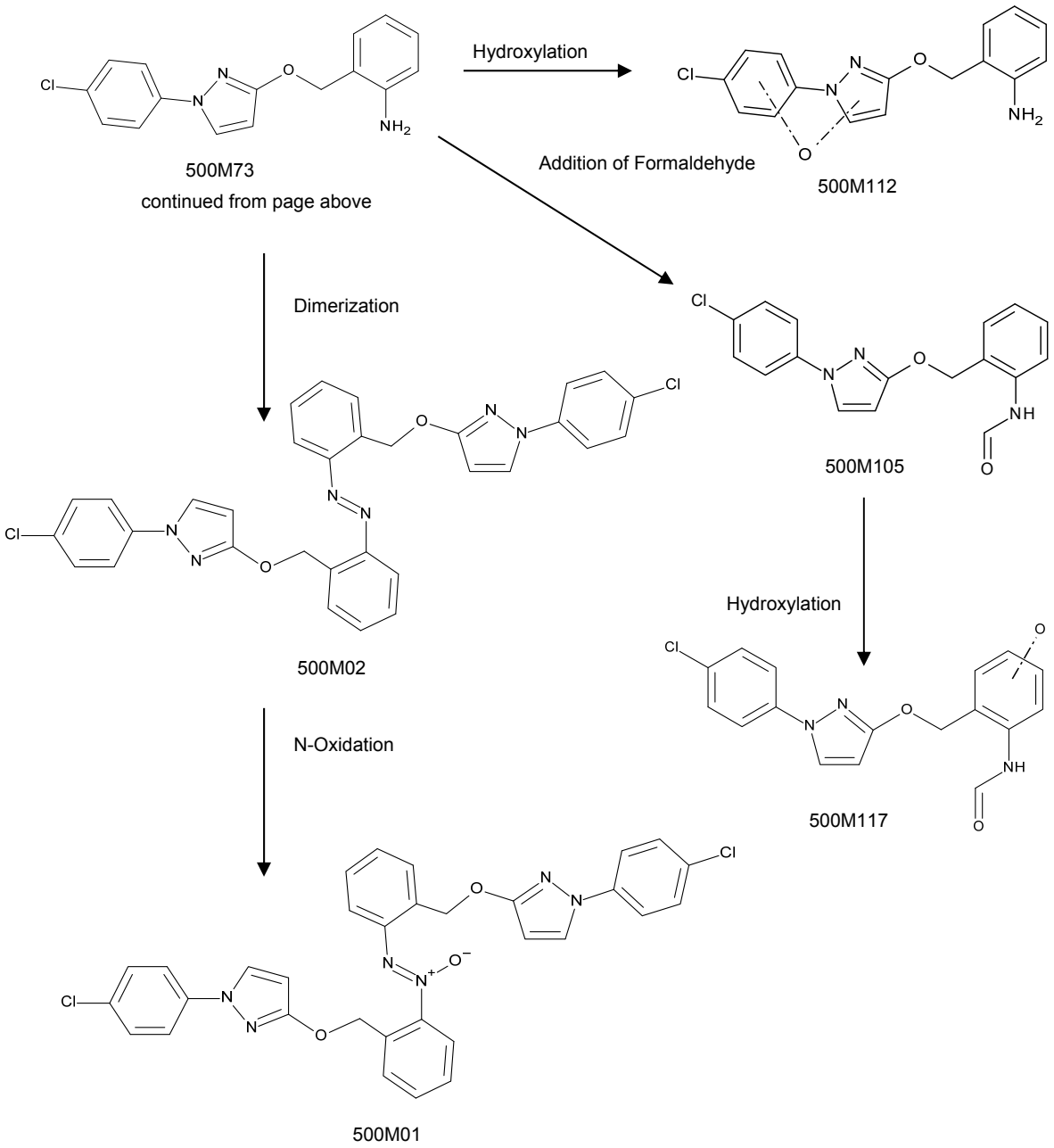


Figure 6.1.1-3: Proposed metabolic pathway of 500M106 in rats

Part 1



Part 2


Hydroxylation
500M73
continued from page above
Addition of Formaldehyde
500M112
Dimerization
500M105
500M02
Hydroxylation
500M117
N-Oxidation
500M01

6.1.2 Summary of acute toxicity

Since the last evaluation new acute inhalation toxicity studies and a NRU-Phototoxicity study have been conducted. In the dossier update of 27-Feb-2017 the range finding toxicity study of the in-vivo mouse micronucleus assay has been added. The mortality data in this study are suitable to derive an acute oral toxicity value in mice, which is considerably lower than the value derived from the acute oral rat study. Reasons are discussed in M-CA 5.2.

Pyraclostrobin is characterized by a rather low dermal toxicity. The toxicity by the inhalation route is to a great extent dependent on the vehicle/solvent used to generate an inhalable aerosol. Whereas for pyraclostrobin dissolved in acetone LC_{50} values of 0.31 mg/L < LC_{50} < 1.07 mg/L and 0.58 mg/L were derived, indicating that pyraclostrobin is toxic by inhalation, the generation of an aerosol with pyraclostrobin dissolved in Solvesso revealed a value of 4.07 mg/L < LC_{50} < 7.3 mg/L. Pyraclostrobin is irritant to the skin, but not irritant to the eye. In a Maximisation test pyraclostrobin was not sensitising.
No indications for a phototoxic potential was observed in an in vitro NRU Phototoxicity Test in Balb/c 3T3 cells.

6.1.3 Summary of short-term toxicity

The short-term toxicity of pyraclostrobin was investigated in dietary 3-month studies in rats, mice, and dogs. Additionally, a dietary 28-day study in rats and a dietary 1-year dog study were performed. The short-term dermal toxicity was determined in a 28-day study in rats. Based on the acute toxicity of BAS 500 F, two 28-day inhalation studies in rats were performed. The results of the short-term toxicity studies are shown in Table 6.1.3-1.

The signs of toxicity observed in the three species tested were overall similar and consisted of reduction of absolute body weight and body weight gain at the high dose levels. The observed clinico-chemical findings can be linked to the (severely) reduced body weight gain. The effects observed typically included reduced total protein, globuline, glucose, tryglycerides and creatinine. The increase in urea may suggest a slightly impaired kidney function at the high dose level in rats and mice. In addition, in rats and mice haematological changes indicative of an anaemic process with compensatory reactions were observed. In rats a higher incidence and/or severity of extramedullary hematopoiesis in the spleen was noted at higher dose levels. In rats and mice, the hemosiderin deposition in spleen seemed to be of slightly lower severity at higher dose levels. Concerning white blood cell parameters, rats demonstrated an increase in white blood cells at the higher dose levels tested, whereas in mice a leukopenia was noted at higher dose levels. The latter was accompanied by effects in lymphatic organs (thymus atrophy, increased apoptotic bodies in mesenteric lymph nodes).
With the exception of the dietary 1-year dog study, the pathology indicated the duodenum mucosa to be the only consistent target organ in all three species tested. The changes (mucosal hypertrophy) were characterised by an increased ratio of cytoplasm to the nuclei in the villi, and a hyperplastic aspect in the epithelial cells.
Only in rats a slight hepatocellular hypertrophy was observed. Secondary to the substantial effects on body weight in rats and mice at higher dose levels, a lower incidence and/or severity of hepatocellular fat storage was noted.

Feeding of BAS 500 F to dogs for one year resulted in clinical signs of toxicity, initially vomitus and diarrhea at the high dose level. At this dose level body weight and food consumption were impaired. Additionally, slight haematological and clinico-chemical changes were observed. There were no specific signs of toxicity or changes in histopathological parameters.

In a 4-week dermal toxicity study in rats no substance-related systemic toxicity was detected up to the highest dose level tested of 250 mg/kg bw. Due to the irritant properties of pyraclostrobin dose dependent signs of local dermal irritation were noted at all dose levels tested.

After subchronic inhalatory exposure of rats to pyraclostrobin dissolved in acetone (6 hours per day on a 5 days/week basis), mortality was observed at 0.3 mg/L. This is in the same range as observed for pyraclostrobin dissolved in acetone in acute inhalation studies, which revealed a 4h LC_{50} of 0.58 mg/L. The respiratory tract (nasal cavity, larynx and lungs) and the duodenum were identified as target organs as indicated by dose dependent atrophy/necrosis and regeneration/repair of the olfactory epithelium, hyperplasia of the respiratory epithelium of nasal cavity and the larynx, perivascular inflammation of the lungs as well as hyperplasia / weight increase of the duodenal mucosal. The reversibility of the observed findings was confirmed after a 28-day treatment-free period.

Table 6.1.3-1: Summary of short-term NOAELs and LOAELs observed in rats, mice and dogs

Study	NOAEL	LOAEL
4-week, oral rat	100 ppm (9.0 mg/kg bw/day)	500 ppm (42.3 mg/kg bw/day)
13-week, oral rat	150 ppm (10.7 mg/kg bw/day)	500 ppm (34.7 mg/kg bw/day)
13-week, oral mouse	< 50 ppm (9.3 mg/kg bw/day) (NOAEL ~ 4 mg/kg bw/day (30 ppm) based on effects observed after 90-day in the mouse carcinogenicity study)	50 ppm (3.5 mg/kg bw/day)
13-week, oral dog	200 ppm (5.8 mg/kg bw/day)	450 ppm (5.8 mg/kg bw/day)
52-week, oral dog	200 ppm (2.7 mg/kg bw/day)	400 ppm (10.8 mg/kg bw/day)
28-day, dermal rat	250 mg/kg bw/day	No systemic toxicity observed
28-day, inhalation rat	0.01 mg/L	0.03 mg/L

6.1.4 Summary of genotoxicity

Pyraclostrobin was evaluated for its potential genotoxicity in vitro using bacterial and mammalian cell mutagenicity tests, a chromosome damage (clastogenicity) test and an unscheduled DNA synthesis test. The results of these studies demonstrated the absence of a genotoxic effect.
In vivo, the test substance was assessed for the induction of micronuclei in mice. The result of this study showed that BAS 500 F has no chromosome-damaging potential. In order to prove the exposure of the target organ in the mouse micronucleus assay (i.e. the bone marrow), a kinetic study using radioactive pyraclostrobin was conducted in mice and the report summary has been added in M-CA 5.4.

It is therefore concluded, that pyraclostrobin has no mutagenic or genotoxic properties both *in vitro* and *in vivo*.

6.1.5 Summary of long-term toxicity and carcinogenicity

The results of a two-year chronic toxicity study and a two-year carcinogenicity study in rats indicate that a maximum tolerated dose was achieved at the high dose level. This is demonstrated by a body weight gain depression of approximately 10% in both sexes. Food consumption was marginally affected at the high dose level and there were slight reductions of clinical chemistry parameters. In high dose males an increased incidence in liver cell necrosis was observed.
There was no evidence of a carcinogenic effect of pyraclostrobin in rats at any dose level.

A carcinogenicity study in mice was conducted up to a maximum tolerated dose as evidenced by significant body weight depression (13% in both sexes) and a reduced body weight gain of 27 – 29%. There were no other signs of toxicity and no test substance related effects were noted in the differential blood count as well as gross- and histopathology investigations.
There was no evidence of a carcinogenic effect of pyraclostrobin in mice at any dose level.

In summary, long-term feeding studies with pyraclostrobin in rats and mice resulted in reduction of body weight and body weight gain. Occasionally clinical chemistry parameters were affected. In high dose male rats an increased incidence of liver cell necrosis was observed. In mice no specific target organ was identified. Pyraclostrobin was not carcinogenic.

6.1.6 Summary of reproductive toxicity

The reproduction toxicity of pyraclostrobin was investigated in a 2-generation reproduction study in rats as well as in prenatal toxicity studies in rats and rabbits.

Parental toxicity was indicated by impaired body weight development and/or reduced food consumption. Reproductive function was not affected in the two generation study in rats including sperm and ovarian parameters as well as the estrus cycle of the rats of both generations. Developmental effects in F1 and F2 pups consisted of a slightly impairment of body weight development. Secondary to the effects on pup body weight development, organ weight changes were noted for a number of organs. Most probably for the same reason a slight, but statistically significant delay of vaginal opening was observed at the high dose. However, the mean number of days for vaginal opening was well within the historical control range and no high dose pup reached the criterion vaginal opening later than the latest control pup.
Accordingly, the NOAEL for reproductive function was identified at the highest dose tested of (300 ppm equivalent to 32.6 mg/kg bw/day). The NOAEL concerning general systemic toxicity for the parental animals was 75 ppm (8.2 mg/kg bw). The NOAEL for developmental toxicity in the F1 and F2 litters also was 75 ppm (8.2 mg/kg bw).

In the prenatal toxicity study in rats no teratogenicity was observed up to the highest dose tested (50 mg/kg bw/day). A slight increase of visceral and skeletal variations at the high dose – even though within the historical control range – was considered to be related to treatment. Maternal toxicity was indicated by reduced food consumption and impaired absolute and corrected body weight development. In conclusion, pyraclostrobin was not teratogenic in rats.
The NOAEL for maternal toxicity was 10 mg/kg bw/day, whereas the developmental NOAEL was set at 25 mg/kg bw/day.

In the rabbit prenatal toxicity study severe maternal toxicity was observed as indicated by dose- and time-dependent decrease of mean food consumption down to 11% of the control, body weight loss and clinical signs. Secondary to the severe maternal toxicity during and shortly after implantation, fetal toxicity at the high dose consisted of an increased post-implantation loss due to an increased number of early resorptions. A slight increase of skeletal malformations of the axial skeleton was within the historical control range and considered unlikely to represent a specific effect of pyraclostrobin treatment.
The NOAEL for developmental toxicity was set at 5 mg/kg bw. The NOAEL for maternal toxicity was established in a supplemental rabbit maternal toxicity study and set at 3 mg/kg bw/d, based on reduced feed consumption and slightly reduced body weight observed at the next higher dose level of 5 mg/kg bw/day. Pyraclostrobin was not teratogenic in rabbits.

The lowest NOAEL for maternal/parental toxicity was 3 mg/kg bw (maternal toxicity study in rabbits) and 5 mg/kg bw/d for developmental toxicity (rabbit prenatal toxicity study).

6.1.7 Summary of neurotoxicity

Pyraclostrobin was tested for potential neurotoxicity in acute and subchronic neurotoxicity studies in rats. These studies included extensive functional observation batteries as well as specific neurohistopathological investigations. In these studies, as well as in other studies with pyraclostrobin, no indications for a neurotoxic potential were observed.

6.1.8 Summary of further toxicological studies on the active substance

6.1.8.1 Supplemental studies in the active ingredient

Additionally, to the studies available for Annex I inclusion of pyraclostrobin, immunotoxicity studies in mice were conducted upon request of US EPA. The studies investigated the cellular mediated immunity as assessed by the functional NKC assay (cellular immune response) as well as the T-cell dependent IgM antibody response (TDAR) to sheep red blood cells (humoral immune response). Like in the subchronic mouse study, lower spleen and thymus weights were observed. For the thymus a stress related reduction of thymus weights can be assumed based on the substantial effects on body weight development. The lower spleen weights correlated to a lower number of spleen cells, which however did not result in an adverse effect on the cellular immune response. In the TDAR study the number of haemolytic plaques per spleen was not affected by treatment up to the highest dose tested. However, based on the lower number of spleen cells per organ, the specific activity (plaques/10^6 spleen cells) as statistically increased compared to the control. While a decrease in the AFC response has been recognized as an indicator that a test substance has the potential of being immunosuppressive; at the present time, the consequences of an increase in the specific AFC response have not been established and thus the adversity of this change cannot be assessed. The NOEL in this study was therefore set at 200 ppm, which was equivalent to a daily dose of 55 mg/kg bw/day.

The effects of pyraclostrobin on serum and urine levels of iron were determined in a 14 day study. A dose- and time dependent decrease of serum iron concentrations was noted. In contrast, serum transferrin levels or urinary iron excretion were not affected by treatment. Based on the decrease of serum iron concentration, the no observed effect level was determined at 3.8 mg/kg bw/day.

6.1.8.2 Endocrine disrupting properties

There is no indication for an endocrine disruption potential of pyraclostrobin from pivotal animal studies. The organ weight changes of male and female sexual organs and endocrine glands observed in subchronic studies at higher dose levels were secondary to the observed decrease of terminal body weights and typically not accompanied by histopathological findings. The decrease of absolute and relative adrenal weights in female mice was accompanied by a decrease of lipid deposition in the x-zone, which is specific to mice and of unknown function. The decreased lipid content is considered secondary to the impaired body weight development. No histopathological effects on the thyroid were observed in rodents and dogs. Likewise, long-term administration of pyraclostrobin did not indicate treatment-related effects on neoplastic or non-neoplastic findings in sexual organs or endocrine tissues. In the 2-generation study administration of pyraclostrobin had no effect on the estrous cycle, the number, morphology and motility of sperm as well as on male or female fertility.
The only potential indication for a sex-hormone mediated effect is a slight delay of vaginal opening in the 2-generation study. However, the mean day of vaginal opening at the high dose level (33.3 days) was well within the historical control range of 29.9 to 34.9 days. The slight delay of vaginal opening is considered to be secondary to a delay of physical development due to a lower body weight gain at the high dose.

In a study by Orton et al. pyraclostrobin was tested for anti-androgenic activity in the MDA-kb2 assay. No anti-androgenicity was observed. As many other pesticides and chemicals, pyraclostrobin is part of the US ToxCast program. Several assays react to varying doses of pyraclostrobin, however, no conclusive picture has emerged.

As a conclusion, there is no evidence for endocrine disruption properties of pyraclostrobin.

6.1.9 Summary of toxicological data on impurities and metabolites

There are three groups of plant or livestock animal metabolites, which were considered no to be adequately covered by the metabolism of pyraclostrobin in rats and thus investigated toxicologically.

Metabolite 500M04 (and its conjugation derivatives; Group 2 of 6) is a metabolite found in all compartments, i.e. soil, water, plants, livestock, fish, rat as well as in in-vitro metabolism studies. 500M04 is of low acute toxicity, is not irritant to the rabbit skin or eye and was negative in a Maximization assay for skin sensitizing effects. In a subchronic rat study at the high dose level an increased water consumption accompanied by a higher volume of discharged urine was noted, which contained high amounts of unidentified crystals. The above changes were in line with the histopathological findings observed in the kidneys at this dose level. However, there was no indication of renal dysfunction as indicated by the absence of respective clinical chemistry alterations. Additionally, a slight regenerative anemia accompanied by an adaptive increase of extramedullary hematopoiesis in the spleen was observed. The NOAEL in this study was 103 mg/kg bw/day and thus about 10-fold higher than the respective NOAEL for pyraclostrobin. 500M04 is not genotoxic as evident from in vitro and in vivo genotoxicity studies. Based on the above findings 500M04 is not considered to be toxicologically relevant. An ADI of 0.52 mg/kg bw/day was calculated.

Metabolites 500M24, 500M49 and 500M51 (Group 3) were tested negative for genotoxicity in a battery of 3 in vitro and one in vivo genotoxicity studies (500M24 only). The metabolites are therefore considered not to be toxicologically relevant. This essentially holds true for Metabolite 500M76 (synonym: BF 500-14; Group 6).

Metabolites 500M02 and 500M106 were found in newly performed metabolism studies. Metabolite 500M02 did not show any genotoxic potential in-vitro, while metabolite 500M106 showed the same outcome in two in-vivo genotoxicity studies. A 28-day rat study with 500M106 showed a NOAEL of 300 mg/kg bw/day. An ADI of 0.5 mg/kg bw/day was calculated covering also 500M02. Based on the toxicological data an ARfD is not needed. Consequently, both metabolites are considered not to be of toxicologically relevance.

The water photolysis metabolites 500M60 (synonym BF 500-11) and 500M62 (synonym BF 500-13) are not considered to be toxicologically relevant based on the results of genotoxicity studies.

Impurities and theoretical impurities have been evaluated for their toxicological relevance in J-CA.

6.1.10 Summary of medical data and information

The information on human data was updated by a recent literature search as well as by inclusion of all internal medical information, which became available since the last submission

All persons handling crop protection products at BASF are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of pyraclostrobin. Thus, the medical monitoring program is designed as a general health check-up.

Some cases of eye irritation deriving from pyraclostrobin in combination with other substances in the course of production, transportation, formulation and packaging have been reported to BASF. Literature research revealed a report on 5 cases of accidental exposure to pyraclostrobin containing aerial spray mist. The most severe incident pertained 27 Hispanics, which were exposed to off-target drift originating from an air craft treating a nearby field. The most common symptom reported by the exposed workers was upper respiratory tract pain or irritation (26 patients), followed by chest pain (20 patients). These observations in combination with supportive evidence from animal studies led to the proposal to classify pyraclostrobin with H335 (STOT SE Category 3, May cause respiratory irritation).

No epidemiological studies are available. No specific signs of poisoning or specific antidotes are available.

For first aid measures and medical treatment following intoxication by pyraclostrobin or pyraclostrobin containing products, it is advised to follow the instructions given in the Safety Data Sheets.

6.2 Toxicological end point for assessment of risk following long-term dietary exposure - ADI

Pyraclostrobin:

No additional data have been provided for renewal of pyraclostrobin that would affect the derivation of reference value agreed upon for Annex I inclusion of pyraclostrobin as laid down in the EU Review Report (SANCO/1420/2001-final, 8 September 2004) and approved in the Commission Directive 2004/30/EC of 10 March 2004.

NOAEL from the chronic dietary toxicity study in the rat was considered to be most relevant for establishing the Acceptable Daily Intake (ADI). The NOAEL in the chronic dietary toxicity study was 75 ppm, which is equivalent to a mean daily dose of 3 mg/kg bw. Pyraclostrobin has no genotoxic or carcinogenic potential, is not teratogenic and has no effects on fertility. Therefore, a safety factor of 100 is applied, resulting in an **ADI of 0.03 mg/kg bw for pyraclostrobin**.

Metabolite 500M04:

Metabolite 500M04 is not genotoxic and has a substantially lower subchronic toxicity compared to pyraclostrobin. As it is a major metabolite consumers may be exposed to, an ADI was derived from the NOAEL observed in the 90-day rat study (103 mg/kg bw/day) and a safety factor of 200. The use of an extra two fold safety factor for extrapolation from subchronic to chronic exposure is in line with the EFSA Scientific Opinion 'Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data' and also with the values discussed in the ECHA 'Guidance on information requirements and chemical safety assessment, Chapter R.8: Characterization of dose [concentration]-response for human health' (Version 2.1, Nov. 2012).

Accordingly, the proposed **ADI for 500M04** is calculated as **0.52 mg/kg bw/day.**

6.3 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

Pyraclostrobin:

No additional data have been provided for renewal of pyraclostrobin that would affect the derivation of reference values agreed upon for Annex I inclusion of pyraclostrobin as laid down in the EU Review Report (SANCO/1420/2001-final, 8 September 2004) and approved in the Commission Directive 2004/30/EC of 10 March 2004.

The ARfD was derived based on the NOAEL of 3 mg/kg bw/d for maternal toxicity from the rabbit prenatal toxicity study. Reduced food consumption and slightly reduced body weight were seen at the next higher tested dose level. Using the NOAEL of 3 mg/kg bw/d and the standard assessment factor of 100, the acute reference dose **(ARfD) is 0.03 mg/kg bw for pyraclostrobin.**

Metabolite 500M04:

For the determination of an ARfD, results for oral studies that used acute or sub-acute (up to 28-day) short-term exposure are the most relevant studies to be considered, provided the study design is sufficient to derive a NOAEL. No such study is available for 500M04. The only study suitable for deriving a NOAEL for short-term exposure effects is the 90-day feeding study. However, as there were no effects on body weight or food consumption, no effects were observable. The only clinically observable effect was an increased water consumption, which was first noted after about 35 days of exposure. It can be assumed that the histopathological changes in the kidneys and ureters likewise did not occur earlier. As hematology and clinical chemistry parameters were determined at study termination only, it is unknown when change of the affected red blood cell and clinical chemistry parameters took place. As a conclusion, no effects of a single or a few administrations of 500M04 can be identified. Accordingly, no ARfD is derived for 500M04.

6.4 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL

There are new data that affect the derivation of the AOEL. These consisted of the newly submitted 28-day inhalation studies in rats and the immunotoxicity studies in female mice.

According to the principles of Annex VI to Directive 91/414 EEC, the proposed AOEL should be based on the highest level at which no adverse effect is observed in tests in the most sensitive relevant animal species.

For the risk assessment of the operator or worker as well as bystander and resident the results of the short-term toxicity including neurotoxicity and immunotoxicity studies and reproduction/developmental toxicity studies are considered the most relevant in order to calculate an AOEL.

The NOAELs and LOAELs of up to 90-day repeated-dose studies including endpoint specific studies for neurotoxicity and immunotoxicity and reproductive toxicity studies are summarized in the following table:

Table 6.4-1: NOAELs and LOAELs from repeated dose studies potentially relevant for AOEL setting

Study	NOAEL	LOAEL
4-week, oral rat	100 ppm (9.0 mg/kg bw/day)	500 ppm (42.3 mg/kg bw/day)
13-week, oral rat	150 ppm (10.7 mg/kg bw/day)	500 ppm (34.7 mg/kg bw/day)
13-week, oral mouse	< 50 ppm (9.3 mg/kg bw/day) (NOAEL ~ 4 mg/kg bw/day (30 ppm) based on effects observed after 90-day in the mouse carcinogenicity study)	50 ppm (3.5 mg/kg bw/day)
13-week, oral dog	200 ppm (5.8 mg/kg bw/day)	450 ppm (5.8 mg/kg bw/day)
52-week, oral dog	200 ppm (2.7 mg/kg bw/day)	400 ppm (10.8 mg/kg bw/day)
28-day, dermal rat	250 mg/kg bw/day	No systemic toxicity observed
28-day, inhalation rat	0.01 mg/L	0.03 mg/L
2-generation rat	75 ppm (8.2 mg(dg bw/day)	300 ppm (32.6 mg/kg bw/day)
Acute neurotox rat	1000 mg/kg bw	Not obtained
90-day neurotox rat	49.9 mg/kg bw/day	Not obtained
Developmental toxicity rat	10 mg/kg bw/day (maternal) 25 mg/kg bw/day (developmental)	25 mg/kg bw/day (maternal) 50 mg/kg bw/day (developmental)
Developmental toxicity rabbit	3 mg/kg bw/day (maternal) 5 mg/kg bw/day (developmental)	5 mg/kg bw/day (maternal) 10 mg/kg bw/day (developmental)
28-day immunotoxicity mouse	200 ppm (50 mg/kg bw/day)	750 ppm (191 mg/kg bw/day)

In the Annex I inclusion process the **AOEL of 0.015 mg/kg bw/day** was set based on the NOAEL of 3 mg/kg bw/day for the maternal toxicity in the rabbit developmental toxicity studies applying a safety factor of 100 and considering a 50% systemic absorption (see Pyraclostrobin Review Report (SANCO/1420/2001-final, 8 September 2004)).

The new immunotoxicity studies revealed a NOEL of 50 mg/kg bw/day which is considerably higher than the NOAEL of 3 mg/kg bw/day used for the derivation of the AOEL. Thus, the immunotoxicity studies in mice do not affect the AOEL setting.

The 28-day inhalation studies revealed a systemic NOAEC of 0.03 mg/L (30 mg/m³) and a local NOAEC of 0.01 mg/L (10 mg/m³). Thus these studies may affect the AOEL setting.

According to Snipes (Crit. Rev. Toxicol., 20(3):175-211, 1989) a respiratory minute volume of 0.2 L for a 250 g rat. Using the standard assumption of a 100% systemic absorption after inhalative exposure, a systemic inhalative dose of 2.88 mg/kg bw/day can be calculated for a 6 hour (360 min) exposure period. Using the safety factor of 100 an inhalative AOEL of 0.029 mg/kg bw/day can be calculated. As this inhalative AOEL is about two fold higher than the AOEL derived in the Annex I inclusion process, i.e. the AOEL of 0.015 mg/kg bw/day is protective for inhalative exposure, too.

Thus it is proposed to set only one **AOEL of 0.015 mg/kg bw/day**.

6.5 Summary of product exposure and risk assessment

Two representative formulations were evaluated in support of the renewal process of pyraclostrobin: **BAS 500 06 F**, an EC formulation containing 200 g/L pyraclostrobin, and **BAS 516 07 F**, a WG formulation containing 67 g/kg pyraclostrobin and 267 g/kg boscalid.
Operator, bystander, resident and worker risk evaluations for both formulations are presented below. Exposure and risk assessments were performed considering the maximum application rate of 0.25 kg/ha pyraclostrobin (1.25 L/ha BAS 500 06 F) for cereals covering the less critical use in maize. For BAS 516 07 F the considered maximum application rate was 0.25 kg/ha corresponding to 0.017 and 0.067 kg/ha for pyraclostrobin and boscalid, respectively.

6.5.1 Operators

BAS 500 06 F
BAS 500 06 F will be applied in field crops (cereals and maize), which is professional use only. The relevant application scenario is outdoor tractor operated boom sprayer with hydraulic nozzles. For operators exposed to pyraclostrobin when applying BAS 500 06 F a safe use could be demonstrated based on BBA model assessment if protective equipment (gloves during mixing/loading as well as gloves, coverall and sturdy footwear during application) is used, but no safe use could be demonstrated based on UK POEM assessments. Therefore, a refined risk assessment with higher tier data on the basis of two field exposure studies was performed.
For the refined assessment the predicted systemic exposure is within the acceptable limit thus demonstrating a safe use for pyraclostrobin when applied in cereal crops and maize at the maximum recommended application rate (predicted systemic exposure: 0.31% of AOEL).

BAS 516 07 F
BAS 516 07 F will be applied in potatoes, which is professional use only. The relevant application scenario is outdoor tractor operated boom sprayer with hydraulic nozzles. For operators exposed to pyraclostrobin and boscalid when applying BAS 516 07 F a safe use could be demonstrated based on BBA model and UK POEM assessments if no protective equipment is used. The predicted exposure corresponds to 23% of the AOEL for pyraclostrobin and 12% of the AOEL for boscalid based on UK POEM assessments, for BBA assessments the predicted exposure is less.
In conclusion operators are considered to be at acceptable risk when exposed to pyraclostrobin and boscalid under the use conditions of BAS 516 07 F in potatoes.

6.5.2 Bystander and resident exposure

The exposure assessment presented in the following is based on the German guidance paper for evaluation of bystander and resident exposure (Martin et al., 2008) for bystander and residents with adopted drift distances of 1 m for field crops.

BAS 500 06 F

For the exposure of a **bystander** passing by a cereal field treated with BAS 500 06 F, the systemic exposure to pyraclostrobin was assessed based on 5 minutes exposure applying generic spray drift deposits of 2.77% of the application rate at 1 meter distance. This estimate results in a predicted systemic exposure for adults of 5.4% of the AOEL for pyraclostrobin. For children the estimate is 4.2% of the AOEL for pyraclostrobin.

Thus, adult and child bystanders passing by a cereal or maize field treated with BAS 500 06 F are considered to be at acceptable risk.

For the exposure of **residents** located next to a cereal field treated with BAS 500 06 F the systemic exposure to pyraclostrobin was assessed based on 2 hour exposure to spray drifts via dermal route. Oral exposure of children and toddlers was considered in addition for ingestion of contaminated plant material and hand to mouth transfer of residues. Exposure to airborne concentration of vapour for non-volatile compounds such as pyraclostrobin is not considered.

This estimate results for adults in 0.7 and for children in 1.5% of the AOEL for pyraclostrobin. Thus, the exposure of adult and child residents living next to a field treated with BAS 500 06 F is considered to be safe.

In conclusion, residents are not at risk if exposed to spray drift of pyraclostrobin under the use conditions of BAS 500 06 F in cereals and maize.

BAS 516 07 F

For the exposure of a **bystander** passing by a field of potatoes treated with BAS 516 07 F, the systemic exposure was assessed based on 5 minutes exposure applying generic spray drift deposits of 2.77% of the application rate at 1 meter distance. This estimate results in a predicted systemic exposure for adults of 0.26 and of 0.12% of the AOEL for pyraclostrobin and boscalid, respectively. For children the estimate is 0.21% of the AOEL for pyraclostrobin and 0.1% of the AOEL for boscalid.

Thus, adult and child bystanders passing by a field of potatoes treated with BAS 516 07 F are considered to be at acceptable risk.

For the exposure of **residents** located next to a field of potatoes treated with BAS 516 07 F, the systemic exposure was assessed based on 2 hour exposure to spray drifts via dermal route. Oral exposure of children and toddlers was considered in addition for ingestion of contaminated plant material and hand to mouth transfer of residues. Exposure to airborne concentration of vapour for non-volatile compounds such as pyraclostrobin and boscalid is not considered.

This estimate results for adults in 0.033 and in 0.02% of the AOEL for pyraclostrobin and boscalid, respectively. For children the estimate is 0.09% of the AOEL for pyraclostrobin and 0.04% of the AOEL for boscalid. Thus, the exposure of adult and child residents living next to a field of potatoes treated with BAS 516 07 F is considered to be safe.

In conclusion, residents are not at risk if exposed to spray drift of pyraclostrobin under the use conditions of BAS 516 07 F in potatoes.

6.5.3 Workers

Estimations of potential worker exposure have been undertaken for both products based on the intended use and the following guidance for exposure prediction: EUROPOEM - Re-entry exposure model final draft, amended by more specific US EPA agricultural transfer coefficients.

BAS 500 06 F

For field crop re-entry in cereals and maize, crop inspection and scouting with a re-entry period of 2 hours was considered to be the adequate scenario. The predicted systemic exposure is about 35% of the AOEL.

It is concluded that there is no unacceptable risk anticipated for the worker wearing adequate working clothing when re-entering cereal crops and maize treated with BAS 500 06 F after the spray dilute has dried.

BAS 516 07 F

For field crop re-entry in potatoes crop inspection and scouting with a re-entry period of 2 hours was considered to be the adequate scenario. The predicted systemic exposure is about 3.3% of the AOEL for pyraclostrobin and 1.6% of the AOEL for boscalid.

It is concluded that there is no unacceptable risk anticipated for the worker wearing adequate working clothing when re-entering potatoes treated with BAS 516 07 F after the spray dilute has dried.

7 RESIDUES

7.1 Summary of storage stability of residues

Plant matrices

Freezer storage stability investigations are available for plant matrices and processed fractions. In the relevant study, the stability of pyraclostrobin and its metabolite 500M07 (synonym: BF 500-3) was investigated up to a period of 18 – 25 months. As representative commodities, peanut nutmeat, peanut oil, wheat grain, wheat straw, sugar beet tops, sugar beet roots, tomatoes and grape juice were fortified with the test items at levels of 1 mg/kg. Pyraclostrobin and 500M07 were found to be stable up to 18 – 25 months.

Animal matrices

In order to investigate the storage stability of the of BAS 500 F residues in matrices of animal origin, investigations were performed in liver, muscle and milk. These matrices are considered as representative for the matrices stored during the residue transfer study in cows, but also to a great extent for poultry.
For fortification experiments the parent compound BAS 500 F and a mixture of BAS 500 F and BF 500-10 were used. The test items selected allow analyzing according to the residue definitions for monitoring and for risk assessment purposes. Whereas for method 439/0 the parent compound is the analyte, for method 446/1, BAS 500 F and BF 500-10 are the "model" substances for the analytes 500M04 (BF 500-5) and 500M85 (BF 500-8). Samples were spiked at a 0.5 mg/kg level (liver, muscle) and at a 0.1 mg/kg level (milk) and stored below -18°C. BAS 500 F residues proved to be stable in all matrices (demonstrated by application of two methods), while BF 500-10 showed some degradation during the course of the study. As all samples from the cow feeding study were analysed within 6 months, residues were sufficiently stable during that period of storage.

7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

7.2.1 Plant Metabolism Studies

In the context of the previous submission, plant metabolism studies have been submitted in grapes, potatoes and wheat covering the crop categories of fruits, root & tuber vegetables and cereals (all foliar application). According to the results of these studies, BAS 500 F is intensively metabolized by mainly three key transformation steps:

- N-Desmethoxylation and O-desmethylation of the side chain
- Hydroxylation of the aromatic ring systems
- Cleavage between the ring systems

The contribution of these reactions followed by subsequent conjugation leads to a large number of metabolites.

For covering a broader use spectrum and a different application type, two new crop metabolism studies have been conducted. They were performed in wheat (seed treatment application) and in paddy rice. Besides these new studies, a cabbage metabolism study is newly submitted in the EU. This study was performed for achieving the registration in Japan.

Chinese cabbage (foliar application)
For the investigation of the metabolism of BAS 500 F, Chinese cabbage plants were treated 3 x at rates of 130 g as/ha using material labeled either in the tolyl or the chlorophenyl ring system. Leaf-ball and outer leaves samples of both labels were mainly extracted with methanol. The extractable radioactivity was characterized and quantified by radio HPLC. In addition, liquid/liquid partitioning experiments using benzene and methanol were carried out. The metabolites were identified by comparison with reference substances. Where possible, they were isolated by HPLC and their structures elucidated by LC-MS/MS.
Predominant residue in outer leaves and leaf ball consists of the parent compound pyraclostrobin and its metabolite 500M07. Some other components which were identified as products formed by cleavage of the molecule, O-glucosylation or methoxylation turned out to be of minor importance, because their respective amount is far below 10% of the TRR (amounts ranged between <0.16 and 3.65% of the TRR). The pathway in Chinese cabbage is presented in Figure 7.2.1-1.

Wheat (seed treatment)
The active substance was applied to the seeds using BAS 662 XI F blank formulation, for each label at a nominal rate of 1 x 10 g as/ha. In general, the levels of total radioactive residues (TRR) were very low (<0.010 mg/kg) in all wheat matrices (straw, hay, forage, grain) for both labels (chlorophenyl and tolyl label) indicating low translocation behavior of parent and its metabolites. Due to the low radioactivity levels only straw samples were extracted with solvent. The extractability of the radioactive residues from straw with methanol and water was moderate for both labels (63.3% TRR for the chlorophenyl label and 46.4% TRR for the tolyl label). Due to low levels of radioactivity in the Residual Radioactive Residues (RRR) after solvent extraction no further solubilization steps were performed. The nature of the residues was examined by HPLC analyses of fractions isolated from the methanol extracts of straw (both labels). Retention time comparison with authentic reference compounds suggests that the only significant peak detected most probably represents the parent compound pyraclostrobin and/or its desmethoxylated metabolite 500M07, which are co-eluting using the applied HPLC methods.
As only parent and metabolite 500M07 were detected in context of this study, no separate pathway is presented.

Paddy rice (foliar application)
For investigating the metabolism in paddy rice, two foliar spray applications were performed with BAS 500 GG F (CS formulation), each with a nominal application rate of 100 g a.s./ha. The first application was carried out at growth stage BBCH 39, the second at BBCH 69. Forage samples of both labels were taken one day before the second application. Straw, grain and spelts were sampled from mature rice plants at BBCH 89. The highest levels of total radioactive residues (TRR) were found in rice straw, in rice grain lower levels were detected for both labels. The lowest values were found in rice forage, sampled one day before the second application. The extractability with methanol and water was high in rice forage; for rice straw and grain, it was slightly lower. From all matrices the major part of the radioactivity was extracted with methanol. The nature of the residues was examined by HPLC analysis of the methanol and water extracts. Metabolites were identified by mass spectrometric analysis and/or co-chromatography experiments.

The by far predominant residues of ^{14}C-pyraclostrobin in rice consisted of the unchanged parent compound and its desmethoxy metabolite 500M07. In rice forage, straw and grain of both labels pyraclostrobin was the main component identified, ranging from 41.5% - 73.3% TRR. Metabolite 500M07 was found in all matrices as the second most prominent compound (from 8.0% to 17.3% TRR). Some further polar and medium polar components were characterized by their HPLC elution behavior and were all clearly below 10% TRR. The Residual Radioactive Residue (RRR) after solvent extraction was characterized using a sequential solubilization procedure with aqueous ammonia solution and enzymes that released further portions of 4.9% - 16.7% TRR. The ammonia and enzyme solubilizates of straw (both labels) were analyzed by HPLC. Like in the water extracts several components eluting in the range between 15 min and about 30 min were detected and characterized by their HPLC elution behavior. Higher levels of non-extractables found in straw and grain, are partially incorporated in or associated with cellulose, lignin, starch or protein. The pathway in rice is shown in Figure 7.2.1-2.

The newly performed studies did not result in the formation of any new metabolite. The metabolic pathways are indicating the same degradation behavior with BAS 500 F as key component, followed by the desmethoxy metabolite 500M07 (synonym: BF 500-3).
An overall proposed pathway for all the plant matrices analyzed (foliar application: grapes, potatoes, Chinese cabbage, rice, wheat; seed treatment: wheat) is presented in Figure 7.2.1-3.

Besides these new studies, a supplementary document (non-GLP) to the grape metabolism study was prepared upon request of Japanese authorities. Purpose of the document was to clarify the way of metabolite identification, which is based on extracts from grape leaves.

Figure 7.2.1-1: Proposed metabolic pathway of pyraclostrobin in Chinese cabbage after foliar application

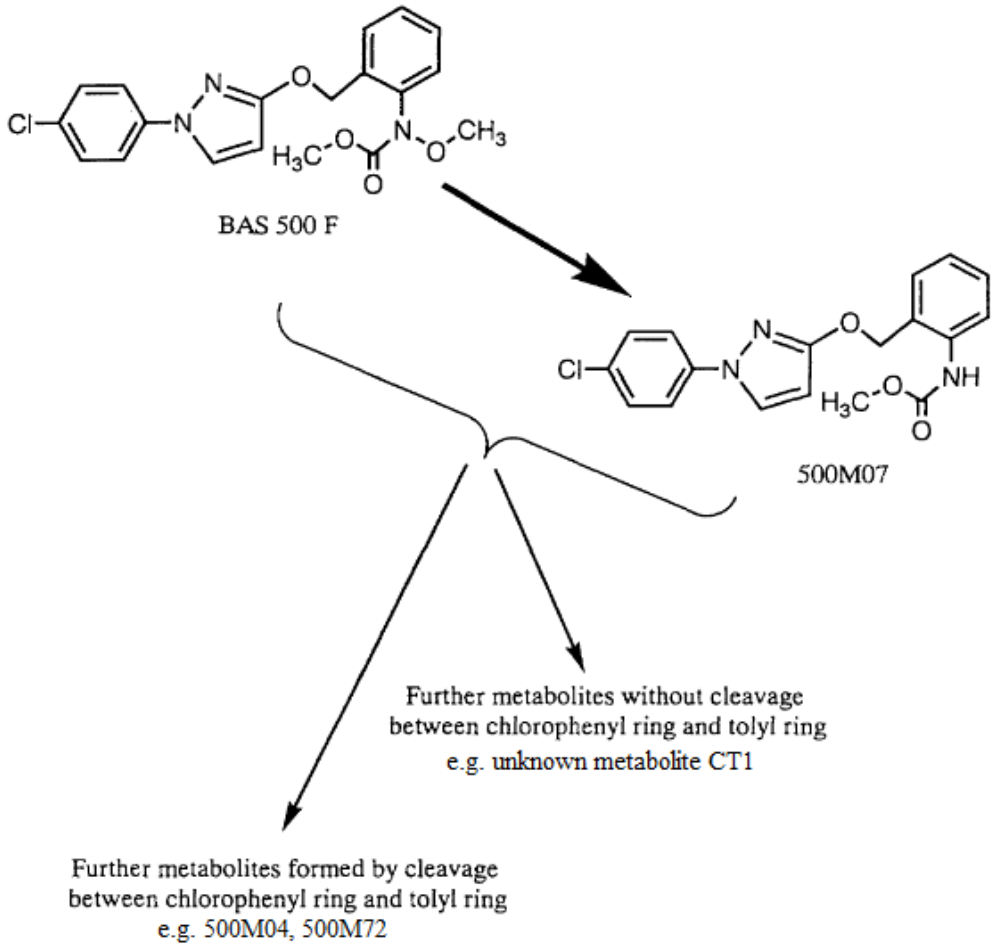
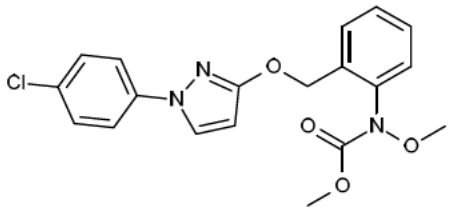
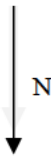


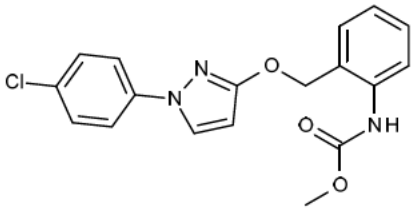
Figure 7.2.1-2: Proposed metabolic pathway of pyraclostrobin in paddy rice after foliar application



BAS 500 F



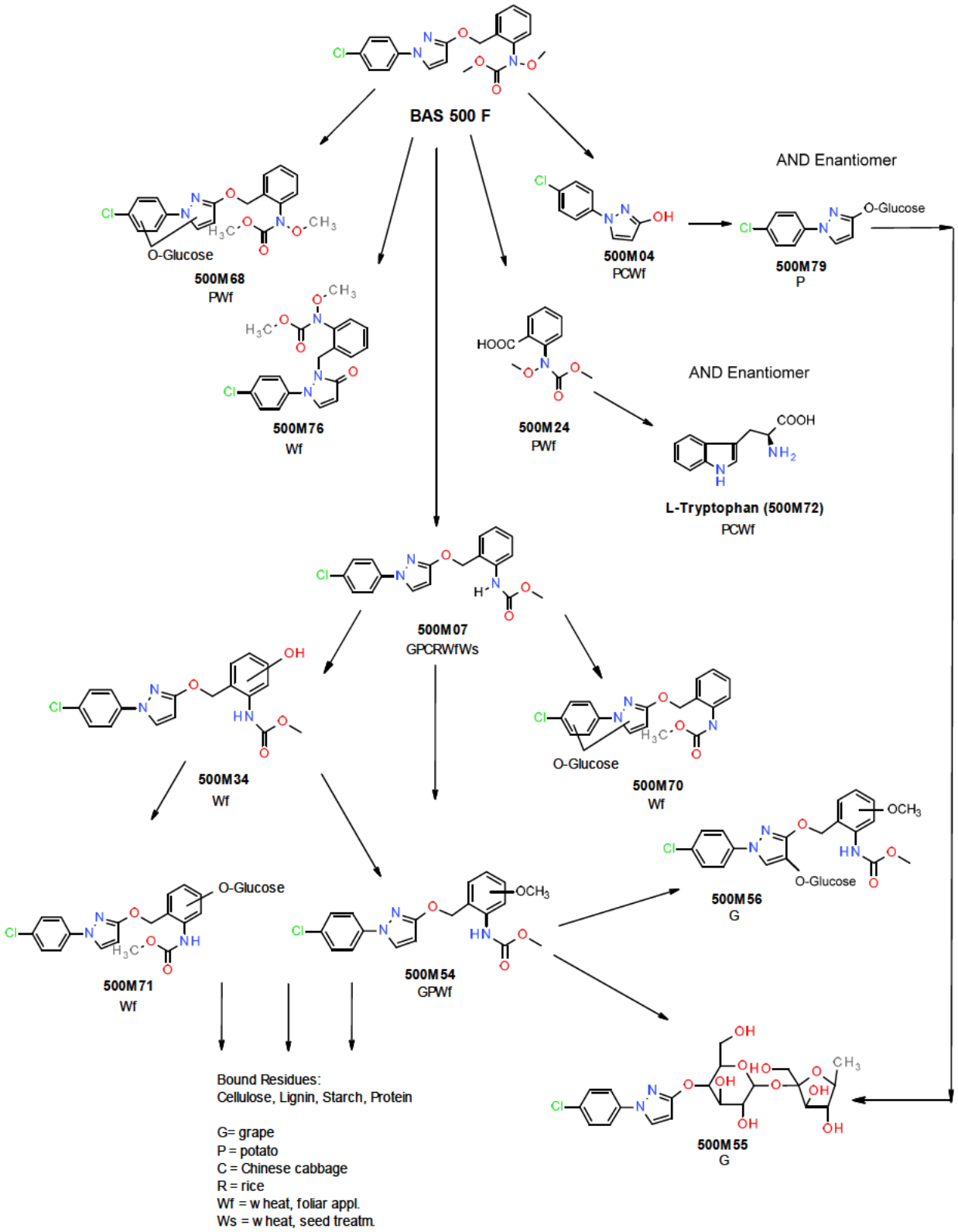
N-desmethoxylation



500M07

Bound Residues:
Cellulose, lignin, starch

Figure 7.2.1-3: Proposed metabolic pathway of pyraclostrobin in grapes, potatoes, Chinese cabbage, rice and wheat after foliar application and in wheat after seed treatment



7.2.2 Livestock Metabolism Studies

Poultry

For Annex I inclusion (Directive 91/414/EEC) a poultry metabolism study was carried out after dosing of tolyl-^{14}C and chlorophenyl-^{14}C labeled pyraclostrobin.

The metabolism and distribution of ^{14}C-BAS 500 F in laying hens was investigated following repeated oral administration of ^{14}C-BAS 500 F at one dose level. The test items were administered to two groups of hens using two different radiolabels. The chlorophenyl label was applied at a dose of 12 mg/kg feed, and the tolyl label was fed at a dose of 13 mg/kg feed, both by gavage and on seven consecutive days. The dose levels corresponded to 0.70 mg/kg body weight (chlorophenyl label) and 0.88 mg/kg body weight (tolyl label).

Pyraclostrobin (BAS 500 F) is rapidly absorbed, distributed and excreted. In hens dosed with 12 mg/kg feed, the residues in muscle were below 0.010 mg/kg. Consequently, no further investigations were carried out. In fat and eggs, pyraclostrobin and the desmethoxy metabolite 500M07 formed the major part of the residue, whereas in liver hydroxylation reactions partly followed by conjugation were predominant.

In detail, five routes of biotransformation were detected: First, the predominant transformation was the desmethoxylation step. Second, the desmethoxylated metabolite 500M07 was hydroxylated at the tolyl ring followed by conjugation with glucuronic acid. Third, the desmethoxylated metabolite was hydroxylated at the chlorophenyl ring or at the pyrazole ring again followed by a conjugation reaction with glucuronic acid. Fourth, the parent compound was hydroxylated at the chlorophenyl ring in para-position whereby the Cl-atom was shifted to the meta-position. Fifth, the parent compound was cleaved at the methylene ether bridge. A specific variation was the substitution of the Cl-atom by glucuronic acid.

These metabolic degradation steps are in accordance with the metabolic transformation steps known from plants but also from other species. The metabolic pathway in hen is included in Figure 7.2.2-4.

Lactating ruminants

For Annex I inclusion (Directive 91/414/EEC) a goat metabolism study was carried out after dosing of the tolyl-^{14}C and chlorophenyl-^{14}C labeled test substance.

The metabolism and distribution of ^{14}C-BAS 500 F was investigated in lactating goats following repeated oral administration of ^{14}C-BAS 500 F at two dose levels. The test compound was administered daily on 5 consecutive days at nominal dose levels of 12 mg/kg and 50 mg/kg feed. The intention of the low dose level was to simulate a dose which would be close to the expected residue level in plant parts to be used as feed items. This dose level would allow judgement whether residues are likely to occur in ruminants under normal agricultural practice. The additional high dose level was added in order to facilitate metabolite identification and characterisation. Following administration of ^{14}C-BAS 500 F to lactating goats, the radioactivity was rapidly and almost completely excreted; excretion mainly occurred via faeces. There was no indication of accumulation of ^{14}C-BAS 500 F in goat milk and tissues. The total radioactive residues in the edible portions of the 12 mg/kg dose group, which is close to the residue level in plant parts to be fed, were low.

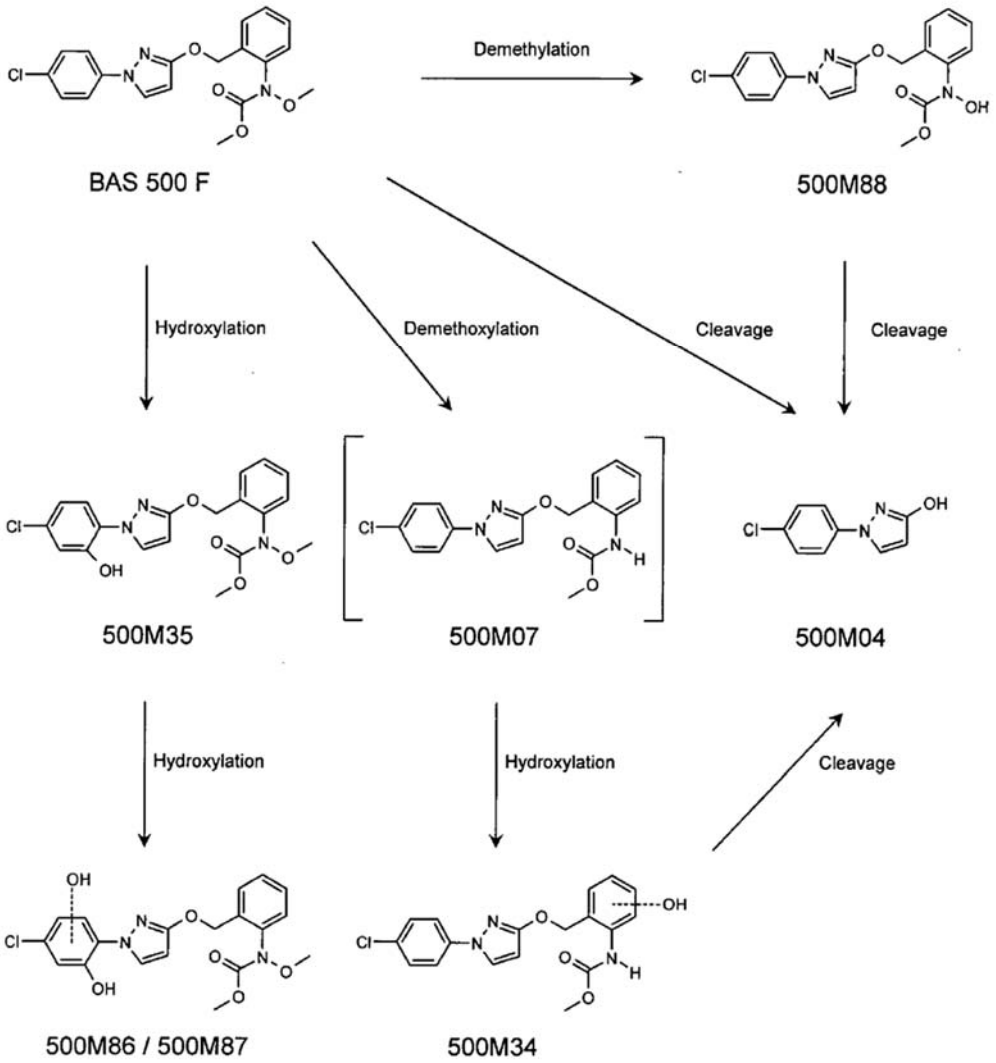
In goats, ^{14}C-BAS 500 F is metabolised by three key transformation steps which are identical to the ones found in hen: (1) desmethoxylation at the oxime ether bond (2) hydroxylation of the chlorophenyl, the pyrazole and/or the tolyl ring system and (3) cleavage between both ring systems and subsequent oxidation of the two resulting molecule parts.

In muscle and fat, BAS 500 F and the desmethoxy metabolite 500M07 were detected as the only residue. In milk, liver and kidney the situation was more complex. In milk, metabolites derived from the cleavage reaction (as 500M04, 500M05 and 500M85) were predominant besides BAS 500 F. In liver and kidney, hydroxylation reaction in various positions of the aromatic ring systems led to the metabolites 500M08, 500M39, 500M45, 500M64, 500M66 and 500M67. In liver the cleavage reaction is less pronounced, whereas in kidney the cleavage product 500M51 was found. The metabolic pathway in goat is included in Figure 7.2.2-4. The non-extractable residues of liver and kidney (high dose) were further characterised by pronase treatment and acid hydrolysis.

Upon request of the Australian authority, an *in vitro* comparison study was performed for goat and cow cell cultures. In order to allow a comprehensive overview, the study summary of these supplemental investigations are provided in context of this dossier.

Goat and cow microsomes and hepatocytes were incubated with the chlorophenyl-^{14}C labeled test substance. BAS 500 F was similarly metabolized by goat and cow microsomes. All metabolites identified in samples from incubations with cow microsomes were also found in the experiments with goat microsomes. The appearance of the main metabolite 500M04 was independent from the microsomes` origin and resulted from cleavage of the ether bond. It was already identified in a previous *in vivo* goat study as one of the key degradation products of BAS 500 F. The metabolites 500M88 (demethylation of the N-methoxycarbamate group) and 500M34 (desmethoxylation of the N-methoxycarbamate group and hydroxylation of the benzylic ring) were further common metabolites found after incubation of BAS 500 F with goat or cow microsomes. Hydroxylation reactions of the chlorophenyl moiety played only a minor role in the *in vitro* biotransformation route of BAS 500 F. The pathway in cow and goat microsomes is presented in Figure 7.2.2-1.

Figure 7.2.2-1: Proposed metabolic pathway of pyraclostrobin in *in vitro* investigations of goat and cow



Pigs

According to Reg. 283/2013, pig metabolism studies shall be provided where the plant protection product is used in crops whose parts or products, also after processing, are fed to pigs and where it becomes apparent that metabolic pathways differ significantly in the rat as compared to ruminants and where the intake is expected to exceed 0.004 mg/kg bw/day.
For pyraclostrobin, no metabolism study was performed in pigs, since the metabolite patterns in rodents (rats) and ruminants (goats) did not show any difference.

Fish

In order to fulfill the requirements of Reg. 283/2013, two studies are submitted in this dossier. According to Reg. 283/2013, metabolism studies on fish may be required where the plant protection product is used in crops whose parts or products, also after processing, are fed to fish and where residues in feed may occur from the intended applications. Pyraclostrobin is registered in the majority of crops being intended as fish feed item. In most of these crops residue levels above LOQ occur.

The bioaccumulation study in bluegill sunfish has been performed in 1999 and was already evaluated as part of the ecotox section during the previous Annex I inclusion process. In the study the tolyl-^{14}C and chlorophenyl-^{14}C labeled test substance was applied to water. The study includes the identification of metabolites in edible and inedible portions of bluegill sunfish.
After exposure of fish to BAS 500 F at a nominal exposure level of 300 ng/L, apparent steady state was reached after 2 - 4 days. After termination of the exposure, radioactivity levels in fish tissues decreased rapidly with a half-life of *ca.* 0.7 – 1.0 days. Bioconcentration factors based on total radioactivity were relatively low in edibles (232 - 262) and relatively high in inedible (1169 - 1221). For unchanged parent compound, the BCF values were considerably lower in all tissues, which is an indication for an intensive metabolic clearance of BAS 500 F. Only minor differences were observed between the two labelled forms of the test compound with regard to the kinetic parameters.
In the edible tissues pyraclostrobin formed the major part of the residue, followed by 500M07. Cleavage of the ether bond resulting in the formation of the metabolite 500M04 occurred to a minor extent and mainly in the inedible portion of fish (see Figure 7.2.2-2).

For fulfilling Reg. 283/2013, a fish metabolism study was performed in which rainbow trouts were dosed orally via feed. During the study, the test item was dosed to the fish for 10 consecutive days for both labels at a nominal level of 10 mg/kg. After 10 days, the fish were killed and edible tissues (liver, filet and filet skin) and pyloric caeca removed post mortem.
The investigation of the samples of both labels showed comparable TRR levels. In all matrices (filet, filet skin, liver) and in both labels (chlorophenyl and tolyl) most of the extracted residues consisted of unchanged parent (70.4-93.1% TRR; 0.210-0.409 mg/kg). Only minor amounts of the metabolite 500M89 (Reg.no. 334089) were detected in extracted residue (1-4% TRR; 0.006-0.011 mg/kg). Taking the actual feed burden and the overdosing factor (8x) into account, no significant residues of 500M89 will occur when fish are fed with commodities being treated with pyraclostrobin. Reactions leading to a cleavage of the molecule between the ring systems did not occur. A degradation to polar residues occurred to a minor extent and at ranges of 1-7% TRR (0.005-0.037 mg/kg). In liver ca. 8-11% TRR (0.044-0.054 mg/kg) were incorporated in the tissue and just releasable with digestive enzymes. The pathway is presented in Figure 7.2.2-3. The results are in accordance with those obtained during the BCF study and they are included in Figure 7.2.2-4.

Figure 7.2.2-2: Proposed metabolic pathway of pyraclostrobin in fish (bioaccumulation study)

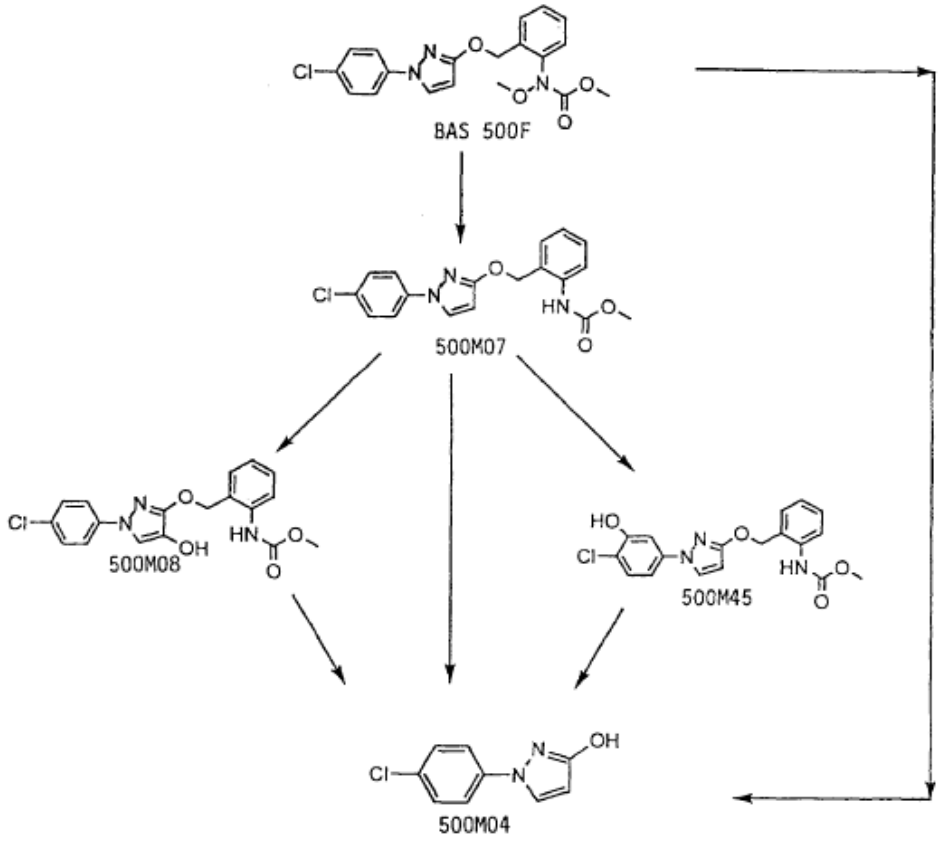


Figure 7.2.2-3: Proposed metabolic pathway of pyraclostrobin in fish after oral dosing

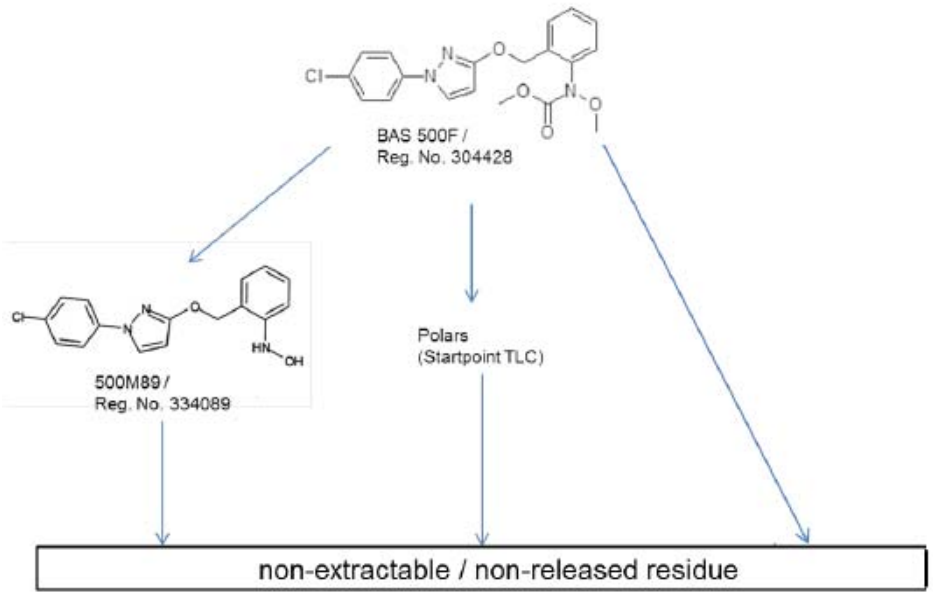
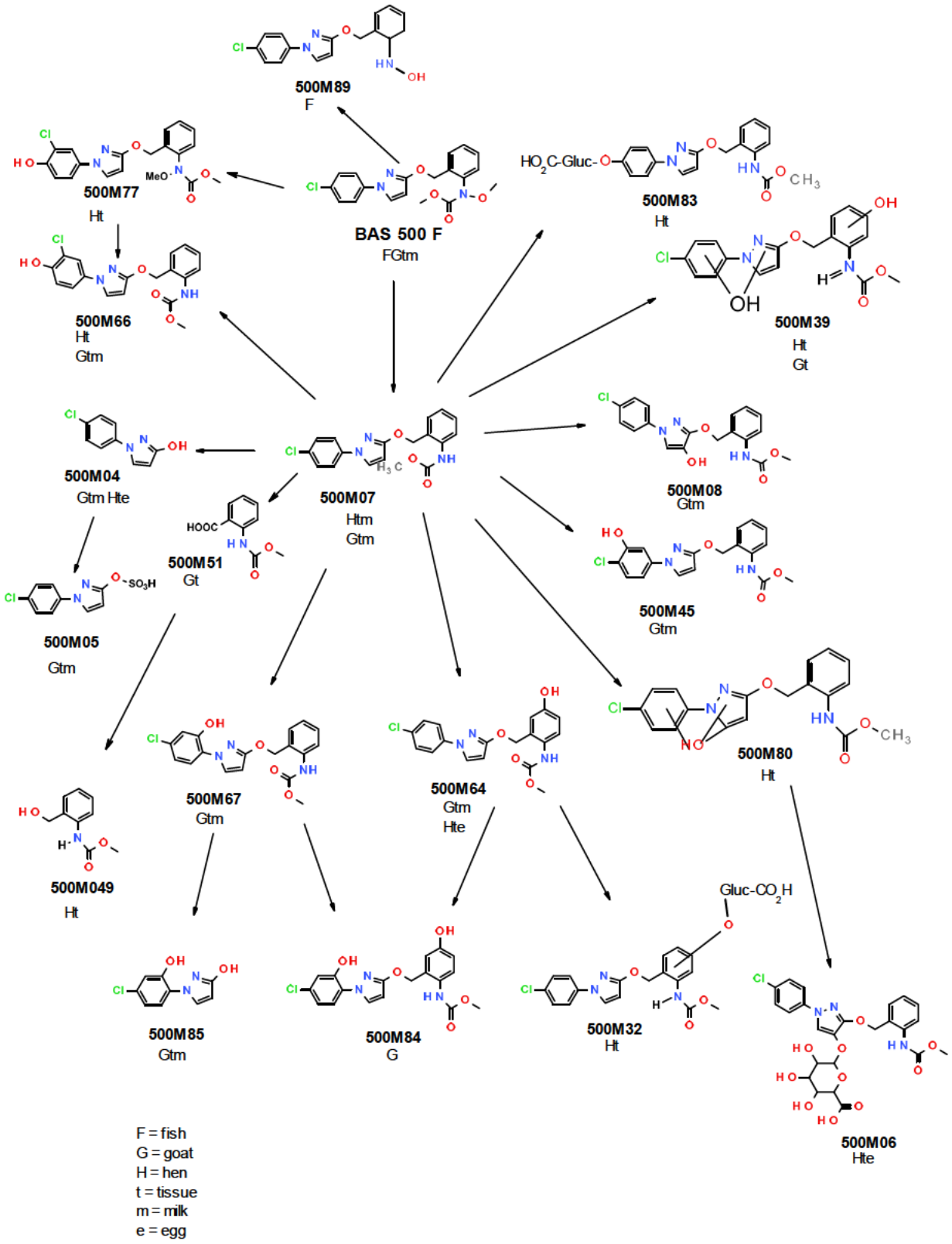


Figure 7.2.2-4: Proposed metabolic pathway of pyraclostrobin in poultry, goats and fish (after oral dosing)



7.3 Definition of the residue

The residue definitions currently established in the EU, but also supported in future, are compiled in the table below. In sections M-CA 6.7 and M-CA 6.9, detailed justifications for BASF's proposal are provided. The proposal is based on a careful evaluation of all studies being available at the time point of submission. Consequently, it includes considerations for all those crops for which an EU MRL is established. It is not limited to the intended uses in potatoes, cereals and maize.

Endpoint	**Active Substance: Pyraclostrobin**	
	EU agreed endpoints (SANCO/ 1420/2001; Monograph12945/ ECCO/BBA/01, Vol. 1, list of endpoints)	Residue definitions proposed in context of this dossier
Residue definition in plant matrices for risk assessment	Pyraclostrobin (parent)	Pyraclostrobin (parent)
Residue definition in plant matrices for monitoring	Pyraclostrobin (parent)	Pyraclostrobin (parent)
Residue definition in animal matrices for risk assessment	Pyraclostrobin (parent) except: Liver (except poultry liver) and milk fat only: Pyraclostrobin and its metabolites analysed as the hydroxypyrazoles BF 500-5* and BF 500-8**, sum expressed as pyraclostrobin	Pyraclostrobin and its metabolites analysed as the hydroxypyrazoles BF 500-5* and BF 500-8**, sum expressed as pyraclostrobin
Residue definition in animal matrices for monitoring	Pyraclostrobin (parent)	Pyraclostrobin (parent)
Conversion factors between residue definitions (animal)		Liver (w/o poultry): 4 All other: 1

* synonym: 500M04
** synonym: 500M85

7.3.1 Plant Matrices

For pyraclostrobin, plant metabolism studies have been performed in fruits, root & tuber vegetables, leafy vegetables and cereals (wheat, paddy rice) covering both, foliar and seed treatment applications. During these investigations, samples/matrices were analyzed which serve either as animal feed or human food. Two confined rotational crop studies were additionally conducted. After application of pyraclostrobin to bare soil, representative crops (typically small grain, leafy crops and root vegetables) were investigated at different replant intervals (emergency plant back, fall plant back, annual plant back).

In general, pyraclostrobin follows a common pathway in all crops and independent from the type of application. The following metabolic conversion steps were observed in the plant metabolism, hydrolysis (simulating processing) and the succeeding crop studies:

- Desmethoxylation of the side chain resulting in the metabolite 500M07
- Cleavage between the ring systems resulting in the metabolites 500M04 (followed by conjugation reactions)
- Cleavage between the ring systems resulting in the metabolites 500M24, 500M49 and 500M72 (tryptophan)
- Hydroxylation of the chlorophenyl pyrazole moiety (followed by conjugation)
- Hydroxylation of the tolyl moiety (followed by conjugation)
- Photolytic rearrangement reaction resulting in the metabolite 500M76

In almost all samples investigated from metabolism studies, the parent molecule forms by far the predominant residue followed by the metabolite 500M07. All other metabolites are present in significantly lower amounts, but also not consistently in all crops or commodities.

Residue definition for monitoring purposes

The analyte(s) to be selected for monitoring purposes should occur in large quantities, and should be common to all commodities in which residues are expected. Ideally, the monitoring method should be based on one single analyte (‘marker or indicator compound’).

In case of pyraclostrobin and its metabolites in food of plant origin, there is only one component which meets all criteria listed in the OECD guidance document. Based on the studies available, the following residue definition is proposed for monitoring purposes in plant commodities (including processing fractions thereof):

Pyraclostrobin, parent only

Residue definition for data generation / risk assessment purposes

In order to propose a suitable residue definition for risk assessment purposes, the pyraclostrobin metabolites found in the plant metabolism studies and during processing were grouped in total into six different groups.

- Group 1: Desmethoxy metabolite 500M07 (synonym: BF 500-3)
- Group 2: Chlorphenyl pyrazole derivatives
- Group 3: Anthranilic acid derivatives 500M24 and 500M49
- Group 4: Hydroxylated metabolites (chlorphenyl pyrazole moiety)
- Group 5: Hydroxylated metabolites (tolyl moiety)
- Group 6: Photo metabolite 500M76

The dietary exposure for each group was assessed separately for identifying the contributions of the plant metabolites to the total dietary risk. Due to obvious reasons no assessment has been performed for the metabolite 500M72, which has been identified as the natural amino acid tryptophan. Due to their absence in succeeding and root crops even at exaggerated rates, the same applies for the aerobic soil metabolites 500M01 and 500M02. The assessments were limited to those target crops from which a contribution to the dietary risk could be expected.

For deriving residue levels to be used in chronic and acute exposure assessments, the following studies were additionally considered:

- Magnitude of residue studies in target crops (supervised field trials)
- Magnitude of residues in processed commodities

Table 7.3.1-1: Contribution of pyraclostrobin plant metabolites to chronic dietary risk

Metabolite group - Metabolites	ADI used [mg/kg bw / day]	ADI utilization [%]	Comment
1 - 500M07	0.03	< 2%	Indicative assessment covering all target crops (worst case)
2 - 500M04 and conjugated	0.52	< 0.1%	
3 - 500M24	0.0015 (TTC)	< 9%	Cereal grain
3 - 500M49	0.0015 (TTC)	0.1%	Oilseeds
3 - 500M51	0.0015 (TTC)	n.a.	Metabolite only found in livestock metabolism studies
4 - Hydroxylated metabolites (chlorphenyl pyrazole moiety)	0.03	0.6%	
5 - Hydroxylated metabolites (tolyl moiety)	0.03	0.6%	
6 - 500M76	0.0015 (TTC)	0.2%	

Table 7.3.1-2: Contribution of pyraclostrobin plant metabolites to acute dietary risk

Metabolite group - Metabolites	ARfD used [mg/kg bw / day]	ARfD utilization [%]	Comment
1 - 500M07	0.03	max. 30% (celery, onions)	Refined for table grapes, apples and scarole
2 - 500M04 and conjugated	n.a.	n.a.	
3 - 500M24	0.005 (TTC)	not relevant	Metabolite only found in wheat metabolism study (grain)
3 - 500M49	0.005 (TTC)	0%	
3 - 500M51	0.005 (TTC)	n.a.	Metabolite only found in livestock metabolism studies
4 - Hydroxylated metabolites (chlorphenyl pyrazole moiety)	0.03	8% (melons) 7% (water melons) 5% (table grapes)	Refined for table grapes, apples and scarole
5 - Hydroxylated metabolites (tolyl moiety)	0.03	11% kale 8% (melons) 7% (water melons)	Refined for table grapes, apples and scarole
6 - 500M76	0.005 (TTC)	15% (kale) 9% (globe artichoke) 8% (Chinese cabbage)	Refined for scarole

The data show that the contributions of the metabolites to the dietary risk are small even under unrealistic worst case assumptions. None of the groups should be included in the residue definition for dietary risk assessment. Due to the favorable outcome, no further refinement than indicated above was performed (e.g. by inclusion of processing factors for vegetable crops, which are always cooked prior to consumption).

Following this indicative assessment based on an in-depth analysis of all metabolism, residue and processing fraction studies, the residue definition shown below is proposed for risk assessment in plant commodities (including processing fractions thereof):

Pyraclostrobin, parent only

7.3.2 Animal matrices

In general, pyraclostrobin follows a common pathway in different livestock species, which is also comparable to the one observed in rats. As in plant matrices, the following metabolic conversion steps were observed in the relevant studies:

- Desmethoxylation of the side chain resulting in the metabolite 500M07
- Cleavage between the ring systems resulting in the metabolites 500M04, 500M05 and 500M85
- Cleavage between the ring systems resulting in the metabolites 500M49 and 500M51
- Hydroxylation of the chlorophenyl pyrazole moiety (followed by conjugation)
- Hydroxylation of the tolyl moiety (followed by conjugation)

Besides these steps, metabolite 500M89 was identified in fish in very low amounts; it is formed by desmethylation.

In the majority of samples investigated (poultry: fat and eggs, goat: milk, meat and fat), the parent molecule forms a predominant part of the residue followed by the metabolite 500M07. In milk, the cleavage products 500M04 and 500M05 are present in the same amounts as parent. Especially in goat liver, but also kidney, relatively high amounts of radioactivity were incorporated into tissues, which could be at least partly released by enzymatic techniques. After applying harsh conditions (refluxing with hydrochloric acid), the metabolites 500M04 and 500M85 were detected in the hydrolyzate. All other metabolites are present in significantly lower amounts, but also not consistently in all livestock commodities.

Residue definition for monitoring purposes

For the residue definition in animal commodities the same criteria apply as for plants. The analyte(s) to be selected for monitoring purposes should occur in large quantities, and should be common to all commodities in which residues are expected. Ideally, the monitoring method should be based on one single analyte (‘marker or indicator compound’).

In case of pyraclostrobin and its metabolites in food of animal origin, there is only one component which meets most of the criteria listed in the OECD guidance document. Based on the studies available, where unchanged pyraclostrobin formed a considerable part of the residue in food items being highly consumed (milk for small children, meat and fat for all subpopulation groups), the following residue definition is proposed for monitoring purposes in animal commodities:

Pyraclostrobin, parent only

Residue definition for data generation / risk assessment purposes

In order to propose a suitable residue definition for risk assessment purposes, the pyraclostrobin metabolites found in livestock metabolism studies were grouped in total into five different groups. The grouping followed the same principles as for plants, but without group 6 ("photo metabolite").

- Group 1: Desmethoxy metabolite 500M07 (synonym: BF 500-3)
- Group 2: Chlorphenyl pyrazole derivatives
- Group 3: Anthranilic acid derivatives 500M49 and 500M51
- Group 4: Hydroxylated metabolites (chlorphenyl pyrazole moiety)
- Group 5: Hydroxylated metabolites (tolyl moiety)

The dietary exposure for each group was assessed separately for identifying the contributions of the livestock metabolites to the total dietary risk. For deriving residue levels to be used in chronic and acute exposure assessments, the following studies were additionally considered:

- Magnitude of residue study in cows
- Magnitude of residue study in hens

Table 7.3.2-1: Contribution of pyraclostrobin livestock metabolites to chronic dietary risk

Metabolite group - Metabolites	ADI used [mg/kg bw / day]	ADI utilization [%]	Comment
1 - 500M07	0.03	< 0.1%	
2 - 500M04 and conjugates	0.52	< 0.1%	
3 - 500M24	0.0015 (TTC)	n.a.	Metabolite only found in wheat metabolism study (grain)
3 - 500M49	0.0015 (TTC)	0%	
3 - 500M51	0.0015 (TTC)	0%	
4 - Hydroxylated metabolites (chlorphenyl pyrazole moiety)	0.03	0.1%	
5 - Hydroxylated metabolites (tolyl moiety)	0.03	< 0.1%	

Table 7.3.2-2: Contribution of pyraclostrobin livestock metabolites to acute dietary risk

Metabolite group - Metabolites	ARfD used [mg/kg bw / day]	ARfD utilization [%]	Comment
1 - 500M07	0.03		
2 - 500M04 and conjugates	n.a.	n.a.	
3 - 500M24	0.005 (TTC)	n.a.	Metabolite only found in wheat metabolism study (grain)
3 - 500M49	0.005 (TTC)	max. 0.2% (poultry liver)	
3 - 500M51	0.005 (TTC)	max. 0.2% (bovine kidney)	
4 - Hydroxylated metabolites (chlorphenyl pyrazole moiety)	0.03	< 1% (milk)	
5 - Hydroxylated metabolites (tolyl moiety)	0.03	< 0.5% (milk)	

The data demonstrate that the contributions of the individual metabolites to the dietary risk at realistic dose levels are considerably lower than for parent.

The proposal below is based on the indicative assessment, but also takes the findings of the cow feeding study into account, where residues of 500M04 and 500M85 were found in liver (realistic feed burden), milk and kidney (10x to 20x).

Accordingly, the following residue definition is proposed for risk assessment in animal commodities:

Pyraclostrobin and its metabolites analysed as the hydroxypyrazoles 500M04 (synonym BF 500-5) and 500M85 (synonym BF 500-8), sum expressed as pyraclostrobin

7.4 Summary of residue trials in plants and identification of critical GAP

Pyraclostrobin is registered in multiple crops belonging to different EU crop groups. Within this dossier, residue data are only provided for the representative uses supporting the renewal process. As formulations the solo EC formulation BAS 500 06 F and the mixed WG formulation BAS 516 07 F (containing pyraclostrobin and boscalid) were selected.

In this dossier section the relevant data for the following crops are summarized:

- Potatoes (BAS 516 07 F)
- Wheat, barley (BAS 500 06 F)
- Maize (BAS 500 06 F)

According to the European Commission guidance on extrapolation and group tolerances (SANCO 7525/VI/95-rev 9, March 2011), the data provided allow extrapolation to other crops such as triticale, rye and oats.

The studies provided for potatoes and cereals in this dossier have been submitted before in context of national or zonal registration dossiers. They have not been evaluated within the MRL re-evaluation process according to Reg. 396/2005, Art. 12. Consequently, they are not considered as peer-reviewed. In case of maize and sweet corn and driven by the high importance of maize silage as feed item, parts of the studies had been provided to EFSA in context of the MRL evaluation process. The studies, which had been assessed before, are clearly indicated so that a differentiation between “old” and new data should be possible.

In chapter M-CA 6.7 the residue levels found in samples destined for human food or for animal feed are compared with the data already being evaluated by EFSA. For the desmethoxy metabolite, the codes 500M07 and BF 500-3 are used synonymously.

7.4.1 Target crops

7.4.1.1 Root and tuber vegetables: Potatoes

The formulation BAS 516 07 F is registered in the Northern part of the EU. Due to the favorable residue behavior in potatoes with residues consistently below the LOQ of 0.02 mg/kg at all sampling intervals, only a reduced number of residue data is required.

In order to allow a more comprehensive evaluation in potatoes, the residue trials with the formulation BAS 536 01 F are additionally summarized. As shown in the table below, the mixed formulation containing pyraclostrobin and dimethomorph is registered at higher rates (100 g BAS 500 F / ha compared to 17 g BAS 500 F / ha) and also in more countries. The results obtained confirm the favorable residue behavior since even at the higher rates the residues found in tuber were well below the LOQ of 0.02 mg/kg.

Table 7.4.1.1-1: Summary of the critical GAP for the proposed use in potatoes for BAS 516 07 F

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum Number of Applications	Minimum Application Interval (days)	Maximum		Minimum PHI (days)
					Rate* (kg as/ha)	Water (L/ha)	
Potatoes (N-EU)	O	41 – 89	4	5 - 10	0.017	150-1000	0-3

* amount of pyraclostrobin per ha and application

Table 7.4.1.1-2: Summary of the critical GAP for the proposed use in potatoes for BAS 536 01 F (supplemental information)

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum Number of Applications	Minimum Application Interval (days)	Maximum		Minimum PHI (days)
					Rate* (kg as/ha)	Water (L/ha)	
Potatoes (N-EU)	O	10 – 89	3	5	0.100	100-400	7
Potatoes (S-EU)	O	10 - 89	3	5	0.100	100-1000	3

* amount of pyraclostrobin per ha and application

In 2003, in total six trials were performed with the formulation BAS 516 00 F. In these trials, an application rate of 20 g as/ha was applied 4 times.

In the growing season 2005, in total ten field trials were performed in Northern Europe (Germany, Belgium, Denmark and Northern France) and Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulation BAS 536 01 F according to the critical GAP of this formulation (± 25%).

In the growing season 2015, in total four field trials were performed in Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulation BAS 516 07 F according to the critical GAP of this formulation (± 25%).

For deriving a MRL proposal, the more critical GAP which is applied with BAS 536 01 F was considered.

Northern Europe (n=5): 5 x <0.02 mg/kg

Southern Europe (n=5): 5 x <0.02 mg/kg

In none of the trials any detectable residue above the limit of quantitation (LOQ) of 0.02 mg/kg was found.

7.4.1.2 Cereals

Multiple residue studies had been performed in cereals for the active substance pyraclostrobin using solo and mixed formulations. The residue data were evaluated during the previous Annex I inclusion process, various national submissions, but also in context of the EU MRL re-evaluation according to Reg. 396/055 Art. 12. They were considered as suitable for establishing an EU MRL for wheat (including triticale), rye, barley and oats. Between 1998 and 2005 more than 70 trials were conducted in representative growing areas of the Northern and Southern part of Europe. In the trials, two applications were performed at 250 g as/ha.

Despite the broad coverage, new residue trials in cereals have been performed between 2009 and 2012. The purpose of these trials was to provide a consistent residue data package for the formulation BAS 500 06 F, but also to ensure that the residue levels in grain are covered by the established EU MRLs. The trials were performed in accordance with the most critical registered GAP (see table below).

Table 7.4.1.2-1: Summary of the critical GAP for the proposed use in cereals for BAS 500 06 F

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum Number of Applications	Minimum Application Interval (days)	Maximum		Minimum PHI (days)**
					Rate* (kg as/ha)	Water (L/ha)	
Barley	O	25 - 69	2	21	0.25	100 – 400	35
Oats	O	25 - 69	2	21	0.25	100 – 400	35
Wheat	O	25 - 69	2	21	0.25	100 – 400	35
Triticale	O	25 - 69	2	21	0.25	100 – 400	35
Rye	O	25 - 69	2	21	0.25	100 – 400	35

* amount of pyraclostrobin per ha and application
** for cereal crops the actual preharvest interval is defined by the latest growth stage of application (which is BBCH 69)

Cereals: Barley

In the growing seasons 2009 - 2012, in total 28 barley field trials comprising 36 plots were performed in Northern Europe (Germany, The Netherlands, Denmark, Northern France and United Kingdom) and Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulations BAS 500 06 F, BAS 702 01 F and BAS 703 04 F according to the critical GAP of formulation BAS 500 06 F (± 25%).

The following parent compound residues were found in barley grain samples at a PHI of 35 days or later:

Northern Europe (n=18): 0.02, 0.039, 0.043, 0.056, 0.06, 0.065, 0.07 (2x), 0.08, 0.09 (2x), 0.10, 0.11, 0.21, 0.22, 0.25, 0.35, 0.82 mg/kg

Southern Europe (n=18): <0.01 (3x), 0.02 (2x), 0.029, 0.03 (3x), 0.039, 0.06, 0.09, 0.11, 0.13, 0.14, 0.15, 0.27, 0.54 mg/kg

Cereals: Wheat

In the growing seasons 2010 - 2012, in total 24 wheat field trials comprising 28 plots were performed in Northern Europe (Germany, The Netherlands, Denmark, Northern France and United Kingdom) and Southern Europe (Italy, Spain, Greece and Southern France). The trials were conducted with formulations BAS 500 06 F and BAS 703 04 F according to the critical GAP of formulation BAS 500 06 F (± 25%).

The following parent compound residues were found in wheat grain samples at a PHI of 35 days or later:

Northern Europe (n=14): <0.01 (7x), 0.016, 0.02 (3x), 0.03, 0.039, 0.14 mg/kg

Southern Europe (n=14): <0.01 (7x), 0.01 (2x), 0.011, 0.015, 0.017, 0.02 (2x) mg/kg

7.4.1.3 Maize

The use in maize was not part of the previous Annex I inclusion process. It was submitted for the first time in Europe in summer 2010. The dRR was intended to achieve the registration of the mixed formulation BAS 512 16 F (containing the active ingredients pyraclostrobin and epoxiconazole) in maize. Subsequently to this submission, further draft Registration Reports were provided to countries acting as zonal RMS. The trials supporting a registration of the solo formulation BAS 500 06 F were partly included since the trials were performed side-by-side at the same locations.

After submission, Germany has prepared an evaluation report on the MRLs for maize, which was provided to EFSA in summer 2011. Based on the evaluation report EFSA has published a Reasoned Opinion (Feb 2012), in which the use in maize and its impact on the dietary feed burden calculations were investigated.

In order to allow a comprehensive evaluation of the formulation BAS 500 06 F, the old and the new data are summarized in M-CA 6.3.3.

Table 7.4.1.3-1: Summary of the critical GAP for the proposed use in maize and sweet corn for BAS 500 06 F

Crop	Outdoor/ Protected	Growth stage (BBCH)	Maximum Number of Applications	Minimum Application Interval (days)	Maximum		Minimum PHI (days)
					Rate (kg as/ha)	Water (L/ha)	
Maize	O	65	1	n.a.	0.2	100 - 400	F
Sweet corn	O	65	1	n.a.	0.2	100 - 400	F

n.a. = not applicable
F = defined by application at last growth stage

In the period from 2008 to 2009, in total 16 residue trials were performed in maize in the EU; 8 trials were located in the Northern Region and 8 in the Southern Region. The formulations BAS 512 04 F (133 g/L pyraclostrobin and 50 g/L epoxiconazole, SE) and BAS 500 06 F (200 g/L of pyraclostrobin, EC) were applied once at a rate of 1.5 L/ha and 1 L/ha, respectively, according to GAP ± 25%. Maize grain was collected at crop maturity (BBCH 89) corresponding to 49 - 99 DALA.

Additionally, in the growing seasons 2012 and 2013, five trials were performed in Northern Europe (Germany, The Netherlands, United Kingdom, Northern France and Belgium) and four were performed in Southern Europe (Italy, Spain, Greece and Southern France). In these trials, formulation BAS 500 06 F was applied according to the critical GAP. Maize grain was collected at crop maturity (BBCH 89) corresponding to 34 - 85 DALA.

The trials fulfill the requirements concerning the geographical and the seasonal distribution. The evaluation of the residue data led to the conclusion that there is no significant difference of the residue behavior regarding different seasons, formulations or regions in North and South EU.

In none of the trials any detectable residue above the limit of quantitation (LOQ) was found.

Sweet corn, barley straw, maize (as feed item) and wheat straw
For all these commodities sufficient residue values were determined and MRLs or pseudo-MRLs could be calculated. The collected relevant residue values and MRL calculations can be found in chapter M-CA 6.7.2.

7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

Poultry

A hen feeding study was performed for registration purposes in the US. In the study hens were dosed at three different dose levels (0.3, 0.9 and 3.0 mg/kg feed) corresponding to approximately 0.5x, 1.5x and 5x of the expected maximum EU feed burden. The samples were analysed using the common moiety approach which covers parent type metabolites and metabolites hydroxylated in the chlorophenyl ring system. The study is only considered as supplemental information as the results from the hen metabolism study indicate a low likelihood of any detectable residues in hen tissues or eggs. The outcome of the study confirmed the conclusions drawn from the metabolism study. Even at the highest dose level (3.0 mg/kg), pyraclostrobin total residues were below the level of quantitation of the common moiety approach applied (LOQ, 0.05 mg/kg per analyte). As the parent molecule is fully covered by the common moiety approach, the residues of pyraclostrobin in hen tissues and eggs were also <0.05 mg/kg. Based on these results, the samples from the 0.5x, 1.5x and depuration groups were not further analyzed.

Ruminants

A residue transfer study with BAS 500 F was conducted in cows. The animals were dosed with 7, 21 and 70 mg/kg feed (dry matter) equal to 140, 420 and 1400 mg/animal and day for a period of 28 days. The samples were analyzed with two methods.

- The analysis according to the single analyte method covering exclusively pyraclostrobin (as proposed for enforcement purposes), did not result in any detectable residue even at the 10 x dose.
- Analysis was also performed according to the common moiety approach, which corresponds to the proposed residue definition for risk assessment. In the dose group relevant under normal agricultural conditions (7 mg/kg feed), no residues could be detected in milk, meat, fat and kidney. Low residues of BAS 500 F metabolites may occur in liver. In the 10 x group (70 mg/kg feed) residues could be detected; the highest levels occurred in liver. The hydroxylated metabolites (pyrazole ring system) formed the major part of residues.

Pig

In case of pyraclostrobin, the metabolism in rats and ruminants are similar. Therefore, no pig feeding study is required.

Fish

According to the Commission Regulations (EU) No 283/2013 (active substances) and 284/2013 (plant protection products) as of 1 March 2013, metabolism studies on fish and fish feeding studies might be required in future (latest by 31 Dec 2015), if residues occur in crops that are intended as feed items for fish. As pyraclostrobin is intended to be used in several crops being fed to fish, a fish metabolism study was performed. The study is reported in section M-CA 6.2. It is considered as suitable for deriving a MRL proposal, despite MRLs will not be set in close future for fish matrices. Due to this fact, but also driven by the lack of any suitable EU guideline / guidance document for the conduct of fish feeding studies, the study is not required.

7.6 Summary of effects of processing

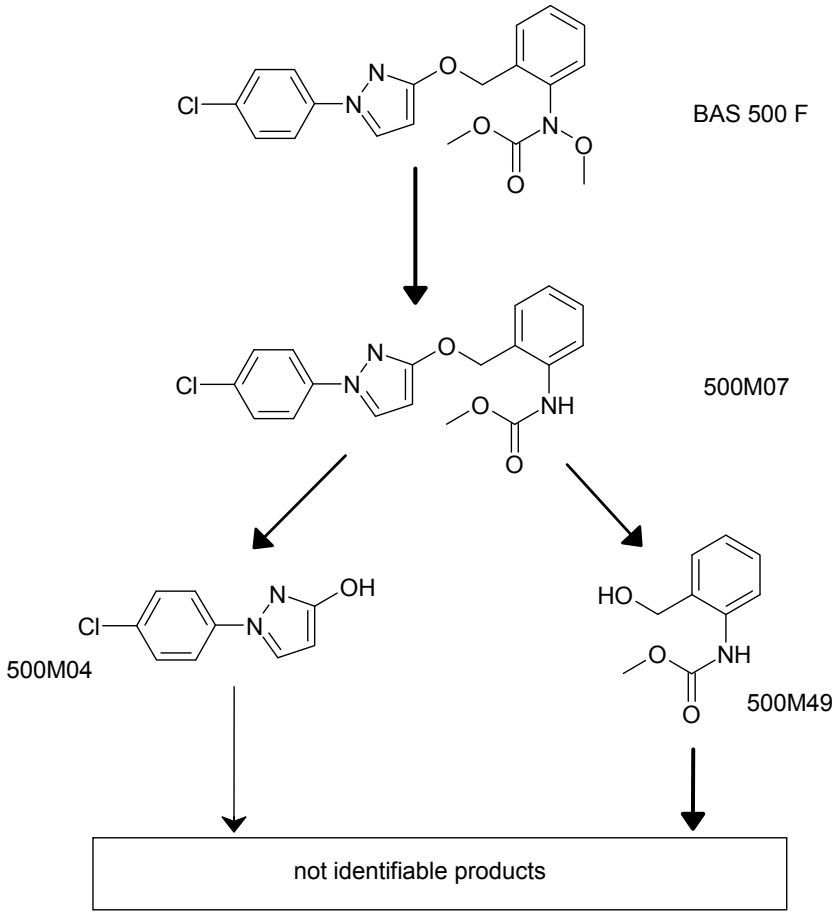
7.6.1 Nature of residues

During the registration according to Directive 91/414/EEC, a study was conducted simulating representative hydrolytic conditions for pasteurization (20 minutes at 90°C, pH 4), boiling/brewing/baking (60 minutes at 100°C, pH 5) and sterilization (20 minutes at 120°C, pH 6). This study, which was evaluated during the previous Annex I inclusion process, demonstrates that food processes such as brewing, cooking, sterilization or pasteurization, will not impact the nature of pyraclostrobin residues. Pyraclostrobin was found to be stable under the test conditions applied.

Upon request of Italy, a ^{14}C study was performed simulating processing conditions of oil purification (deodorization step from raw to refined oil). The hydrolytic degradation of pyraclostrobin was investigated at high temperatures in an olive oil / water mixture to simulate the process of olive oil raffination (deodorization step from raw oil to refined oil). The study was performed with ^{14}C-pyraclostrobin labeled at the chlorophenyl and at the tolyl ring. The test substance was applied into a mixture of olive oil and aqueous sodium chloride solution (in a ratio of 2:1) at a rate of 1 mg/mL and heated up to 190°C ± 5°C and 240°C ± 5°C for 30 minutes. After the incubation, the mixture was analyzed for the parent compound and possible transformation products. Phases and extracts of them were analyzed by LSC, HPLC-MS/MS and radio TLC.

In the study, an extensive degradation of the test item was observed at both temperatures. In almost all samples the same metabolites were found in different compositions. Hydrolytic degradation of the parent compound at 190°C resulted in the formation of the metabolite 500M07, which was then degraded to 500M49 and 500M04. At 240°C further degradation occurred. Parent and the metabolite 500M07 were only found in minor amounts. While for the chlorophenyl label the metabolite 500M04 was the major component, for the tolyl label most of the radioactivity present could be only characterized. Intensive characterization attempts were undertaken using different enzymes (lipase, pepsin, pancreatin), but also chromatographic techniques. They did not indicate the presence of any individual or distinct metabolite. The metabolite 500M07 was still the most abundant peak, which could be identified, followed by metabolite 500M49.

Figure 7.6.1-1: Proposed metabolic pathway of pyraclostrobin during oil processing



The results of this study were considered for proposing suitable residue definitions in plant matrices.

7.6.2 Magnitude of residues

During the past 15 years, multiple processing studies have been performed with pyraclostrobin containing products covering industrial production, but also household preparations. For the crops below, processing studies exist. The information on the processing steps covered is limited to those steps being considered as relevant for human consumption.

Table 7.6.2-1: Overview on available processing studies supporting registered use of pyraclostrobin containing formulations

EU Crop Group	Crops	Processing steps covered
Citrus fruits	Oranges	Peeling, canning, juice and marmalade production
Pome fruits	Apples	Washing, juice and sauce production
Stone fruits	Cherries	Washing, canning, juice and fruit syrup production
	Plums	Washing, puree and prune production
Berries and small fruits	Grapes	Wine, juice, raisins
	Strawberries	Washing, canning, jam, fruit syrup and distillate production
	Currants	Washing, canning, juice, jam and jelly production
Root and tuber vegetables	Carrots	Washing, peeling, cooking, juice production, canning
Fruiting vegetables	Tomatoes	Washing, canning, juice, puree and paste production
	Melons	Peeling
Brassica vegetables	Cabbage	Washing, cooking, Sauerkraut production, juice production
Leaf vegetables and fresh herbs	Lettuce, spinach	Washing, blanching, cooking
Legume vegetables	Peas	Washing, canning
Oilseeds and oilfruits	Soybean, oilseed rape, cotton, sunflower	Oil production (raw, refined)
Cereals	Barley	Pot barley, beer production
	Wheat	Flour, bran and germ production
Tea, coffee, herbal infusions and cocoa	Coffee	Roasting, brewing of coffee
Hops	Hops	Beer production
Sugar plants	Sugar cane	Sugar production (brown, refined)

Some of the studies were conducted according to the EU guidance document whereas the investigations performed in the USA are following US EPA OPPTS guidelines. The more recent studies are fulfilling the relevant OECD GD and GL.

The majority of processing factors is included in the data bases of the German BfR and Dutch RIVM (BfR webpage: BfR compilation of processing factors for pesticide residues (October 2011), http://chemkap.rivm.nl/groente-fruit/processing-factors/). In almost all studies, pyraclostrobin residues did not show any accumulation in food destined for human consumption. Exceptions are prunes and raisins, which can be explained by loss of water.

New processing studies are submitted in this dossier covering the intended uses in wheat, oats and maize. The results of these studies confirmed the previous findings with no or low indication for accumulation of residues in food destined for human consumption.

Wheat:
Pyraclostrobin was concentrated in wilted silage, bran, middlings, shorts and gluten, whereas the metabolite 500M07 (BF 500-3) was concentrated in wet silage, wilted silage, bran, germ, middlings, shorts, gluten and whole meal flour. The concentration is most likely based on the weight ratio and different water contents between RAC and processing fractions. In all further matrices the residues declined during processing.

Oats:
In husks and dust, the residue levels of pyraclostrobin and its metabolite 500M07 (BF 500-3) indicated concentration (pyraclostrobin: TF 2.34 and 5.23, 500M07: TF 2.42 and 5.45), whereas in all further matrices the residues declined during processing.

Maize:
The concentration factors for residues of pyraclostrobin and its metabolite 500M07 (BF 500-3) from maize grain to its processed fractions were 1, indicating that residues did not concentrate. Residues in the RAC grain samples were ≤ LOQ.

7.7 Summary of residues in rotational crops

7.7.1 Nature of residues in rotational crops

A confined rotational crop study was evaluated during the previous Annex I inclusion process. The metabolism of pyraclostrobin in rotational crops was studied in lettuce, radish and wheat after application of [tolyl-U-14C]-pyraclostrobin and [chlorophenyl-U-14C]-pyraclostrobin. The radiolabelled active substance was applied once to bare soil at an application rate of 0.9 kg a.s./ha; the respective crops were sown or planted at 30, 120 and 365 DAT.
Based on the results of the study, no accumulation of pyraclostrobin or its degradation products in the parts of plants used for human or animal consumption was observed. For succeeding crops, the proposed metabolic pathway involves desmethoxylation, a further degradation to various medium polar and polar metabolites and afterwards, most likely, conjugation reactions and final incorporation and/ or association into natural products, such as starch, cellulose and/ or lignins. The metabolic pathway of pyraclostrobin in rotational crops is similar to that in primary crops, and no formation of any other metabolite was observed.

In order to prove that no metabolite consisting solely of the pyrazole moiety is formed in soil (see also M-CA 7) and taken up by plants, preliminary investigations with the ^{14}C-pyrazole labelled pyraclostrobin were performed. ^{14}C-pyrazole labelled pyraclostrobin was applied at the maximum seasonal rate of 500 g as/ha to bare soil; crops were planted / sown after an aging period of 30 DAT. The use rate of 500 g as/ha corresponds to a realistic worst case GAP for Europe (2 x 250 g as/ha in cereal crops).

In all rotational crop matrices (radish, wheat, lettuce) low levels of radioactive residues were determined. The calculated Total Radioactive Residues (TRR) in radish leaf accounted for 0.010 mg/kg, for radish root 0.003 mg/kg, for wheat forage 0.014 mg/kg and for lettuce plant 0.016 mg/kg. Despite the low levels confirming the previous findings of the full guideline study, further investigations (extraction, partition, chromatographic analysis) were performed.
HPLC analysis of the concentrated water phases of the methanol extracts of radish leaf, wheat forage and lettuce plant resulted in a metabolite pattern of one polar peak at a retention time of approximately 4.3 min and three peaks with medium polarity (at approximately 20.2 min, 21.7 min and 22.8 min). An assignment of the peaks to structures was not possible due to the low levels of radioactive residues. All peaks were below 0.01 mg/kg. The Residual Radioactive Residues (RRR) were also low with values ranging from 0.002 to 0.006 mg/kg.

The results of both studies indicate that there was no significant translocation of pyraclostrobin and/or its degradation products from soil.

7.7.2 Magnitude of residues in rotational crops

According to Reg. 283/2013, studies on the magnitude of residues in rotational crop are required under the following circumstances:

If the metabolism studies indicate that residues of the active substance or of relevant metabolites or breakdown products either from plant or soil metabolism may occur (> 0.01 mg/kg), limited field studies and, if necessary, field trials shall be carried out.

Studies shall not be required in the following cases:
- no metabolism studies on rotational crops are to be performed, or
- metabolism studies on rotational crops show that no residues of concern are to be expected in rotational crops

Due to the favorable residue behavior of pyraclostrobin in succeeding crops, which has been demonstrated in two studies with different ring labels and under worst case conditions (bare soil application with partly exaggerated rates), no residues are expected. Consequently, no higher tier study is required.

7.8 Summary of other studies

Not relevant as no supplemental studies are provided for pyraclostrobin and its intended uses in potatoes, cereals and maize.

7.9 Estimation of the potential and actual exposure through diet and other sources

Chronic and acute assessments were conducted for parent pyraclostrobin and the metabolites of concern.

7.9.1 Chronic exposure

7.9.1.1 Pyraclostrobin (parent)

With the current EFSA model the chronic risk assessment ranges from 10 to 77% of ADI. The diet with the highest TMDI is "DE child" with 77% of ADI. For this diet, the highest contributors are oranges with 25.4% of ADI. The diet with the second highest TMDI is “NL child” with 60.1% of ADI, in which also oranges are the major contributor with 20.8% of ADI.

In order to check the contribution of fish, a dietary exposure assessment was performed using the German consumption data as published in 2012. Besides the child, the revised spreadsheet contains two additional consumer groups (total population: age group 14 – 80 years, females in child bearing age: 14 – 50 years).

Three assessments are performed. In accordance with the EFSA PRIMo model, the % ADI utilizations are based on mean body weights.

- In the first assessment, the same input values as for PRIMo were entered into the calculation.
- In the second assessment, the contribution of fish (at 0.05 mg/kg) was investigated.
- In the third assessment, the overall assessment was performed.

The NEDI calculation showed the low contribution of fish commodities to the chronic dietary risk for pyraclostrobin. The data clearly demonstrates that the presence of pyraclostrobin in fish does not result in any consumer risk. Compared to all other commodities, the contribution of fish to the chronic exposure is even under worst case assumptions rather small.

Table 7.9.1.1-1: NEDI calculations using the German model (NVS2_V_0-9_DE1)

Subpopulation group	% ADI utilization based on current MRLs	% ADI utilization fish commodities	% ADI utilization (total)
German child (VELS)	77	0.1	77.1
General population	36.8	0.1	36.9
Women in child bearing age	39.2	0.1	39.3

7.9.1.2 Metabolites

Metabolites identified in crop and livestock metabolism studies

For performing indicative assessments, the metabolites were grouped according to sub-structures (see M-CA 6.9). In a second step, the exposure was estimated based on all available data (combination of metabolism information and data from residue field trials / feeding studies). Subsequently, chronic dietary exposure assessments were performed for identifying the contributions of the metabolites to the total dietary risk.

Desmethoxy metabolite 500M07:
With the current EFSA model the chronic risk assessment ranges from 0.3 to 1.7% of ADI. The diet with the highest TMDI is "WHO Cluster Diet B" with 1.7% of ADI. For this diet, the highest contributor is wheat with 0.6% of ADI. The diet with the second highest TMDI is “DE child” with 1.7% of ADI, in which pome fruits are the major contributor with 0.8% of ADI.

Chlorophenyl pyrazole derivatives (500M04 and conjugates, 500M85):
With the current EFSA model the chronic risk leads to no values above 0.0% of ADI.

Anthranilic acid derivative 500M24:
With the current EFSA model the chronic risk assessment ranges from 0 to 8.9% of ADI. The diet with the highest TMDI is "IE Adult" with 8.9% of ADI. For this diet, the highest contributor is barley with 8.3% of ADI. The diet with the second highest TMDI is “WHO Cluster Diet E” with 6.3% of ADI, in which also barley is the major contributor with 5.4% of ADI.

Anthranilic acid derivative 500M49:
With the current EFSA model the chronic risk assessment ranges from 0.0 to 0.1% of ADI. The diet with the highest TMDI is "WHO Cluster Diet E" with 0.1% of ADI. For this diet, the highest contributor is rape seed with 0.1% of ADI. The diet with the second highest TMDI is “WHO Cluster Diet B” with 0.1% of ADI, in which sunflower seed is the major contributor with 0.1% of ADI.

Anthranilic acid derivative 500M51:
The estimation of the metabolite in food of animal origin resulted in median residue levels far below 0.001 mg/kg. Due to negligible exposure, no chronic exposure assessment was performed.

Hydroxylated metabolites (chlorphenyl pyrazole moiety):
With the current EFSA model the chronic risk assessment ranges from 0.1 to 0.6% of ADI. The diet with the highest TMDI is "DE child" with 0.6% of ADI. For this diet, the highest contributors are pome fruits with 0.3% of ADI. The diet with the second highest TMDI is “NL child” with 0.5% of ADI, in which also pome fruits are the major contributor with 0.2% of ADI.

Hydroxylated metabolites (tolyl moiety):
With the current EFSA model the chronic risk assessment ranges from 0.1 to 0.6% of ADI. The diet with the highest TMDI is "DE child" with 0.6% of ADI. For this diet, the highest contributors are pome fruits with 0.3% of ADI. The diet with the second highest TMDI is “WHO Cluster Diet B” with 0.4% of ADI, in which wine grapes are the major contributor with 0.2% of ADI.

Photo metabolite 500M76:
With the current EFSA model the chronic risk assessment ranges from 0 to 0.2% of ADI. The diet with the highest TMDI is "WHO Cluster Diet B" with 0.2% of ADI. For this diet, the highest contributor is lettuce with 0.1% of ADI. The diet with the second highest TMDI is “FR toddler” with 0.2% of ADI, in which leek is the major contributor with 0.1% of ADI.

The exposure estimates for metabolites applying worst case assumptions (for vegetables: no consideration of processing) did not indicate any dietary concern. The calculation of the % ADI utilizations resulted in values far below 100% and – even more important - clearly below the ones of the parent molecule. No significant contribution to the chronic exposure can be expected from any plant or livestock metabolite.

Metabolites identified in the *in-vitro* comparison study

Based on the study results of an *in-vitro* comparison study with cell cultures from different species, it cannot be excluded that humans might be exposed to metabolites 500M02, 500M106 and 500M107 (glucuronic acid conjugate of 500M106) after consumption of food containing pyraclostrobin residues. For the time being no guidance document exists how to perform a dietary risk assessment for such metabolites. However, as the metabolite 500M106 showed a considerable lower toxicity in the 28-day rat study compared to parent, no separate dietary risk assessment should be needed. The assessment is covered by the chronic exposure estimate provided for the parent molecule.

7.9.2 Acute exposure

7.9.2.1 Pyraclostrobin (parent)

The evaluation resulted in ARfD utilizations well below 100% for all representative crops. The highest utilization using default variability factors was calculated for sweet corn (17.1 %).
In the context of this dossier, MRLs for fish are proposed for the first time. In order to check the contribution of fish to acute exposure, an indicative assessment has been performed. The proposed MRL of 0.05 mg/kg was entered into the German model as it includes the relevant consumption data. The maximum % ARfD utilizations are comparable for all subpopulation groups (child, total population, women in child bearing age); maximum ARfD utilizations of about 4.5% are reached for shell fish and freshwater fish, respectively.

7.9.2.2 Metabolites

Metabolites identified in crop and livestock metabolism studies

The acute dietary exposure was also assessed for the metabolites following the same grouping principles.

Desmethoxy metabolite 500M07:
For the assessment, an ARfD of 0.03 mg/kg bw/day was used. The calculation was done with default variability factors, except of pome fruits and table grapes in the IESTI2 calculation, for which a variability factor of 3 was applied (already accepted by EFSA, see EFSA Journal 2011;9(8):2344). The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for celery (29.1%), followed by onions (18.1%) and cucumbers (17.5%).

Anthranilic acid derivative 500M49:
For the assessment, an ARfD of 0.005 mg/kg bw/day was used. The calculation was done with default variability factors. As a result, no ARfD utilization above 0.0% was determined.

Anthranilic acid derivative 500M51:
For the assessment, an ARfD of 0.005 mg/kg bw/day was used. The calculation was done with the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for bovine kidney (0.2%), followed by swine kidney (0.1%).

Hydroxylated metabolites (chlorphenyl pyrazole moiety):
For the assessment, an ARfD of 0.03 mg/kg bw/day was used. The calculation was done with default variability factors, except of pome fruits and table grapes in the IESTI2 calculation, for which a variability factor of 3 was applied (already accepted by EFSA, see EFSA Journal 2011;9(8):2344). The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization (IESTI2) was observed for melons (8.1%), followed by watermelons (6.5%) and table grapes (5.2%).

Hydroxylated metabolites (tolyl moiety):
For the assessment, an ARfD of 0.03 mg/kg bw/day was used. The calculation was done with default variability factors, except of pome fruits and table grapes in the IESTI2 calculation, for which a variability factor of 3 was applied (already accepted by EFSA, see EFSA Journal 2011;9(8):2344). The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization (IESTI2) was observed for melons (8.1%), followed by kale (7.9%) and watermelons (6.5%).

Photo metabolite 500M76:
For the assessment, an ARfD of 0.005 mg/kg bw/day was used. The calculation was done with default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization (IESTI2) was observed for kale (10.6%), followed by Chinese cabbage (8.2%) and globe artichokes (6.3%).

The exposure estimates for metabolites applying worst case assumptions (for vegetables: no consideration of processing) did not indicate any dietary concern. The calculation of the % ARfD utilizations resulted in values far below 100% and – even more important - clearly below the ones of the parent molecule. No significant contribution to the acute exposure can be expected from any plant or livestock metabolite.

Metabolites identified in the *in-vitro* comparison study

Based on the study results from an *in-vitro* comparison study with cell cultures from different species, it cannot be excluded that humans might be exposed to the metabolites 500M02, 500M106 and 500M107 (glucuronic acid conjugate of 500M106) after consumption of food containing pyraclostrobin residues. As metabolite 500M106 showed a considerable lower acute toxicity compared to parent, no separate dietary risk assessment should be needed. The assessment is covered by the exposure estimates provided for the parent molecule.

7.10 Proposed MRLs and compliance with existing MRLs

7.10.1 Plant matrices

In the context of this dossier, the residue data summarized in M-CA 6.3 were compared with those being already evaluated by EFSA within previous submissions (EU MRL Regulation 396/2005, Art. 6-10 or Art. 12).

Potatoes
In none of the trials any detectable residue above the limit of quantitation (LOQ) was found.
As all residue levels in potato tubers were below the LOQ of 0.02 mg/kg, it is not needed to perform any statistical calculation to derive an MRL. Based on the findings of the residue trials it is proposed **to keep the established EU MRL** for potato tubers at the limit of quantitation of the enforcement method, which corresponds to 0.02 mg/kg.

Code number 0211000 (potatoes): 0.02 mg/kg (default MRL)

Barley
The data show, that the calculated MRL for pyraclostrobin in barley grain is **covered** by the current MRL of 1.0 mg/kg. And therefore BASF proposes **to keep the current EU MRL** of pyraclostrobin at 1.0 mg/kg for barley grain.

Code number 0500010 (barley): 1.0 mg/kg

Based on the extrapolation rules of the EU guidance document "Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs" (SANCO 7525/VI/95 rev. 9, March 2011), **the same MRL should apply** for oat grain:

Code number 0500050 (oats): 1.0 mg/kg

Barley straw (feed item)
The data show, that the calculated MRL for pyraclostrobin in barley straw is covered by the current *"pseudo MRL"* of 15 mg/kg (see MRL review for pyraclostrobin according to Article 12, EFSA Journal 2011;9(8):2344). Therefore, BASF proposes **to keep the current value** of pyraclostrobin at 15 mg/kg for barley and oat straw.

Sweet corn [extrapolation from maize "cobs without husks"]
The data show, that the calculated MRL for pyraclostrobin in sweet corn after application of formulation BAS 500 06 F is not covered by the current MRL of 0.02 mg/kg (default MRL). Therefore, BASF **proposes to establish an EU MRL** of pyraclostrobin at 0.07 mg/kg for sweet corn.

Code number 0234000 (sweet corn): 0.07 mg/kg

Maize
In none of the trials any detectable residue above the limit of quantitation (LOQ) was found.
As all residue levels in maize grain were below the LOQ of 0.01 mg/kg, it is not needed to perform any statistical calculation to derive an MRL.

Based on the findings of the residue trials **it is proposed to keep** the MRL for maize grain at the limit of quantitation of the enforcement method, which corresponds to 0.02 mg/kg. This MRL also covers potential imports of maize grain from the NAFTA region.

Code number 0500030 (maize): 0.02 mg/kg (default MRL)

Maize (feed item)
As a conclusion it is proposed **to keep the established pseudo MRL** for pyraclostrobin of 1.5 mg/kg in maize -feed items (silage), see EFSA Journal 2012;10(3):2606. The residue situation is very comparable in the Northern and in the Southern EU.

Wheat
The data show, that the calculated MRL for pyraclostrobin in wheat grain is covered by the current MRL of 0.2 mg/kg. Therefore, BASF proposes **to keep the current EU MRL** of pyraclostrobin at 0.2 mg/kg for wheat grain.

Code number 0500090 (wheat): 0.2 mg/kg

Based on the extrapolation rules of the EU guidance document "Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs" (SANCO 7525/VI/95 rev. 9, March 2011), **the same MRL should apply** for rye grain:

Code number 0500070 (rye): 0.2 mg/kg

Wheat straw (feed item)
The data show, that the calculated MRL for pyraclostrobin in wheat straw is covered by the current *"pseudo MRL"* of 10 mg/kg (see MRL review for pyraclostrobin according to Article 12, EFSA Journal 2011;9(8):2344). Therefore, BASF proposes **to keep the current value** of pyraclostrobin at 10 mg/kg for wheat and rye straw.

7.10.2 Animal matrices

In the context of this dossier, revised dietary feed burden calculations were conducted (see M-CA 6.7.2) using the EU and the OECD agreed methodology and the input values derived in this dossier. The following maximum feed burdens were derived for dairy ruminants, meat ruminants, poultry and pigs using the agreed EU methodology. As supplemental information the main contributor is also provided below.

Table 7.10.2-1: Summarised results of the livestock dietary burden calculations for dairy ruminants

Dietary burden of dairy ruminants	
Maximum dietary burden (mg/kg bw/d):	0.145683
Maximum dietary burden (mg/kg feed DM):	4.006279
Highest contributing commodity:	Barley straw

Table 7.10.2-2: Summarised results of the livestock dietary burden calculations for meat ruminants

Dietary burden of meat ruminants	
Maximum dietary burden (mg/kg bw/d):	0.296061
Maximum dietary burden (mg/kg feed DM):	6.908081
Highest contributing commodity:	Barley straw

Table 7.10.2-3: Summarised results of the livestock dietary burden calculations for poultry

Dietary burden of poultry	
Maximum dietary burden (mg/kg bw/d):	0.041119
Maximum dietary burden (mg/kg feed DM):	0.651047
Highest contributing commodity:	Barley grain

Table 7.10.2-4: Summarised results of the livestock dietary burden calculations for pigs

Dietary burden of pigs	
Maximum dietary burden (mg/kg bw/d):	0.047270
Maximum dietary burden (mg/kg feed DM):	1.181744
Highest contributing commodity:	Kale

The following results in the context of the feed burden calculations were derived for cattle, sheep, swine and poultry using the OECD methodology. For the time being, the calculation according to OECD is considered as supplemental information. In general, the results are in good accordance with the feed burden calculations according to EU.

Table 7.10.2-5: Summary of the results for RWCFL (EU)

	Cattle Beef	Cattle Dairy	Sheep Ram/Ewe	Sheep Lamb	Swine Breeding	Swine Finishing	Poultry Broiler	Poultry Layer	Poultry Turkey
Regions	EU	EU	EU	EU	EU	EU	EU	EU	EU
Body weight (kg)	500	650	75	40	260	100	1.7	1.9	7
Daily intake (kg DM)	12	25	2.5	1.7	6	3	0.12	0.13	0.5
Dietary burden (mg/kg bw)	**0.083**	**0.134**	**0.212**	**0.271**	**0.028**	**0.024**	**0.036**	**0.079**	**0.031**
Feed burden (mg/kg DM)	**3.461**	**3.483**	**6.365**	**6.365**	**1.225**	**0.798**	**0.515**	**1.149**	**0.435**

Based on these feed burden calculations, the livestock metabolism and feeding studies were carefully re-evaluated.

The established EU MRLs were reconfirmed.

0.05 mg/kg for all animal matrices (except milk)
0.01 mg/kg for milk

For fish, a preliminary proposal for a future EU MRL was derived based on the estimated feed burden and the results of the fish metabolism study (after oral dosing):

0.05 mg/kg for all fish commodities

The established and proposed MRLs for commodities being relevant in context of this dossier are summarized in the following table:

Table 7.10.2-6: Maximum residue levels for pyraclostrobin in the EU and changes proposed by BASF

Plant/animal commodity (Code number)	**Established EU MRL [mg/kg]**	**Proposed EU MRL [mg/kg]**
Potatoes (0211000)	0.02*	0.02*
Sweet corn (0234000)	0.02*	**0.07**
Barley (0500010)	1.0	1.0
Maize (0500030)	0.02*	0.02*
Oats (0500050)	1.0	1.0
Rye (0500070)	0.2	0.2
Wheat (0500090)	0.2	0.2
Products of animal origin-terrestrial animals, except milk (1000000)	0.05*	0.05*
Milk (1020000)	0.01*	0.01*
Fish, fish products, shell fish, molluscs and other marine and freshwater food products (1100000)	n.a.	**0.05**

* default MRL
n.a. not applicable
proposed MRL changes are highlighted in **bold**

7.11 Proposed import tolerances and compliance with existing import tolerances

The MRLs listed in M-CA 6.7.2 include domestic uses, but also values for imported crops. Prior to final approval, the import tolerances have been evaluated by Germany (RMS) and EFSA. Parts of them are resulting from the adoption of CODEX MRLs. In the meantime, pyraclostrobin and its crops have been assessed three times by JMPR.

8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

8.1 Summary of fate and behaviour in soil

Laboratory soil degradation studies

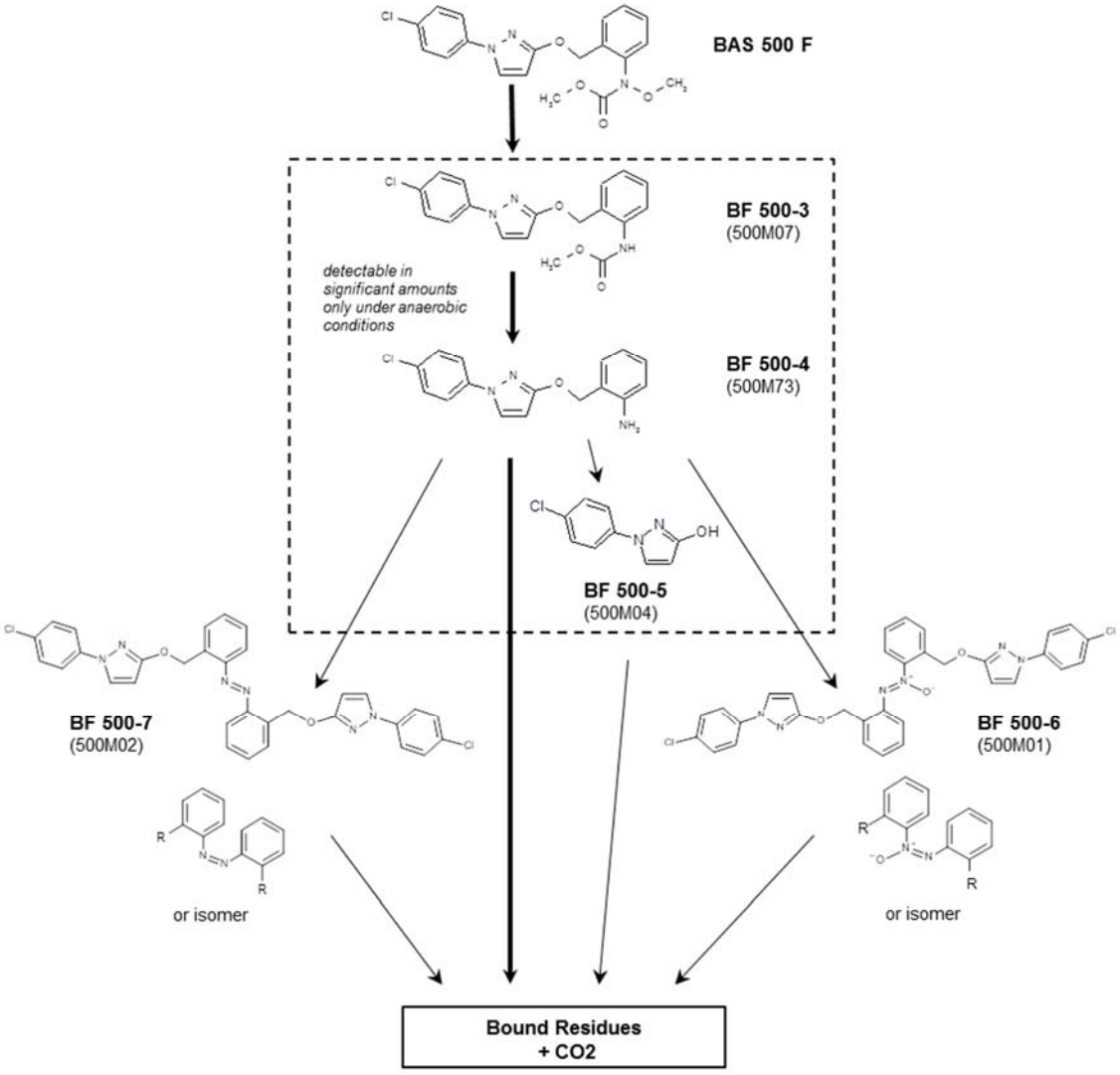
Route of degradation

The aerobic soil degradation of pyraclostrobin was evaluated in GLP laboratory studies conducted with either tolyl-, chlorophenyl-, or pyrazole-labelled test item. The degradation of pyraclostrobin occurs by stepwise degradation of the carbamate group via des-methoxylation (BF 500-3) to an anilinic intermediate (BF 500-4), which then reacts rather fast with organic (humic) substances in soil (major pathway) forming non-extractable residues up to ~56% TAR (total applied radioactivity) after 100 days. The anilinic intermediate can also react with each other (minor pathway) forming the dimeric metabolites BF 500-6 (max. 31% TAR) and BF 500-7 (max. 13% TAR). Cleavage of the pyraclostrobin molecule by formation of metabolite BF 500-5 is also observed, however, only to a very minor extent (~2.8% TAR). Mineralization is obtained with all three ^{14}C-ring labels (tolyl-label up to 7% TAR, chlorophenyl-label ~5% TAR, pyrazole-label ~2.2% TAR), indicating that the pyraclostrobin molecule is finally attacked at each ring system leading to degradation to CO_2.

Under anaerobic conditions, the degradation follows in principle the same pathway. Since oxygen demanding reactions are slowed down considerably, the des-methoxy metabolite BF 500-3 as well as the anilinic metabolite BF 500-4 are formed in higher amounts than under aerobic conditions. BF 500-3 reaches up to 96% TAR and BF 500-4 up to 11% TAR under water-logged and nitrogen-flooded conditions. BF 500-4, which is never detected in aerobic soil, showed some isomerization (500M74 max. 11.4 and 500M75 max. 7.3% TAR, respectively) in the anoxic water phase, which is however considered unlikely to occur under agricultural field conditions. Pyraclostrobin will not be sprayed on water-logged soils, and even in water-logged soil after heavy rain at least some oxygen is supposed to be present, which immediately would lead to BF 500-4 degradation.

Under photolytic conditions, the same metabolites are detected as under dark conditions. No special photo-degradates are formed.

Figure 8.1-1: Proposed route of pyraclostrobin degradation in soil



Rate of degradation

Using the data from both older and new studies, the laboratory $DegT_{50}$ values for pyraclostrobin and its metabolites were (re-)calculated according to the current guidance of the FOCUS workgroup on degradation kinetics (FOCUS 2006).

Pyraclostrobin non-normalized half-lives under laboratory conditions (i.e. persistence endpoints) ranged from 11 to 98 days in soils (n=10) incubated at 20ºC in the dark with soil moistures maintained at ~40-50% maximum water holding capacity.

The kinetic endpoints for metabolites BF 500-4, BF 500-5, BF 500-6 and BF 500-7 were derived either from the laboratory soil degradation studies with pyraclostrobin or from studies performed with the metabolites as test substance. The non-normalized laboratory $DegT_{50}$ values (i.e. persistence endpoints) were calculated to be 0.2 to 1.8 days for BF 500-4, 0.1 to 0.2 days for BF 500-5, 96 to 971 days for BF 500-6, and 81 to > 1000 days for BF 500-7. The transient metabolite BF 500-3 was also included in the kinetic evaluation of soil degradation studies with pyraclostrobin, however, reliable endpoints could not be derived.

Under anaerobic conditions, the $DegT_{50}$ values were calculated to be 1.5 - 2.3 days for pyraclostrobin, 57 - 70 days for BF 500-3, 8 - 11 days for BF 500-4, 37 - 65 days for isomer 500M74, 15 - 24 days for isomer 500M75 and 32 days for BF 500-5.

Field studies

Field soil dissipation

In the previous DAR considering studies conducted prior to 2000, dissipation of pyraclostrobin in soil under field conditions was shown to be rapid with non-normalized DT_{50} values from 6 trials ranging from 2 to 37 days. The 6 trials were distributed in Germany (3), Spain (2), and Sweden (1). Applications were made in spring at 250 g a.s. ha^{-1} on bare soil. Metabolites BF 500-3, BF 500-6 and BF 500-7 were, if at all, only sporadically observed close to the LOQ of 0.01 mg/kg.

A kinetic re-evaluation of the original 6 field dissipation datasets was conducted according to current FOCUS (2006) kinetics guidance to determine persistence (trigger) endpoints. The pyraclostrobin best-fit $DisT_{50}$ values ranged from 5 to 56 days and the corresponding $DisT_{90}$ ranged from 80 – 348 days. There was no dependence of pyraclostrobin dissipation on soil pH. No endpoints could kinetically be derived for the metabolites due to the low frequent occurrence. These results support that pyraclostrobin and its metabolites are not persistent under field conditions.

Field soil degradation

The old field soil dissipation study was analyzed following EFSA (2010) [*Guidance for evaluating laboratory and field dissipation studies to obtain DegT50 values of plant protection products in soil.* EFSA Journal 2010; 8(12):1936] in conjunction with FOCUS (2006) to obtain degradation rates in the soil matrix. A total of four sites could be analyzed with normalized $DegT_{50}$ values (20°C and pF2) that range from 15.5 – 32.2 days.

A new field soil degradation study was conducted in 2011-2012 following the EFSA (2010) opinion by excluding soil surface processes (e.g. volatilization, photolysis) by covering the soil with a 4 – 10 mm sand layer. The four trials were located in Denmark, Germany, Italy and France. The nominal application rate at each site was 250 g a.s. ha^{-1} on bare soil. Soil samples were analyzed for pyraclostrobin and the metabolites BF 500-3, BF 500-6 and BF 500-7. Pyraclostrobin residues were almost exclusively found in the top 0-10 cm soil layer (residues $\leq$ 0.004 mg kg^{-1}) and only sporadically detected in the 10-20 cm layer. Below 20 cm no residues above LOQ (0.001 mg kg^{-1}) were found. The metabolites BF 500-3, BF 500-6 and BF 500-7 and were detected at all sites. They were found above LOQ only in the top 0-20 cm soil layer.

Data were normalized to 20°C and pF2 moisture and evaluated according to FOCUS (2006) kinetics guidance. The $DegT_{50}$ values for pyraclostrobin ranged from 15 to 181 days. For the metabolites $DegT_{50}$ values ranged from 163 – 361 days for BF 500-6 and from 184 - 460 days for BF 500-7. No endpoints could be derived for metabolite BF 500-3.

Mobility in soil

Pyraclostrobin mobility in soil was evaluated using data from studies considered in the previous EU evaluation ($K_{f,oc}$, column leaching, aged soil column leaching). The $K_{f,oc}$ values determined in 3 EU, 2 US and 1 Canadian soil ranged from 6000 to 16000 mL g^{-1} (n = 6) with an arithmetic mean of 9304 mL g^{-1}. No radioactivity could be found in the leachates of the column and aged soil column leaching studies. Pyraclostrobin can thus be considered to be immobile in soil.

Adsorption isotherms were determined for metabolite BF 500-3 in 3 EU, 2 US and 1 Canadian soil during the previous EU evaluation. The $K_{f,oc}$ values ranged from 4240 - 12000 mL g^{-1} with an arithmetic mean of 9315 mL g^{-1}.

Adsorption values were determined for metabolite BF 500-6 in 3 EU, 2 US and 1 Canadian soil during the previous EU evaluation. Due to the extremely low water solubility, the adsorption could be determined for one concentration only. The K_{oc} values ranged from 3360 - 126800 mL g^{-1}. Since the reported recoveries of the test substance were partly very low, a new study was performed with 4 German soils and 1 Spanish soil. The K_{oc} values of this new study, providing an acceptable substance recovery, ranged from 107301 to 311704 mL g^{-1}. Since the second study confirmed the high values of the first, both data sets were combined resulting in a median K_{oc} of 107301 mL g^{-1}.

Adsorption values were determined for metabolite BF 500-7 in 3 EU, 2 US and 1 Canadian soil during the previous EU evaluation. Due to the extremely low water solubility, the adsorption could be determined for one concentration only. The K_{oc} values of ranged from 4020 - 149900 mL g^{-1}. Since the reported recoveries of the test substance were partly very low, a new study was performed with 4 German soils and 1 Spanish soil. The K_{oc} values of this study, providing an acceptable substance recovery, ranged from 242564 to 801927 mL g^{-1}. Since the second study confirmed the high values of the first, both data sets were combined resulting in a median K_{oc} of 149900 mL g^{-1}.

Adsorption values were determined for metabolite BF 500-4 in 4 German soils and 1 Spanish soil. Due to the extremely low water solubility, the adsorption could be determined for one concentration only. The K_{oc} values ranged from 6871 - 15748 mL g^{-1} with an arithmetic mean (n=5) of 9819 mL g^{-1}.

Adsorption isotherms were determined for metabolite BF 500-5 in 4 German soils and 1 Spanish soil. The K_{foc} values ranged from 400 - 831 mL g^{-1} with an arithmetic mean (n=5) of 705 mL g^{-1}.

The groundwater risk assessment, which considers conservative assumptions on sorption and degradation, supports that pyraclostrobin and its metabolites are unlikely to enter groundwater.

8.2 Summary of fate and behaviour in water and sediment

Pyraclostrobin was hydrolytically stable at pH 5 and 7. Only with pH 9, a very slight degradation could be observed.

In the aqueous photolysis study, pyraclostrobin degraded quickly to numerous breakdown and rearrangement products (in total 38 compounds identified or at least characterized by mass). The half-life of pyraclostrobin was calculated to be 0.06 days (when considering continuous irradiation).

According to a Manometric Respiratory Test (OECD 301), pyraclostrobin has to be considered as not readily biodegradable.

In a water/sediment study under dark conditions, no metabolites were formed in water, but a very fast binding to the sediment occurred. In sediment, the metabolite BF 500-3 was detected in amounts > 10% TAR and BF 500-6 and BF 500-7 were detected in amounts > 5% TAR. The degradation rates of pyraclostrobin were re-evaluated according to FOCUS (2006). The evaluation at P-I level resulted in $DisT_{50}$ values for the water of 0.4 - 2.3 days (2 labels, 2 systems). Reliable $DegT_{50}$ values for the total system could be obtained only from one w/s system and were calculated to be 23 - 27 days.

Since under outdoor conditions, photolysis as well as sediment adsorption are simultaneously influencing the degradation/dissipation of pyraclostrobin in water, already for the previous EU evaluation a higher tier study was performed simulating the light and temperature regime of the major application period for the active substance (May-July).

This study showed that besides about 15 minor degradation products, 3 photo-metabolites were formed in the water in amounts >10% TAR (BF 500-11, BF 500-13, and BF 500-14). In the sediment, only metabolite BF 500-3 was detected (max. 16.9% TAR). This study was performed without volatile collection and no material balance could be provided. Therefore, a new irradiated water/sediment study was initiated with an adapted test design (very similar to the OECD 308 guideline, but with irradiation), and with collection of volatiles to enable a full material balance.

The overall route of degradation in water/sediment under realistic outdoor conditions was confirmed by this new study. The metabolites BF 500-11, BF 500-13 and BF 500-14 were again detected, however, in much lower amounts than in the previous study (all < 6% TAR). In sediment, besides high amounts of non-extractable residues (max. 63 - 66% TAR after 42 days), the metabolites BF 500-3, BF 500-6 and BF 500-7 were formed in max. amounts of 6, 4 and 2% TAR, respectively. Mineralization to CO_2 amounted to 3 - 4% TAR after 42 days.

Reliable dissipation and degradation rates for pyraclostrobin were re-calculated according to FOCUS 2006. The SFO kinetic model (P-I level) revealed $DisT_{50}$ values of 4.5 and 5.9 days for water and sediment, respectively, and a $DegT_{50}$ of 7.2 days for the total system. Calculations at the P-II level resulted in $DegT_{50}$ values of 7.5 and 6.5 days for the water and the sediment compartment, respectively. Total system $DisT_{50}$ values could be calculated for metabolites BF 500-3, BF 500-11 and BF 500-14 reaching 93, 23 and 17 days, respectively.

All these results show that in case pyraclostrobin reaches the water, it quickly disappears from the water phase by fast photolytic reactions and by precipitation to the sediment and further binding to the organic matrix.

Aerobic mineralization of pyraclostrobin in surface water was tested with and without suspended sediment. Without suspended sediment, the degradation of pyraclostrobin was characterized by a low mineralization rate irrespective of test concentration or label position (< 1% TAR). With suspended sediment, the mineralization reached 2.8 - 4.6% TAR (2 labels, 2 test concentrations).

In the pelagic test without suspended sediment, pyraclostrobin degraded only very slowly. More than 78 - 97% TAR unchanged parent were still detectable in the water phase after 63 days. Only the des-methoxy metabolite BF 500-3 (max. 6.7% TAR) and the cleavage product BF 500-5 (max. 5.9 - 10.9% TAR) appeared in significant amounts. The $DegT_{50}$ of pyraclostrobin were 410 and 458 days (high and low test conc.) and 11 - 29 days for BF 500-3.

In the suspended sediment test, the behavior of pyraclostrobin was very similar to the dark water/sediment study. Pyraclostrobin quickly disappeared from the water phase with $DisT_{50}$ values of 1.7 - 2.3 days. Although the amount of sediment was only 1 g L^{-1}, 73 - 80% of the applied radioactivity was associated with the solid particles after 63 days and 47 - 58% could not be extracted with solvents. The $DegT_{50}$ (total system) was calculated with SFO kinetic to be 26 - 28 days.

8.3 Summary of fate and behaviour in air

Pyraclostrobin is characterized by a low vapor pressure (2.6 x 10^{-8} Pa at 20°C) and a low volatilization from soil and plant surfaces (< 3% in 24h). The photochemical oxidative degradation in air (Atkinson) was calculated to be 0.62 hours, indicating that pyraclostrobin is rapidly degraded in air. Consequently, there is no risk of long-range transport of pyraclostrobin.

8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

No relevant monitoring data are available.

8.5 Definition of the residues in the environment requiring further assessment

According to the results presented in M-CA 7.1 – 7.3 the following compounds have to be considered for the environmental risk assessment:

Soil:	pyraclostrobin, BF 500-6, BF 500-7
Ground Water:	pyraclostrobin, BF 500-3, BF 500-4, BF 500-5, BF 500-6, BF 500-7
Surface Water:	pyraclostrobin, BF 500-5, BF 500-11, BF 500-13, BF 500-14
Sediment:	pyraclostrobin, BF 500-3, BF 500-6, BF 500-7
Air:	pyraclostrobin

For monitoring purposes, the residue definition is parent only.

8.6 Summary of exposure calculations and product assessment

Soil:

PEC_{soil} following application of BAS 500 06 F (to cereals and maize) and BAS 516 07 F (to potatoes) were calculated for worst-case application scenarios. The calculations were carried out according to the recommendations of the FOCUS working group on degradation kinetics, assuming a soil bulk density of 1.5 g cm^{-3} and a soil layer depth of 5 cm. Maximum, actual and time-weighted average concentrations in soil ($PEC_{soil,max}$, $PEC_{soil,act}$, $PEC_{soil,twa}$) were calculated for pyraclostrobin and its soil metabolites BF 500-6 and BF 500-7. Additionally, the plateau concentration of BF 500-6 and BF 500-7 in soil at steady state ($PEC_{soil,plateau}$) and the overall accumulation PEC in soil ($PEC_{soil,accu}$) after application of the parent pyraclostrobin over many years, was calculated.

Results can be found in CP 9.1.3/2.

Groundwater:

Predicted environmental concentrations in groundwater (PEC_{gw}) of pyraclostrobin, its aerobic soil metabolites BF 500-6 and BF 500-7 and its anaerobic soil metabolites BF 500-3, BF 500-4 and BF 500-5 following application of BAS 500 06 F (to cereals and maize) and BAS 516 07 F (to potatoes) were calculated in accordance with the guidance of the FOCUS groundwater scenarios work group.

PEC_{gw} of pyraclostrobin and its aerobic and anaerobic soil metabolites were calculated with the model FOCUS-PEARL 4.4.4 and FOCUS-PELMO 5.5.3 for all available FOCUS scenarios for all crops with an annual application pattern (continuous cropping over a period of 26 years).

The maximum PEC_{gw} for all compounds was < 0.001 µg L^{-1} and thus clearly below the 0.1 µg L^{-1} threshold value.

Based on the exposure assessment, no risk mitigation measures are needed.

Surface water and sediment:

Predicted environmental concentrations (PEC) in surface water and sediment were calculated for pyraclostrobin, its water metabolites BF 500-5, BF 500-11, BF 500-13, BF 500-14 and its sediment metabolites BF 500-3, BF 500-6 and BF 500-7 after a spray application of pyraclostrobin to cereals (single and twofold application), maize (single application) and potatoes (single and fourfold application). Calculations were performed according to the recommendations of the FOCUS working group on surface water scenarios in a stepwise approach considering the pathways spray drift, drainage and runoff.

PEC_{sw} and PEC_{sed} of pyraclostrobin were calculated with all available scenarios for all use patterns. The calculations were performed for the FOCUS Steps 1 – 4 using the software packages STEPS1-2 for Step 1 and Step 2, SWASH 3.1, FOCUS-PRZM 3.1.1, FOCUS-MACRO 4.4.2 and TOXSWA 3.3.1 for Step 3. Moreover, the pre- and post-processing tool PECRobot v1.4 was used for setting up the Step 4 calculations, which were finally simulated using TOXSWA 3.3.1. Step 4 calculations were performed using no-spray buffer zones (up to 20 m for cereals and 10 m for maize) and/or drift reducing nozzles for drift mitigation. Moreover, vegetated filter strips for runoff and erosion were applied (up to 20 m for cereals and 10 m for maize). For the use in potatoes no additional mitigation measures were required beyond edge-of-field (Step 3). PEC_{sw} and PEC_{sed} of the metabolites of pyraclostrobin were only calculated at Step 1 and Step 2.

Results can be found in CP 9.2.5/1.

Air:

No exposure assessment required nor performed.

Other routes of exposure:

No other routes of exposure are relevant for the representative uses of pyraclostrobin.

9 EFFECTS ON NON-TARGET SPECIES

9.1 Summary of effects on birds and other terrestrial vertebrates

BAS 500 06 F and BAS 516 07 F are two new representative formulations, which have not been evaluated during a previous Annex I inclusion. For pyraclostrobin, the EU agreed endpoints plus endpoints from new, additional studies as well as revised endpoints from EU agreed studies are used for the risk assessment on birds and mammals. The risk assessment is based on the proposed uses of BAS 500 06 F and BAS 516 07 F. Note that BAS 516 07 F contains pyraclostrobin and boscalid. No separate risk assessment for boscalid is presented, as the active substance in focus of the evaluation is pyraclostrobin. Boscalid is solely addressed in the context of the assessment of combined effects of formulations containing more than one active substance (i.e. dietary risk assessment for a virtual compound and the formulation).

The risk assessment on birds and mammals considers the principles given in the latest guidance document by EFSA (*Anonymous 2009: Guidance Document on risk assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. European Food Safety Authority*), hereafter cited as EFSA/2009/1438.

Effects on Birds:

Dietary risk assessment

Under the conservative assumptions of a tier 1 assessment all TER_A and TER_{LT} values of pyraclostrobin exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects for applications in cereals and maize (both BAS 500 06 F) and potatoes (BAS 516 07 F).

Food chain behaviour (secondary poisoning) and bioaccumulation

Based on the tier 1 assessment, all TER values for the risk assessments for earthworm- and fish-eating birds for pyraclostrobin as contained in BAS 500 06 F and BAS 516 07 F, exceed the trigger of 5 set by Commission regulation (EU) 546/2011 for acceptability of effects for secondary poisoning. The bioaccumulation potential of pyraclostrobin was considered as low in the respective EU reviews and therefore further evaluation on biomagnification is not necessary. It can therefore be concluded that the application of BAS 500 06 F and BAS 516 07 F does not raise concerns regarding food chain behaviour (secondary poisoning) or biomagnification.

Drinking water risk assessment

Following EFSA/2009/1438, the puddle scenario is the one relevant for cereals, maize and potatoes. Since the ratio of the effective application rate to the relevant toxicity endpoints is below the value of 3000 for pyraclostrobin, conducting a quantitative risk assessment for the proposed use pattern of BAS 500 06 F and BAS 516 07 F is not necessary. In conclusion the proposed use pattern of BAS 500 06 F in cereals and maize and BAS 516 07 F in potatoes does not pose a risk to birds via uptake of contaminated drinking water.

Effects of the formulations

All TER_A values from the tier 1 acute dietary risk assessment of the formulation, calculated for the virtual compound (BAS 516 07 F) as well as for the formulation (BAS 500 06 F and BAS 516 07 F), exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. This indicates a low acute risk for birds from the use of BAS 500 06 F in cereals and maize and BAS 516 07 F in potatoes according to the recommended use pattern.

Overall conclusion

It can be concluded that the application of BAS 500 06 F in cereals and maize and BAS 516 07 F in potatoes according to good agricultural practice will not adversely affect birds under natural conditions.

Effects on Terrestrial Vertebrates Other Than Birds

Dietary risk assessment

Under the conservative assumptions of a tier 1 assessment all TER_A values of pyraclostrobin exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects for applications in cereals and maize (both BAS 500 06 F) and potatoes (BAS 516 07 F).

All TER_{LT} values for potatoes (BAS 516 07 F) and the TER_{LT} values of BAS 500 06 F for two scenarios in cereals (small insectivorous mammal "shrew" at BBCH ≥ 20 and small omnivorous mammal “mouse” at BBCH ≥ 40) and three scenarios in maize (small insectivorous mammal “shrew” at BBCH ≥ 20 and small omnivorous mammal “mouse” at BBCH 30 - 39 and BBCH ≥ 40) are above the relevant trigger.

For the scenarios large herbivorous mammal “lagomorph” (cereals), small omnivorous mammal “mouse” (cereals at BBCH 10-29) and small herbivorous mammal “vole” (cereals at BBCH ≥ 40 and maize at BBCH 30 - 39 and BBCH ≥ 40) the TER_{LT} values for pyraclostrobin are below the trigger and need to be refined. Based on a thorough analysis of existing data for several vole species, BASF believes that the "vole" scenario is not really relevant for higher tier reproductive risk assessments in arable crops like cereals and maize. Nevertheless, as the relevance of the “vole” scenario has been controversially discussed by the European Member States and a harmonized approach is currently lacking, quantitative higher tier risk assessments for the vole in cereals and maize have been added.

The refined reproductive risk assessment for the scenarios large herbivorous mammal “lagomorph”, small omnivorous mammal “mouse” and small herbivorous mammal “vole” considers the following refinement parameters:

- Ecologically relevant reproductive toxicity endpoint for pyraclostrobin
- Residue decline of pyraclostrobin in plants for refinement of MAF x twa
- Identification of representative mammal species including ecological data (PT and PD values and FIR)
- Interception by cereal and maize plants

Taking these refinement steps into account, all relevant TER_{LT} values exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects indicating a low and acceptable risk to mammals from the use of BAS 500 06 F in cereals and maize and BAS 516 07 F in potatoes. A low long-term risk to mammals from applications of BAS 500 06 F is further confirmed by two field effect studies with BAS 500 06 F.

Food chain behaviour (secondary poisoning) and bioaccumulation

Based on the tier 1 assessment all TER values for the risk assessments for earthworm- and fish-eating mammals, for pyraclostrobin as contained in BAS 500 06 F and BAS 516 07 F, exceed the trigger of 5 set by Commission regulation (EU) 546/2011 for acceptability of effects for secondary poisoning. The bioaccumulation potential of pyraclostrobin was considered as low in the respective EU reviews and therefore further evaluation on biomagnification is not necessary. It can therefore be concluded that the application of BAS 500 06 F and BAS 516 07 F does not raise concerns regarding food chain behaviour (secondary poisoning) or biomagnification.

Drinking water risk assessment

Following EFSA/2009/1438, the puddle scenario is the one relevant for mammals. Since the ratio of the effective application rate to the relevant toxicity endpoints is below the value of 3000 for pyraclostrobin, conducting a quantitative risk assessment for the proposed use pattern of BAS 500 06 F and BAS 516 07 F is not necessary. In conclusion the proposed use pattern of BAS 500 06 F in cereals and maize and BAS 516 07 F in potatoes does not pose a risk to birds via uptake of contaminated drinking water.

Effects of the formulation

All TER_A values from the tier 1 acute dietary risk assessment of the formulation BAS 516 07 F, calculated for the virtual compound as well as for the formulation, exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects.

For BAS 500 06 F, the TER_A from the tier 1 acute dietary risk of the formulation was below the trigger for the large herbivorous mammal scenario “lagomorph” (cereals) and the small herbivorous mammal scenario “vole” (cereals at BBCH ≥ 40 and maize at BBCH 30 - 39) and required refinement.

For the refined acute risk assessment the following parameters are considered:

- Residue decline of pyraclostrobin in plants for refinement of MAF_{90}
- Identification of representative mammal species including ecological data (PD values and FIR)

Taking these refinement steps into account, the refined TER_A value for the large herbivorous mammal scenario "lagomorph" and the small herbivorous mammal scenario "vole" in cereals exceed the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. The conservatively refined TER_A value for the small herbivorous mammal scenario "vole" was slightly below the trigger for ~~cereals and~~ maize.

A further higher tier assessment of the small herbivorous mammal scenario based on three lines of evidence (i.e. results from a body burden modelling approach in small mammals, dietary toxicity studies under field-relevant exposure conditions, and results from two field effect studies with BAS 500 06 F) indicates a low and acceptable risk to mammals from the use of BAS 500 06 F in cereals and maize.

Overall conclusion

It can be concluded that the application of BAS 500 06 F in cereals and maize and BAS 516 07 F in potatoes according to good agricultural practice will not adversely affect mammals under natural conditions.

Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

Pyraclostrobin shows inherent toxicity to amphibians and can cause mortality to young and sensitive amphibian metamorphs under worst-case laboratory conditions at and below maximum field rates. However, this is only true if those sensitive metamorphs are exposed to direct overspraying. An additional soil residue study exposing animals on treated soil demonstrate strongly reduced toxicity already shortly after spraying and no toxicity was seen if introduced 4 hours after spraying. Furthermore, two semi-field studies with BAS 500 06 F performed with young metamorphs under realistic worst-case conditions in cereals at BBCH 25-30 using the full field rate of 250 g a.s./ha and higher rates (+25% in the 1st study with frogs and +50% in the 2nd study with toads), showed no mortality and no significant sublethal effects to frogs and no effects on toads were observed (at field rates up to 375 g a.s./ha). The semi-field studies cover on the one side all relevant exposure routes including direct spray, uptake from contaminated surfaces and potential oral uptake of potentially contaminated feed items. On the other side it also includes more realistic exposure considering plant interception and above all amphibian behavioral aspects leading to reduced exposure (such as hiding).
Data from literature indicate that certain formulations produce higher toxicity while others show a reduced toxicity. Particularly formulations with high organic solvent content maximizing immediate and full bioavailability of the active substance (and which may themselves have certain direct toxicity to amphibians) show higher toxicity. The semi-field study was performed with the EC formulated product BAS 500 06 F, having a high content of organic solvents, and thus constituting a respective worst-case formulation.
BAS 516 07 F is applied at much lower rates of pyraclostrobin (17 g a.s./ha). This is about three times below the rate at which even under worst-case laboratory conditions no effects are observed. Furthermore, BAS 516 07 F is a WG-formulated product, which does not contain organic solvents and which is expected to have a reduced toxicity.

There is no indication that reptiles might be particularly at risk from applications of this fungicide. It is considered that the risks to reptiles are fully covered by the risk assessment to other vertebrates (birds, mammals, amphibians) shown above.

Overall Conclusion

Pyraclostrobin and certain pyraclostrobin containing products show inherent toxicity to sensitive amphibian live stages. However, a refined risk assessment and a higher tier semi-field study performed under realistic worst-case conditions showed low risk of BAS 500 06 F applications in cereals under realistic conditions. BAS 516 07 F is applied at much lower rates ensuring low risk to amphibians for applications of this product in potatoes.

9.2 Summary of effects on aquatic organisms

BAS 500 06 F and BAS 516 07 F are two new representative formulations, which have not been evaluated during a previous Annex I inclusion. For pyraclostrobin and its major metabolites, the EU agreed endpoints plus endpoints from new, supplementary studies as well as revised endpoints from EU agreed studies are used for the risk assessment on aquatic organisms. The risk assessment is based on the already registered uses of BAS 500 06 F and BAS 516 07 F.
BAS 500 06 F: 2 x 1.25 L product/ha equivalent to 250 g a.s./ha in cereals and 1x 1.0 L product/ha (equivalent to 200 g a.s./ha) in maize
BAS 516 07 F: 4 x 0.25 kg product/ha; equivalent to 17 pyraclostrobin/ha in potato

The TER values for all metabolites of pyraclostrobin are significantly above the respective trigger values based on worst-case standard assumptions; they are thus considered not to be of ecotoxicological relevance.

The acute and long-term TER values for aquatic insects, aquatic crustaceans, algae and the aquatic plant *Lemna gibba* exposed to pyraclostrobin exceed the required trigger values, indicating low risk to these groups of aquatic organisms. TER values for fish and daphnids, however, do not meet the required trigger value for applications close to sensitive surface water bodies according to standard worst-case assumptions. However, higher tier studies (such as a mesocosm study covering the risk to aquatic invertebrates including *Daphnia* and a SSD for fish) demonstrate the lack of an unacceptable risk from the use of this substance in winter and spring cereals according to good agricultural practice if no-spray buffer zones of 5 m or 50% drift reducing nozzles are considered. Application in potato and maize according to the proposed GAP and good agricultural practice is of low risk to aquatic ecosystems with no need for any additional mitigation measures.

The studies performed with the formulated products BAS 500 06 F and BAS 516 07 F reflect the toxicity of the active substance pyraclostrobin and there is no indication of any higher (or unexpected) toxicity of the products. Therefore, the risk assessment presented based on the data of the active substance also covers the risk to aquatic organisms following the proposed uses of BAS 500 06 F and BAS 516 07 F.

Overall Conclusion
The active substance pyraclostrobin is toxic to aquatic organisms, in particular to fish and contamination of water must be avoided. However, a refined risk assessment has been performed based on a number of additional and higher tier studies. According to the refined risk assessment it can be concluded that applications according to GAP and good agricultural practice will be of low risk to aquatic ecosystems for application of BAS 500 06 F in winter and spring cereals if no-spray buffer zones of 5 m or the use of 50% drift reducing nozzles are considered. For the proposed uses of BAS 500 06 F in maize and BAS 516 07 F in potato, low risk to aquatic organisms can be shown with no need for additional mitigation measures.

9.3 Summary of effects on arthropods

Bees

The calculated HQs for acute oral and acute contact exposure of honeybees to pyraclostrobin and the formulated products BAS 516 07 F and BAS 500 06 F are below the Commission Regulation (EU) 546/2011 trigger value of 50.

Additionally, no unacceptable effects in queen development, queen survival and adult worker bee health at 400 ppm Pristine treated pollen (which is based on analysis equivalent to 22 ppm pyraclostrobin treated pollen) were observed. Furthermore, the potential acute toxicity (oral and contact) of pyraclostrobin to adult bumblebees was addressed in a laboratory study. As currently no risk assessment scheme exists, these results are presented as additional information.

Furthermore, no unacceptable effects were observed in a semi-field tunnel study conducted with BAS 500 06 F on honeybee colonies in flowering *Phacelia tanacetifolia* with focus on bee brood at a rate of 1.25 L/ha. The study was carried out according to EPPO Guideline 170 (4) and the OECD Guidance Document. The test item caused no unacceptable effects on honeybee survival, colony development and colony strength when applied at a rate of 1.25 L/ha under semi-field conditions (tunnel) to *P. tanacetifolia* during active foraging conditions. The brood termination rates, the brood indices as well as the brood compensation indices indicate no test item related effect on the brood development following the labelling of the eggs.

Overall conclusion:
The recommended representative uses of BAS 516 07 F and BAS 500 06 F according to good agricultural practice present a low risk to honeybees and will not adversely affect honeybees or honeybee colonies.

Non-target arthropods other than bees

In the case of the formulated product BAS 516 07 F, the calculated hazard quotient of the first tier risk assessment indicated no in-field and no off-field risk for both tested species. However, in order to have additional information for the risk assessment, Tier II tests were carried out with *C. carnea*, *A. bilineata*, *P. cupreus* and *Pardosa* spec. For these species the Tier II risk assessment indicated no in-field and no off-field risk, too. Additionally, three field studies were carried out to observe effects on natural occurring populations of predatory mites. No unacceptable effects on population development of predatory mites were observed up to 5 x 0.75 kg/ha. Therefore, a potential in-field and off-field risk can be excluded for BAS 516 07 F at the proposed use pattern of 4 x 0.25 kg/ha.

Considering the formulated product BAS 500 06 F, a potential in-field risk to non-target arthropods cannot be excluded (HQ > 2) based on the calculated $HQ_{in\text{-}field}$ of *T. pyri* and *A. rhopalosiphi*. Therefore, higher Tier 2 tests were carried out with *T. pyri* and *A. rhopalosiphi* and additionally with the foliage-dweller *Chrysoperla carnea* and the soil-dweller *Aleochara bilineata*. For *T. pyri*, *A. rhopalosiphi* and *A. bilineata* no risk was indicated for in- and off-field habitats. Based on the results of an extended laboratory study with *C. carnea*, a potential risk for in-field habitats cannot be excluded. Therefore, an aged residue study with *C. carnea* was carried out under more realistic conditions, which resulted in no unacceptable effects on survival and reproduction at DAT 0 up to 1.25 L/ha and DAT 7 up to 2.5 L/ha. Based on the results of the aged residue study, no in-field and off-field risk for *C. carnea* is indicated.

Overall conclusion:

Taking into account all available data, it can be concluded that low risk for non-target arthropods is expected from the use of the representative formulations BAS 516 07 F and BAS 500 06 F at application rates of up to 4 x 0.25 kg/ha and 2 x 1.25 L/ha, respectively. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

9.4 Summary of effects on non-target soil meso- and macrofauna

Acute studies on earthworms were performed with pyraclostrobin, its metabolites (BF 500-6 and BF 500-7) and with the representative formulations (BAS 516 07 F and BAS 500 06 F). The results of the acute studies conducted both products are presented as additional information (please refer to M- CP 10.7). Chronic studies with earthworms and collembolans were carried out with pyraclostrobin (earthworm only), the metabolites BF 500-6 and BF 500-7 as well as with the two formulations. Moreover, chronic studies on soil mites and field studies on earthworms have been conducted with both products.

In the risk assessment, all TER values exceeded the trigger value of 5 for chronic exposure.

A study with BAS 500 06 F on organic matter breakdown is presented as additional information in M-CP 10.7.

Overall conclusion:
It is concluded that the recommended representative uses of BAS 516 07 F and BAS 500 06 F will not pose any unacceptable risks to populations of earthworms or other soil macro-organisms.

9.5 Summary of effects on soil nitrogen transformation

BAS 516 07 F (tested as minor change formulation BAS 516 00 F) had no significant effect on soil micro-organisms at 24 mg BAS 516 07 F/kg dry soil, corresponding to 1.6 mg pyraclostrobin/kg dry soil and 8.0 mg total a.s./kg dry soil. These concentrations are approximately 107 higher than the maximum $PEC_{soil, max}$ of 0.015 mg pyraclostrobin/kg dry soil - and approximately 31-times higher than the sum of the highest PEC-values for both a.s., i.e. 0.260 mg total a.s./kg dry soil. This supports the conclusion that under field conditions, the use of BAS 516 07 F at the proposed rates poses no unacceptable risk to non-target soil micro-organisms.

BAS 500 06 F had no significant effect on soil micro-organisms at 17.3 mg BAS 500 06 F/kg dry soil, corresponding to 3.33 mg pyraclostrobin/kg dry soil. This is approximately 15 times higher than the maximum $PEC_{soil, max}$ of 0.228 mg pyraclostrobin/kg dry soil. This supports the conclusion that under field conditions, the use of BAS 500 06 F at the proposed rates poses no unacceptable risk to non-target soil micro-organisms.

The NOEC values of the pyraclostrobin metabolites BF 500-6 and BF 500-7 are higher than the respective worst-case PEC values.

Overall conclusion:
It is concluded that the recommended representative uses of BAS 516 07 F and BAS 500 06 F will not pose an unacceptable risk to non-target soil micro-organisms, if applied according to the recommended use pattern.

9.6 Summary of effects on terrestrial non-target higher plants

Based on the risk assessments it can be concluded that the use of BAS 516 07 F and BAS 500 06 F poses no unacceptable risk to non-target plants. Particular precautions to reduce the environmental concentrations resulting from BAS 516 07 F and BAS 500 06 F applications are not required for the protection of terrestrial non-target plants.

Overall conclusion:
It is concluded that the uses of BAS 516 07 F and **BAS 500 06 F according to the recommended use pattern will not pose an unacceptable risk to non-target plants.**

9.7 Summary of effects on other terrestrial organisms (flora and fauna)

A study on carbon transformation (BAS 516 07 F tested as BAS 516 00 F), studies on acute earthworm toxicity (both products) and a study with BAS 500 06 F on organic matter breakdown has been carried out. The results are presented as additional information.

9.8 Summary of effects on biological methods for sewage treatment

Disturbances in the bio-degradation process of activated sludge are not to be expected if the test item is correctly introduced into adapted wastewater treatment plants at low concentrations.

9.9 Summary of product exposure and risk assessment

Applications of the formulated products BAS 500 06 F and BAS 516 07 F according to good agricultural practice will not adversely affect birds and mammals and other vertebrates (amphibians) under natural conditions.

The formulated products are toxic to aquatic organisms and contamination of aquatic ecosystems must be prevented. However, according to the risk evaluation presented, low risk to aquatic organisms can be demonstrated. For the proposed uses of BAS 500 06 F, low risk to aquatic organisms can be shown if no-spray buffer zones of 5 m or the use of 50% drift reducing nozzles for winter and spring cereals are considered. For the proposed uses of BAS 500 06 F in maize and BAS 516 07 F in potatoes, low risk to aquatic organisms can be demonstrated with no need for any additional mitigation measures.

Following application of both representative formulations no risk or unacceptable effects are expected for honeybees, non-target arthropods others than bees, non-target meso- and macrofauna, soil nitrogen transformation processes and non-target higher plants. Particular precautions to reduce the environmental concentrations resulting from BAS 500 06 F and BAS 516 07 F applications are not required for the protection of terrestrial non-target organisms.

Comprehensive risk assessments for aquatic organisms and terrestrial vertebrates are presented in M-CP 10.1 and 10.2. For risk assessments of arthropods, soil meso- and macrofauna, soil micro-organisms and non-target terrestrial plants please refer to M-CP 10.3 – 10.6.

10 CLASSIFICATION AND LABELLING

10.1 Classification and labelling of the active ingredient

The following harmonized classification and labelling was adopted for pyraclostrobin:

Legislation	**Classification**	**Labelling**	**Concentration limits**
Directive 67/548/EEC	T, N R: 23-38-50-53	T, N R: 23-38-50/53 S: 1/2-45-60-61-63	None
Regulation (EC) No 1272/2008	Hazard class and category code: Acute Tox. 3 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1 Hazard statement code: H331, H315, H400, H410	Pictogram signal word code: GHS06 GHS09 Danger Hazard statement code: H331, H315, H410	M-factor = 100

Single accidental exposure of humans to spray mist of pyraclostrobin containing formulations resulted in symptoms of respiratory irritation (see M-CA 5.9.3). Based on this direct observation in humans and supportive evidence for respiratory irritation observed in subchronic inhalation studies in rats (see CA 5.3.3/1 and 5.3.3/2), BASF self-classified pyraclostrobin with STOT SE Category 3 (H335, May cause respiratory irritation). Consequently, BASF proposes for pyraclostrobin the classification and labelling as shown below.

Legislation	**Classification**	**Labelling**	**Concentration limits**
Directive 67/548/EEC	T, N R: 23-37-38-50-53	T, N R: 23-37/38-50/53 S: 1/2-45-60-61-63	None
Regulation (EC) No 1272/2008	Hazard class and category code: Acute Tox. 3 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1 STOT SE 3 Hazard statement code: H331, H315, H400, H410, H335	Pictogram signal word code: GHS06 GHS09 Danger Hazard statement code: H331, H315, H410, H335	M-factor = 100

10.2 Classification and labelling

Formulation BAS 500 06 F:

Proposed classification according to Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures:

Labelling:	Signal word:	Danger
	Hazard statements:	H319, H315, H332, H302, H317, H304, H400, H410, EUH401
	Precautionary statements:	please refer to document CP12

Proposed classification according to Dangerous Substances Directive (Directive 67/548/EEC):

Labelling:	Indication of danger:	Harmful, Dangerous for the environment
	R-phrases:	R20/22, 38, 43, 50/53
	S-phrases:	please refer to document CP12

Formulation BAS 516 07 F:

Proposed classification according to Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures:

Labelling:	Signal word:	Warning
	Hazard statements:	H400, H410, EUH401
	Precautionary statements:	please refer to document CP12

Proposed classification according to Dangerous Substances Directive (Directive 67/548/EEC):

Labelling:	Indication of danger:	Dangerous for the environment
	R-phrases:	R50/53
	S-phrases:	please refer to document CP12

11 RELEVANCE OF METABOLITES IN GROUNDWATER

11.1 Summary

None of the pyraclostrobin metabolites poses any risk for groundwater contamination.

11.2 Conclusion

A relevance assessment is not considered necessary.

12 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

Pyraclostrobin does not consist of any isomers.

FURTHER INFORMATION TO BE SUBMITTED

None

Appendix 1: Metabolites formed from Active Substance and their occurrence

Please see Document N3.

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT N2

LISTING OF ENDPOINTS

Compiled by:

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1165142 (version 1)
27-Feb-2017	Chapter 3: The results of a rat metabolism study with metabolite 500M106 and new endpoints derived from toxicity studies with metabolites 500M02 and 500M106 were included. In addition update of information on 500M62. Chapter 4: A new residue trial in potatoes was added. Chapter 6: Update of the mammal risk assessment and consideration of two new amphibia studies as well as a new bumble bee study. New or changed text is marked in yellow.	BASF DocID 2017/1032928 (version 2)

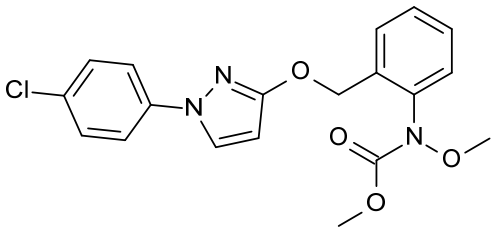
[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

LISTING OF END POINTS FOR *ACTIVE SUBSTANCE*

Chapter 1: Identity, Physical and Chemical Properties, Details of Uses and Further Information, and Proposed Classification and Labelling

Active substance (ISO Common Name)	Pyraclostrobin
Function (*eg.* fungicide)	Fungicide
Rapporteur Member State	Germany

Identity (SANCO/11802 data point 1)

Chemical name (IUPAC)	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-methoxy carbamate
Chemical name (CA)	carbamic acid, [2-[[[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester
CIPAC No	657
CAS No	175013-18-0
EC No (EINECS or ELINCS)	not assigned
FAO Specification (including year of publication)	not assigned
Minimum purity of the active substance as manufactured (g/kg)	975 g/kg
Identity of relevant impurities (of toxicological, environmental and/or other significance) in the active substance as manufactured (g/kg)	Dimethyl sulfate (max. 1 mg/kg)
Molecular formula	$C_{19}H_{18}ClN_3O_4$
Molecular mass	387.8 g/mol
Structural formula	

Physical-chemical properties (SANCO/11802 data point 2)

Melting point and boiling point (state purity)	Melting point: 63.7 - 65.2°C (99.8%) Boiling point: no boiling point up to decomposition at 200°C (99.8%)
Vapour pressure, volatility (in Pa, state temperature and purity)	Vapour pressure: 2.6 x 10^{-8} Pa, 20°C (99.8%) Henry's law constant: 5.307 x 10^{-6} Pa m^3 mol^{-1}
Appearance (physical state, colour)	PAI (99.8%): white to light beige crystalline solid TGAI (amorphous, 96.8%): solid, amber, glass like material TGAI (crystalline, 99.4%): solid, light yellow, odourless, fine crystalline powder
Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	UV-Vis absorption (22°C, 99.8%): 2.5 x 10^4 L mol^{-1} cm^{-1} at 205 nm 2.4 x 10^4 L mol^{-1} cm^{-1} at 275 nm
Solubility in water (in g/l or mg/l, state temperature and purity)	1.9 ± 0.17 mg/L at 20°C (99.8%) in deionised water (pH of 5.8) There is no dissociation in water, therefore pH dependence on solubility is not applicable.
Solubility in organic solvents (in g/l or mg/l, state temperature and purity)	All data at 20°C (purity: 99.8%) n-heptane 3.7 g/L solution 2-propanol 30.0 g/L solution octanol 24.2 g/L solution olive oil 28.0 g/L solution methanol 100.8 g/L solution acetone >500 g/L solution ethyl acetate >500 g/L solution acetonitrile >500 g/L solution dichloromethane >500 g/L solution toluene >500 g/L solution
Partition coefficient n-octanol/water (log POW) (state pH and temperature)	3.99 (20°C, 99.8%)
Dissociation in water (state purity)	not applicable (no indication of dissociation in water)
Flammability and self-heating (state purity)	not highly flammable, not self-heating (99.0 and 99.9%)
Flash point (state purity)	not applicable (test material has a melting point >40°C)
Explosive properties (state purity)	not explosive (99.0 and 99.9%)
Surface tension (state concentration and temperature, state purity)	71.8 mN/m at 0.5 % (w/w) (20°C, 98.5%) 71.5 mN/m at 2.0 % (w/w) (20°C, 98.5%)
Oxidising properties (state purity)	not oxidising (99.0 and 99.9%)

Summary of representative uses registered or under evaluation in the EU for BAS 500 06 F

GAP rev 1.0., date: 2014-Jun-03

PPP (code)	**BAS 500 06 F**	**Formulation type:**	**EC**
active substance 1	**Pyraclostrobin**	**Conc. of as 1:**	**200 g/L**
Applicant:	**BASF SE**	**professional use**	☒
Zone(s):EU		**non professional use**	☐

Verified by MS: **y/n**

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
Cereals												
1	DK	Barley (spring, winter)	F	*Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 69	a) 1 b) 1	a) 1.25 b) 1.25	a) 0.25 b) 0.25	100 - 400	42	
2	DK	Oats Rye	F	*Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	42	
3	DK	Triticale Wheat	F	*Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
4	EE	Barley (spring, winter) Oats Wheat (spring, winter)	F	*Blumeria graminis* *Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis* *Septoria tritici*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
5	FI	Barley (spring, winter) Oats Rye Triticale Wheat (spring, winter, durum)	F	*Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.2 b) 2.4	a) 0.24 b) 0.48	150 - 300	35	A range of 0.3 – 1.2 L/ha is registered.
6	LT, LV, SE	Barley (spring, winter) Oats Rye Triticale Wheat (spring, winter, durum)	F	*Puccinia spp.* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	A range of 0.8 – 1.25 L/ha is registered.
7	AT*	Barley (spring, winter) Rye Triticale	F	*Puccinia recondita* *Puccinia hordei* *Pyrenophora teres* *Rhynchosporium secalis* sunburn injury	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
8	AT*	Wheat (spring, winter, durum, spelt)	F	*Drechslera tritici-repentis* *Puccinia recondita* *Puccinia striiformis*	Spraying	25 – 61 (*P. recondita*: 25-69)	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
9	BE*, LU*	Barley (spring, winter) Oats Rye Triticale Wheat (spring, winter, durum, spelt)	F	*Drechslera tritici-repentis* *Puccinia coronata* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis* sunburn injury	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
10	CZ*, NL*	Barley (spring, winter) Rye Triticale Wheat (spring, winter, durum, spelt)	F	*Drechslera tritici-repentis* *Puccinia coronata* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis* sunburn injury	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
11	DE*	Barley Rye Wheat	F	*Drechslera tritici-repentis* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 – 61 *(P.recondita:* 25-69*)*	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
12	DE*	Barley	F	decrease of nonparasitic leaf spots	Spraying	32 - 61	a) 1 b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	
13	DE*	Triticale	F	*Puccinia recondita*	Spraying	25 – 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
14	HU*, PL*, RO*, SK*, SI*	Barley (spring, winter) Rye Triticale Wheat (spring, winter, durum, spelt)	F	*Drechslera tritici-repentis* *Puccinia coronata* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis* sunburn injury	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	A range of 0.8 – 1.25 L/ha is registered.
15	IE, UK	Barley (spring, winter) Oats (winter, spring)	F	*Puccinia coronata* *Puccinia hordei* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis*	Spraying	25 - 59	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	200 - 400	35	Including physiological effects
16	IE, UK	Wheat (spring, winter, durum, spelt)	F	*Fusarium* (ear) *Puccinia recondita* *Puccinia striiformis* *Septoria nodorum* (UK), *Septoria tritici* (UK)	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.5	200 - 400	35	Including physiological effects
17	IE	Rye Triticale	F	*Puccinia recondita* *Puccinia striiformis* *Puccinia triticina* *Rhynchosporium secalis*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.5	200 - 400	35	Including physiological effects

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
18	BG*, IT, GR	Barley (spring, winter) Triticale Wheat (spring, winter, durum)	F	*Blumeria graminis* *Fusarium spp.* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis* *Septoria spp.*	Spraying	25 - 69	a) 2 (21d) b) 2	a) 1.25 b) 2.50	a) 0.25 b) 0.50	100 - 400	35	Including physiological effects A range of 1 – 1.25 L/ha is registered.
19	ES, FR, PT	Barley Oats Rye Triticale Wheat	F	*Fusarium spp.* *Puccinia coronata* *Puccinia hordei* *Puccinia recondita* *Puccinia striiformis* *Pyrenophora teres* *Rhynchosporium secalis* *Septoria spp.*	Spraying	25 - 69	a) 2 (21d) b) 2)	a) 1.10 b) 2.20	a) 0.22 b) 0.44	100 - 400	35	Maximum 1 application against *Pyrenophora teres* in barley
Maize												
20	DK*, SE	Maize (forage and grain)	F	*Exserohilum turcicum* *Kabatiella zeae*	Spraying	30 - 65	a) 1 b) 1	a) 1.00 b) 1.00	a) 0.2 b) 0.2	100 - 400	n.a.	PHI defined by growth stage at application.
21	UK*	Maize (forage and grain)	F	*Puccinia sorghum* *Kabatiella zeae* *Setosphaeria turcica*	Spraying	30 - 65	a) 1 b) 1	a) 1.00 b) 1.00	a) 0.2 b) 0.2	200 - 400	n.a.	Including physiological effects PHI defined by growth stage at application.
21	BG	Maize (forage and grain)	F	*Exserohilum turcicum* *Puccinia sorghi*	Spraying	30 - 65	a) 1 b) 1	a) 1.00 b) 1.00	a) 0.2 b) 0.2	100 - 400	n.a.	PHI defined by growth stage at application. A range of 0.7 – 1 L/ha is registered.

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
23	ES*, GR, IT, PT*	Maize (forage and grain)	F	*Exserohilum turcicum* *Puccinia sorghi*	Spraying	30 - 65	a) 1 b) 1	a) 1.00 b) 1.00	a) 0.2 b) 0.2	100 - 400	n.a.	Including physiological effects PHI defined by growth stage at application.

*evaluation ongoing

Summary of representative uses registered or under evaluation in the EU for BAS 516 07 F

GAP rev. 1.0, date: 2014-Jun-09

PPP (code)	**BAS 516 07 F**	**Formulation type:**	**WG**
active substance 1	**Pyraclostrobin**	**Conc. of as 1:**	**67 g/kg**
active substance 2	**Boscalid**	**Conc. of as :**	**267 g/kg**
Applicant:	**BASF**	**professional use**	☒
Zone(s):	**EU**	**non professional use**	☐
Verified by MS:	**y/n**		

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:**
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
1	DK	Potatoes	F	*Alternaria* spp.	Spraying	47-75	a) 4 (5d) b) 4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	150	3	
2	EE	Potatoes	F	*Alternaria* spp.	Spraying	47-75	a) 4 (7d) b) 4	a) 0.25 a) 1.00	a) 17 + 67 b) 67 + 267	150	3	
3	LT	Potatoes	F	*Alternaria* spp.	Spraying	47-75	a) 4 (7d) b) 4	a) 0.20 b) 0.80	a) 13 + 53 b) 54 + 214	250-1000	14	
4	LV	Potatoes	F	*Alternaria* spp.	Spraying	first symptoms	a) 4 (7d) b) 4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	250-400	3	
5	SE	Potatoes	F	*Alternaria* spp.	Spraying	47-75	a) 4 (10d) b) 4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	150-400	3	
6	DE, AT	Potatoes	F	*Alternaria* spp.	Spraying	51-89	a) 4 (10d) b)4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	200-400	3	used as critical GAP in risk assessments
7	BE	Potatoes	F	*Alternaria* spp.	Spraying	first symptoms	a) 4 (10d) b) 4	a) 0.20 b) 0.80	a) 13 + 53 b) 54 + 214	300-400	3	

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled**	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
8	NL	Potatoes	F	*Alternaria* spp.	Spraying	40 - 95	a) 4 (14d) b) 4	a) 0.20 b) 0.80	a) 13 + 53 b) 54 + 214	200-400	0	
9	HR	Potatoes	F	*Alternaria* spp.	Spraying	41-89	a) 4 b)4	a) 0.25 b) 1.00	a) 17 + 67 b) 67 + 267	300	14	

Chapter 2: Methods of Analysis

Analytical methods for the active substance (SANCO/11802 data point 4.1)

Technical as (principle of method)	Reverse phase HPLC with UV detection and external calibration
Impurities in technical as (principle of method)	Significant impurities: see Document J Dimethyl sulfate: Solid-phase microextraction (SPME) coupled to GC/MS and external calibration
Plant protection product (principle of method)	Active substance in BAS 500 06 F and BAS 516 07 F: Reverse phase HPLC with UV detection and external standard Dimethyl sulfate in both formulations: Head space GC-MS with internal standard

Analytical methods for residues (SANCO/11802 data point 4.1 to 4.2)

Residue definitions for monitoring purposes

Food/feed of plant origin	Pyraclostrobin (parent)
Food/feed of animal origin	Pyraclostrobin (parent)
Soil	Pyraclostrobin (parent)
Water surface drinking/ground	Pyraclostrobin (parent) Pyraclostrobin (parent)
Air	Pyraclostrobin (parent)
Body fluids and tissues	Pyraclostrobin (parent)

Monitoring/Enforcement methods

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	BASF Method 421/0: LC/MS-MS: LOQ 0.02 mg/kg QuEChERS method: LC/MS-MS: LOQ: 0.01 mg/kg
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	BASF method L0151/01: LC/MS-MS: LOQ: 0.01 mg/kg
Soil (principle of method and LOQ)	BASF method L0166/01: LC/MS-MS: LOQ: 0.001 mg/kg or BASF method L0161/01: LC/MS-MS: LOQ: 0.001 mg/kg
Water (principle of method and LOQ)	BASF method L0182/02: LC/MS-MS: LOQ: 0.003 µg/L
Air (principle of method and LOQ)	BASF method L0197/01: LC/MS-MS: LOQ: 4.44 ng/L
Body fluids and tissues (principle of method and LOQ)	BASF method L0151/01: LC/MS-MS: LOQ: 0.01 mg/kg

Classification and proposed labelling (SANCO/11802 data point 10 and SANCO/11803 data point 12) according to Regulation (EC) No 1272/2008

Active substance

Pictogram:		
Signal words:	Danger	
Hazard statements:	H315	Causes skin irritation.
	H331	Toxic if inhaled.
	H400	Very toxic to aquatic life.
	H410	Very toxic to aquatic life with long lasting effects.
	H335	May cause respiratory irritation.
Precautionary statements:	P271	Use only outdoors or in a well-ventilated area.
	P273	Avoid release to the environment.
	P280c	Wear protective gloves.
	P260h	Do not breathe mist or vapour.
	P260i	Do not breathe dust/gas/mist/vapours.
	P264	Wash with plenty of water and soap thoroughly after handling.
	P311	Call a POISON CENTER or doctor/physician.
	P304 + P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
	P303 + P352	IF ON SKIN (on hair): Wash with plenty of soap and water.
	P391	Collect spillage.
	P332 + P313	If skin irritation occurs: Get medical advice/attention.
	P362	Take off contaminated clothing and wash before reuse.
	P403 + P233	Store in a well-ventilated place. Keep container tightly closed.
	P405	Store locked up.
	P501	Dispose of contents/container to hazardous or special waste collection point.

Product BAS 500 06 F

Pictogram:	
Signal words:	Danger
Hazard statements:	H319 Causes serious eye irritation. H315 Causes skin irritation. H332 Harmful if inhaled. H302 Harmful if swallowed. H317 May cause an allergic skin reaction. H304 May be fatal if swallowed and enters airways. H400 Very toxic to aquatic life. H410 Very toxic to aquatic life with long lasting effects. EUH401 To avoid risks to human health and the environment, comply with the instructions for use.

Precautionary statements:	**P280**	Wear protective gloves/clothing.
	P261	Avoid breathing vapours.
	P264	Wash contaminated body parts thoroughly after handling.
	P270	Do not eat, drink or smoke when using this product.
	P271	Use only outdoors or in a well-ventilated area.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	P301 + P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
	P331	Do NOT induce vomiting.
	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
	P330	Rinse mouth.
	P304 + P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
	P361	Remove/Take off immediately all contaminated clothing.
	P391	Collect spillage.
	P501	Dispose of contents/container to hazardous or special waste collection point.

***** P-statements in **bold** are recommended for product label

Product BAS 516 07 F

Pictogram:		
Signal words:	Warning	
Hazard statements:	H400	Very toxic to aquatic life.
	H410	Very toxic to aquatic life with long lasting effects.
	EUH401	To avoid risks to human health and the environment, comply with the instructions for use.
Precautionary statements:	P391	Collect spillage.
	P501	Dispose of contents/container to hazardous or special waste collection point.

Chapter 3: Impact on Human and Animal Health

Absorption, distribution, excretion and metabolism in mammals (SANCO/11802 data point 5.1)

Rate and extent of absorption:	Parent: Rapid absorption: Tmax ~ 1 hour: 50% (based on urinary and biliary excretion within 48 hours)
Distribution:	Parent: Widely, highest concentrations in the liver
Potential for accumulation:	None
Rate and extent of excretion:	Parent: Complete within 5 d, mainly via feces (80-90%, biliary excretion amounting to 35%), via urine 11-15% Metabolite 500M106: Complete within 5 d, mainly via feces (85-90%), via urine 16-18%
Metabolism in animals	Parent: Extensive (>95%) with more than 50 metabolites Main metabolic pathways included N-desmethoxylation, hydroxylation, cleavage of ether bond and further oxidation of the resulting molecule parts, conjugation with glucuronic acid or sulphate Metabolite 500M106: Extensive with more than 10 metabolites Main metabolic pathways included cleavage of the NO bond followed by dimerization, hydroxylation, cleavage of ether bond, conjugation with glucuronic acid or sulphate
Toxicologically significant compounds (animals and plants)	Parent and metabolites
Toxicologically significant compounds (environment)	Parent

Acute toxicity (SANCO/11802 data point 5.2)

Rat LD_{50} oral	LD_{50} > 5000 mg/kg bw
Rat LD_{50} dermal	LD_{50} > 2000 mg/kg bw
Rat LC_{50} inhalation	LC_{50} = 0.58 mg/L
Skin irritation	Irritating
Eye irritation	Not irritating
Skin sensitization (test method used and result)	Not sensitising (M & K maximisation test)
Phototoxicity	Not phototoxic

Short term toxicity (SANCO/11802 data point 5.3)

Target / critical effect	Reduced body weight, gastrointestinal tract, red blood cells, diarrhoea (dog), hepatocellular hypertrophy (rat), white blood cells and lymphatic organs (mouse), local irritation of the upper respiratory tract (rat inhalation)
Lowest relevant oral NOAEL / NOEL	90-day mouse[#]: 30 ppm (4 mg/kg bw/d)
Lowest relevant dermal NOAEL / NOEL	28-day rat: > 250 mg/kg bw/d (systemic toxicity)
Lowest relevant inhalation NOAEL / NOEL	0.01 mg/L (local toxicity) 0.3 mg/L (systemic toxicity)

[#] based on effects on body weight after 90 days in the carcinogenicity study in male mice

Genotoxicity (SANCO/11802 data point 5.4)

In vitro studies	Weight of evidence suggests no genotoxic concern
In vivo studies in somatic cells	Not genotoxic
In vivo studies in germ cells	No data, not required

Long term toxicity and carcinogenicity (SANCO/11802 data point 5.5)

Target/critical effect	Reduced body weight (rat & mouse); liver cell necrosis (rats)
Lowest relevant NOAEL / NOEL	24-months, rat/mouse: 75/30 ppm (4 mg/kg bw/d)
Carcinogenicity	No evidence of carcinogenicity

Reproductive toxicity (SANCO/11802 data point 5.6)

Reproduction target / critical effect	Reduced pup body weight gain in the presence of parental toxicity
Lowest relevant reproductive NOAEL / NOEL	Rat: 75 ppm (8.2 mg/kg bw/d)
Developmental target / critical effect	Developmental effects in rats and embryotoxicity (including malformations) in rabbits at maternally toxic doses
Lowest relevant developmental NOAEL / NOEL	Rabbit: 5 mg/kg bw/d
Lowest relevant maternal NOAEL / NOEL	Rabbit: 3 mg/kg bw/d

Neurotoxicity / Delayed neurotoxicity (SANCO/11802 data point 5.7)

Acute neurotoxicity	Not neurotoxic; NOAEL > 1000 mg/kg bw
Subchronic neurotoxicity	Not neurotoxic; NOAEL > 50 mg/kg bw/d in males and > 112 mg/kg bw/d in females (highest doses tested)

Other toxicological studies (SANCO/11802 data point 5.8)

Toxicity studies of metabolites	**Plant and livestock metabolites** **Group 2** **500M04** - LD50 oral > 2000 mg/kg bw - not irritating to skin and eye, not a skin sensitizer - 90-day dietary rat study: NOAEL 102 mg/kg bw/day (males), 316 mg/kg bw/day (females), target organs: red-blood cell system, kidney - no evidence for genotoxicity in vitro and in vivo - ADI: 0.52 mg/kg bw/day (200 SF, 90-day rat study) **Conclusion: not toxicologically relevant** **Group 3** **500M24** - no evidence for genotoxicity in vitro and in vivo - ADI: 0.0015 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) - ARfD: 0.005 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) **Conclusion: not toxicologically relevant** **500M49** - no evidence for genotoxicity in vitro - ADI: 0.0015 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) - ARfD: 0.005 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) **Conclusion: not toxicologically relevant** **500M51** - no evidence for genotoxicity in vitro - ADI: 0.0015 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) - ARfD: 0.005 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) **Conclusion: not toxicologically relevant** **Group 6** **500M76** - no evidence for genotoxicity in vitro and in vivo - ADI: 0.0015 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) - ARfD: 0.005 mg/kg bw/day (based on TTC-concept for non-genotoxic compounds) **Conclusion: not toxicologically relevant** **Metabolites identified in new metabolism studies** **500M02** no evidence for genotoxicity in vitro - ADI: 0.5 mg/kg bw/day (600 SF, extended 28-day rat study with metabolite 500M106) - ARfD: not needed **Conclusion: not toxicologically relevant** **500M106** - 28-day oral gavage rat study: NOAEL 300 mg/kg bw/day (both sexes), target organs: duodenum, liver, spleen and the hematological system - no evidence for genotoxicity in vitro and in vivo - ADI: 0.5 mg/kg bw/day (600 SF, extended 28-day rat study) - ARfD: not needed **Conclusion: not toxicologically relevant**

Water photolysis metabolites **500M60** - no evidence for genotoxicity in vitro and in vivo **Conclusion: not toxicologically relevant** **500M62** - by weight of evidence no conclusive alert for genotoxicity - high weight of evidence being not genotoxic based on comparison to 8 already tested metabolites and due to metabolic considerations - not genotoxic in the Ames test **Conclusion: not toxicologically relevant**

Medical data (SANCO/11802 data point 5.9)

.....	Exposure to humans may result in irritation of skin and eyes. Accidental inhalation of pyraclostrobin containing products was reported to result in symptoms of respiratory irritation

Summary	Value	Study	Safety factor
ADI	0.03 mg/kg bw/day	2-year study in rats	100
AOEL	0.015 mg/kg bw/day	Developmental toxicity, rabbit (maternal effects); 50% oral absorption	100
ARfD (acute reference dose)	0.03 mg/kg bw/day	Developmental toxicity, rabbit (maternal effects)	100

Dermal absorption (SANCO/11803 data point 7.3)

.....	**BAS 500 06 F (EC):** Concentrate: 3% Spray dilute: 7% **BAS 516 07 F (WG):** Concentrate: 0.2% Spray dilute 5%

Acceptable exposure scenarios (including method of calculation)

BAS 500 06 F (EC formulation) in cereal crops and maize

Operator	BBA-model with PPE: 6% of AOEL UK-POEM with PPE: 165% of AOEL Refined risk assessment based on field exposure data, with PPE: 0.3% of AOEL		
Workers	Draft EUROPOEM with adopted TC-factor:		
	No PPE:	35% of AOEL	
Bystanders	German model:		
	Adults:	5.4% of AOEL	
	Children:	4.2% of AOEL	
Residents	German model:		
	Adults:	0.7% of AOEL	
	Children:	1.5% of AOEL	

BAS 516 07 F (WG formulation) in potatoes

Operator	BBA-model without PPE:		
	Pyraclostrobin:	3.7% of AOEL	
	Boscalid:	1.9% of AOEL	
	UK-POEM without PPE:		
	Pyraclostrobin:	23% of AOEL	
	Boscalid:	12% of AOEL	
Workers	Draft EUROPOEM with adopted TC-factor:		
	No PPE:		
	Pyraclostrobin:	3.3% of AOEL	
	Boscalid:	1.6% of AOEL	
Bystanders	German model:		
	Pyraclostrobin:	Adults:	0.26% of AOEL
		Children:	0.21% of AOEL
	Boscalid:	Adults:	0.12% of AOEL
		Children:	0.10% of AOEL
Residents	German model:		
	Pyraclostrobin:	Adults:	0.03% of AOEL
		Children:	0.09% of AOEL
	Boscalid:	Adults:	0.02% of AOEL
		Children:	0.04% of AOEL

Chapter 4: Residues

Metabolism in plants (SANCO/11802 data point 6.2.1 and 6.7)

Plant groups covered	Fruits (grapes), root and tuber vegetables (potatoes), leafy vegetables (Chinese cabbage), cereals (wheat, rice)
Rotational crops	Root crop (radish), leafy vegetables (lettuce), cereals (wheat)
Metabolism in rotational crops similar to metabolism in primary crops?	Yes
Processed commodities	Stable under hydrolysis conditions according to OECD 507, degradation to 500M07, 500M04 and 500M49 under conditions of oil processing (deodorization step, 190°C and 240°C)
Residue pattern in processed commodities similar to residue pattern in raw commodities	Yes
Plant residue definition for monitoring	Pyraclostrobin (parent)
Plant residue definition for risk assessment	Pyraclostrobin (parent)
Conversion factor (monitoring to risk assessment)	None

Metabolism in livestock (SANCO/11802 data points 6.2.2 to 6.2.5 and 6.7)

Animals covered	Ruminant (goat), poultry (hen), fish (rainbow trout)
Time needed to reach a plateau concentration in milk and eggs	Goat - milk: about 3 days Poultry - eggs: not reached after 7 days of dosing
Animal residue definition for monitoring	Pyraclostrobin (parent)
Animal residue definition for risk assessment	Pyraclostrobin and its metabolites analysed as the hydroxypyrazoles BF 500-5 (synonym 500M04) and BF 500-8 (synonym 500M85), sum expressed as pyraclostrobin
Conversion factor (monitoring to risk assessment)	Liver (w/o poultry): 4 All other: 1
Metabolism in rat and ruminant similar (yes/no)	Yes
Fat soluble residue: (yes/no)	Yes

Residues in succeeding crops (SANCO/11802 data point 6.6)

30, 120, 365 days plant back interval after application of 0.9 kg as/ha: TRR in the edible parts for human consumption are very low (radish roots, lettuce: < 0.040 mg/kg; wheat grain: < 0.089 mg/kg). No accumulation of pyraclostrobin or its degradation products (radish, lettuce: < 0.0106 mg/kg; wheat straw: < 0.0147 mg/kg; wheat grain: not detectable)

Stability of residues (SANCO/11802 data point 6.1)

Food of plant origin (peanut nutmeat, peanut oil, wheat grain, wheat straw, sugar beet tops, sugar beet roots, tomatoes, grape juice): Pyraclostrobin and metabolite BF 500-3 stable for 18 months (peanut) or 25 months (all other matrices) Food of animal origin: Pyraclostrobin stable for 8 month, metabolite BF 500-10 (model compound) with slow degradation, but sufficiently stable to evaluate the submitted feeding study (analysed within 6 month)

Residues from livestock feeding studies (SANCO/11802 data point 6.4)

Intakes by livestock ≥ 0.1 mg/kg diet (dry weight basis) (yes/no – if yes, specify the level):	Ruminant: Yes 4.0 mg/kg (dairy) 6.9 mg/kg (beef)	Poultry: Yes 0.65 mg/kg	Pig: Yes 1.2 mg/kg
Potential for accumulation (yes/no):	Yes	Yes	Yes
Metabolism studies indicate potential level of residue ≥ LOQ in edible tissue	Yes	No	Yes
Muscle	< 0.05* / < 0.1**		< 0.05*
Liver	< 0.05* / 0.2**		< 0.05*
Kidney	< 0.05* / < 0.1**		< 0.05*
Fat	< 0.05* / < 0.1**		< 0.05*
Milk	< 0.01* / < 0.02**		n.a.
Eggs	n.a.		n.a.

* Residue levels according to the enforcement method used during the cow feeding study
** Residue levels according to the data generation method used during the cow feeding study

Summary of critical residue data (SANCO/11802 data point 6.3)

Crop	Northern/ Southern field or glasshouse	Trial results relevant to the critical GAP (a)	Recommendation/comments	MRL	HR (b)	STMR (c)
Potatoes	Northern EU	<0.02 (10x) mg/kg		0.02	<0.02	<0.02
Potatoes	Southern EU	<0.01 (4x) mg/kg, <0.02 (6x) mg/kg		0.02	<0.02	<0.02
Barley (grain)	Northern EU	0.02, 0.039, 0.043, 0.056, 0.06, 0.065, 0.07 (2x), 0.08, 0.09 (2x), 0.10, 0.11, 0.21, 0.22, 0.25, 0.35, 0.82 mg/kg		1	0.82	0.085
Barley (grain)	Southern EU	<0.01 (3x), 0.02 (2x), 0.029, 0.03 (3x), 0.039, 0.06, 0.09, 0.11, 0.13, 0.14, 0.15, 0.27, 0.54 mg/kg		1	0.54	0.035
Barley (straw)	Northern EU	0.50, 0.93, 1.17, 1.28, 1.4, 1.9, 2.09, 2.1, 2.42, 2.5, 2.61, 2.75, 2.8, 2.9, 3.98, 4.1, 6.3, 8.73 mg/kg		*(15)*	8.73	2.46
Barley (straw)	Southern EU	<0.01, 0.56, 0.77, 0.79, 0.81, 0.98, 1.04, 1.2, 1.3, 1.94, 2.1, 2.42, 2.87, 3.05, 3.36, 3.53, 3.79, 4.24 mg/kg		*(15)*	4.24	1.62
Oats (grain)	Northern EU	0.02, 0.039, 0.043, 0.056, 0.06, 0.065, 0.07 (2x), 0.08, 0.09 (2x), 0.10, 0.11, 0.21, 0.22, 0.25, 0.35, 0.82 mg/kg		1	0.82	0.085
Oats (grain)	Southern EU	<0.01 (3x), 0.02 (2x), 0.029, 0.03 (3x), 0.039, 0.06, 0.09, 0.11, 0.13, 0.14, 0.15, 0.27, 0.54 mg/kg		1	0.54	0.035
Oats (straw)	Northern EU	0.50, 0.93, 1.17, 1.28, 1.4, 1.9, 2.09, 2.1, 2.42, 2.5, 2.61, 2.75, 2.8, 2.9, 3.98, 4.1, 6.3, 8.73 mg/kg		*(15)*	8.73	2.46
Oats (straw)	Southern EU	<0.01, 0.56, 0.77, 0.79, 0.81, 0.98, 1.04, 1.2, 1.3, 1.94, 2.1, 2.42, 2.87, 3.05, 3.36, 3.53, 3.79, 4.24 mg/kg		*(15)*	4.24	1.62
Wheat (grain)	Northern EU	<0.01 (7x), 0.016, 0.02 (3x), 0.03, 0.039, 0.14 mg/kg		0.2	0.14	0.013
Wheat (grain)	Southern EU	<0.01 (7x), 0.01 (2x), 0.011, 0.015, 0.017, 0.02 (2x) mg/kg		0.2	0.02	0.01

Crop	Northern/ Southern field or glasshouse	Trial results relevant to the critical GAP (a)	Recommendation/comments	MRL	HR (b)	STMR (c)
Wheat (straw)	Northern EU	0.30, 0.37, 0.41, 0.51, 1.1, 1.3 (2x), 1.74, 1.9, 1.98, 3.01, 5.43, 5.5, 5.76 mg/kg		*(10)*	5.76	1.52
Wheat (straw)	Southern EU	0.45, 0.46, 0.85, 0.95 (2x), 1.5, 1.55, 1.6, 2.26, 4.08, 4.17, 4.18, 4.40, 6.96 mg/kg		*(10)*	6.96	1.575
Rye (grain)	Northern EU	<0.01 (7x), 0.016, 0.02 (3x), 0.03, 0.039, 0.14 mg/kg		0.2	0.14	0.013
Rye (grain)	Southern EU	<0.01 (7x), 0.01 (2x), 0.011, 0.015, 0.017, 0.02 (2x) mg/kg		0.2	0.02	0.01
Rye (straw)	Northern EU	0.30, 0.37, 0.41, 0.51, 1.1, 1.3 (2x), 1.74, 1.9, 1.98, 3.01, 5.43, 5.5, 5.76 mg/kg		*(10)*	5.76	1.52
Rye (straw)	Southern EU	0.45, 0.46, 0.85, 0.95 (2x), 1.5, 1.55, 1.6, 2.26, 4.08, 4.17, 4.18, 4.40, 6.96 mg/kg		*(10)*	6.96	1.575
Maize (grain)	Northern EU	13x <0.01 mg/kg		0.02	<0.01	<0.01
Maize (grain)	Southern EU	12x <0.01 mg/kg		0.02	<0.01	<0.01
Maize (green plant)	Northern EU	0.11, 0.21, 0.22, 0.25 (2x), 0.28, 0.30, 0.33, 0.36, 0.42, 0.43, 0.44, 0.66, 0.71, 0.84 mg/kg		*(1.5)*	0.84	0.33
Maize (green plant)	Southern EU	0.15, 0.18, 0.21 (2x), 0.26, 0.27, 0.29, 0.34, 0.50, 0.57, 0.60, 0.74, 0.76, 0.89 mg/kg		*(1.5)*	0.89	0.315
Sweet corn	Northern EU	<0.01 (3x), 0.011, 0.015, 0.039 mg/kg		0.07	0.039	0.011
Sweet corn	Southern EU	<0.01 (4x) mg/kg		0.07	<0.01	<0.01

(a) Number of trials in which particular residue levels were reported e.g. "<0.01 (3x), 0.01" means in 3 trials residue levels of <0.01 mg/kg and in 1 trial a residue level of 0.01mg/kg were found

(b) Highest Residue

(c) Supervised Trials Median Residue i.e. the median residue level estimated on the basis of supervised trials relating to the critical GAP

Consumer risk assessment (SANCO/11802 data point 6.9)

ADI	0.03 mg/kg bw/day
TMDI (% ADI) according to EFSA PRIMo model rev 2	77% (German child)
NEDI (% ADI)	Not applicable
Factors included in IEDI and NEDI	MRLs established for food of plant and animal origin (except liver)
ARfD	0.03 mg/kg bw/day
IESTI (% ARfD)	17% (sweet corn), 10% (potatoes, wheat grain), 6% (barley grain)
NESTI (% ARfD) according to national large portion consumption data	Not applicable
Factors included in IESTI and NESTI	Indicative assessment using MRLs

Processing factors (SANCO/11802 data point 6.5), based on BAS 500 F residues

Crop / processed crop	Number of studies	Transfer factor	% Transference*
Barley grain / brewing malt	4	1.2	
Barley grain / beer	4	0.7	
Barley grain / pot barley	1	0.7	
Wheat grain / bran	4	2.72	
Wheat grain / flour	4	<0.30	
Wheat grain / germ	4	0.68	
Wheat grain / middlings	4	1.82	
Wheat grain / shorts	4	2.78	
Wheat grain / gluten	4	1.61	
Wheat grain / gluten feed meal	4	0.64	
Wheat grain / starch	4	0.27	
Wheat grain / whole meal flour	4	0.83	
Wheat grain / whole grain bread	4	0.30	
Oat grain / flour	4	0.12	
Oat grain / groats / rolled oats	4	0.06	
Oat grain / husks	4	2.34	
Oat grain / dust	4	5.23	
Oat grain / bran	4	0.30	

Crop / processed crop	Number of studies	Transfer factor	% Transference*
Maize whole plant / chopped fodder	4	1.22	
Maize whole plant / silage	4	1.38	
Maize grain / refined oil	4	1	
Maize grain / flour	4	1.25	
Maize grain / meal	4	1.5	
Maize grain / starch	4	1	
Maize grain / bran	4	1	
Maize grain / middlings	4	1	
Maize grain / gluten	4	1	
Maize grain / gluten feed meal	4	1	
Maize grain / steep water	4	1	
Maize grain / germs	4	1	
Maize grain / press cake	4	1	

* Calculated on the basis of distribution in the different portions, parts, or products as determined through balance studies

Proposed MRLs (mg/kg) (SANCO/11802 data point 6.7) – for intended uses; for all others see Document E1

Potatoes:	0.02
Sweet corn:	0.07
Barley and oat grain:	1
Maize grain:	0.02
Wheat and rye grain:	0.2
Food of animal origin (including fish, except milk):	0.05
Food of animal origin – milk:	0.01

Chapter 5: Fate and Behaviour in the Environment

Route of degradation (aerobic) in soil (SANCO/11802 data point 7.1.1.1)

Mineralization after 100 days	4-7% (tolyl label) 5% (chlorophenyl label) 2.2% (pyrazole label)
Non-extractable residues after 100 days	37-55% (tolyl label) 56% (chlorophenyl label) 53% (pyrazole label)
Metabolites requiring further consideration - name and/or code, % of applied (range and maximum)	BF 500-6, max. 31% after 120 days BF 500-7, max. 13% after 62 days

Route of degradation in soil (anaerobic and photolysis) (SANCO/11802 data points 7.1.1.2 and 7.1.1.3)

Anaerobic degradation

Mineralization after 100 days	~2% (tolyl label) 0% (chlorophenyl label)
Non-extractable residues after 100 days	61% (tolyl label) 37% (chlorophenyl label)
Metabolites requiring further consideration - name and/or code, % of applied (range and maximum)	BF 500-3, max. 96% after 7 days BF 500-4 + 2 isomers, max. 11.1, 7.3 and 11.4% BF 500-5, max. 7.7%

Soil photolysis

Metabolites requiring further consideration - name and/or code, % of applied (range and maximum)	after 15 days: 64-74% parent, 12% bound residues, 2% CO_2 no photodegradates for further consideration

Rate of degradation in soil (SANCO/11802 data point 7.1.2 and SANCO/11803 data point 9.1.1)

Method of calculation	FOCUS Kinetics (2006)
Laboratory studies (range or median, with n value, with r^2 value) - DT_{50}	Pyraclostrobin DT_{50lab} (aerobic, non-normalised trigger endpoints): 11.2 – 97.9 d (n=10) DT_{50lab} (20°C, pF2, aerobic, normalised modelling endpoints): 15.6 – 95.4 d (n=10) BF 500-3 Transient metabolite, no reliable endpoints could be derived.

Rate of degradation in soil (SANCO/11802 data point 7.1.2 and SANCO/11803 data point 9.1.1)

	BF 500-4 DT_{50lab} (aerobic, non-normalised trigger endpoints): 0.2 – 1.8 d (n=3) DT_{50lab} (20°C, pF2, aerobic, normalised modelling endpoints): 1.6 – 6.1 d (n=3) BF 500-5 DT_{50lab} (aerobic, non-normalised trigger endpoints): 0.09 – 0.24 d (n=3) DT_{50lab} (20°C, pF2, aerobic, normalised modelling endpoints): 0.4 – 0.7 d (n=3) BF 500-6 DT_{50lab} (aerobic, non-normalised trigger endpoints): 95.9 – >1000 d (n=6) DT_{50lab} (20°C, pF2, aerobic, normalised modelling endpoints): 59.2 – 903.2 d (n=8) BF 500-7 DT_{50lab} (aerobic, non-normalised trigger endpoints): 81.5 - >1000 d (n=7) DT_{50lab} (20°C, pF2, aerobic, normalised modelling endpoints): 51.3 - 729 d (n=6)
Laboratory studies (range or median, with n value, with r^2 value) - DT_{90}	Pyraclostrobin DT_{90lab} (aerobic, non-normalised trigger endpoints): 87.7 – >1000 d (n=6) BF 500-3 Transient metabolite, no reliable endpoints could be derived. BF 500-4 DT_{90lab} (aerobic, non-normalised trigger endpoints): 3.9 – 13.5 d (n=3) BF 500-5 DT_{90lab} (aerobic, non-normalised trigger endpoints): 1.77 – 2.95 d (n=3) BF 500-6 DT_{90lab} (aerobic, non-normalised trigger endpoints): 318.7 – >1000 d (n=6) BF 500-7 DT_{90lab} (aerobic, non-normalised trigger endpoints): 270.7 – >1000 d (n=7)

Rate of degradation in soil (SANCO/11802 data point 7.1.2 and SANCO/11803 data point 9.1.1)

Laboratory studies - 10°C	DT_{50lab} (10°C, aerobic, calculated from normalised modeling endpoints at 20°C): 40.3 – 246.1 d (n=10)
Laboratory studies - anaerobic	Pyraclostrobin DT_{50lab} (20°C): 1.5 – 2.3 d (1 soil, 2 labels) BF 500-3 DT_{50lab} (20°C): 56.9 – 70.1 d (1 soil, 2 labels) BF 500-4 DT_{50lab} (20°C): 7.8 – 11.3 d (1 soil, 2 labels) BF 500-5 DT_{50lab} (20°C): 32.2 d (1 soil, 1 label)
Degradation in saturated zone	not relevant
Field studies (state location, range or median with n value)	**Best-fit trigger endpoints** Data from field dissipation study without soil coverage Pyraclostrobin DT_{50f}: 5.2 – 55.8 d 6 locations (3 Germany, 2 Spain, 1 Sweden) **Normalized modelling endpoints (20°C, pF2)** Data from both field studies (with and without sand cover to prevent surface processes) Pyraclostrobin DT_{50f}: 15.5 – 181.2 d 8 locations: uncovered: 3 Germany, 1 Sweden covered: 1 Denmark, 1 Germany, 1 Southern France, 1 Italy BF 500-6 DT_{50f}: 163.5 – 361.4 d 3 locations covered: 1 Denmark, 1 Germany, 1 Southern France BF 500-7 DT_{50f}: 183.7 – 460.1 d 3 locations covered: 1 Denmark, 1 Germany, 1 Southern France

Rate of degradation in soil (SANCO/11802 data point 7.1.2 and SANCO/11803 data point 9.1.1)

	Non-normalized trigger endpoints Data from field dissipation study without soil coverage DT_{90f} : 80.3 – 347.8 d 6 locations (3 Germany, 2 Spain, 1 Sweden)
Soil accumulation and plateau concentration	Not required for pyraclostrobin. Accumulation potential for BF 500-6 and BF 500-7 addressed by modelling.

Soil adsorption/desorption (SANCO/11802 data point 7.1.3)

K_{foc} / K_{oc}	Pyraclostrobin $K_{f,oc}$: 6000 – 16000 mL g^{-1} (arithmetic mean 9304 mL g^{-1}, n=10) 1/n: 0.86 – 1.03 (arithmetic mean 0.95, n=6) BF 500-3 $K_{f,oc}$: 4240 – 12000 mL g^{-1} (arithmetic mean 9315 mL g^{-1}, n=6) 1/n: 0.77 – 0.94 (arithmetic mean 0.83, n=6) BF 500-4 K_{oc} : 6871 – 15748 mL g^{-1} (arithmetic mean 9819 mL g^{-1}, n=5) BF 500-5 $K_{f,oc}$: 400 – 831 mL g^{-1} (arithmetic mean 705 mL g^{-1}, n=5) 1/n: 0.80 – 0.88 (arithmetic mean 0.85, n=5) BF 500-6 K_{oc} : 3360 – 311704 mL g^{-1} (median 107301 mL g^{-1}, n=11) BF 500-7 K_{oc} : 4020 – 801927 mL g^{-1} (median 149900 mL g^{-1}, n=11)

Soil adsorption/desorption (SANCO/11802 data point 7.1.3)

K_f / K_d	Pyraclostrobin K_f: 30 – 368 mL g^{-1} (n=6) BF 500-3 K_f: 47 – 354 mL g^{-1} (n=6) BF 500-4 K_d: 64 – 291 mL g^{-1} (n=5) BF 500-5 K_f: 5 – 15 mL g^{-1} (n=5) BF 500-6 K_d: 84 – 1985 mL g^{-1} (n=11) BF 500-7 K_d: 100.5 – 5025 mL g^{-1} (n=11)
pH dependence (yes / no) (if yes type of dependence)	No (for parent and all metabolites)

Mobility in soil (SANCO/11802 data points 7.1.4 and SANCO/11803 data points 9.1.2)

Column leaching	0% in leachate, all radioactivity in top soil layer
Aged residues leaching	0% in leachate, all radioactivity in top soil layer
Lysimeter/field leaching studies	based on K_{oc} and DT_{50} values no leaching expected

PEC (soil) (SANCO/11803 data point 9.1.3)

Parent

Method of calculation	SFO kinetic model after FOCUS Kinetics (2006) Depth of the soil layer: 5 cm Soil bulk density 1.5 g cm^{-3} <u>Substance parameters:</u> $DT_{50,soil}$ (d): 55.8 worst-case of field dissipation studies (n=4, non-normalised)
Application rate	2 x 250 g a.s. ha^{-1} to cereals (BBCH 25), 21 day interval (worst case scenario) Plant interception: 50% (first application), 70% (second application)

$PEC_{(s)}$		Multiple application Actual	Multiple application Time weighted average
Initial		0.228	-
Short term	24h	0.226	0.227
	2d	0.223	0.226
	4d	0.217	0.223
Long term	7d	0.209	0.219
	14d	0.192	0.210
	21d	0.176	0.201
	28d	0.161	0.193
	50d	0.123	0.174
	100d	0.066	0.146

BF 500-6

Method of calculation	SFO kinetic model after FOCUS Kinetics (2006) Depth of the soil layer: 5 cm Soil bulk density 1.5 g cm^{-3} <u>Substance parameters:</u> $DT_{50,soil}$ (d): 1000 worst-case default Max. occurrence in soil from lab and field data: 31% (lab) Molar mass correction factor: 0.788 (metabolite is a dimer)
Application rate	2 x 250 g a.s. ha^{-1} to cereals (BBCH 25), 21 day interval (worst case scenario) Plant interception: 50% (first application), 70% (second application)
Potential accumulation ($PEC_{soil,accu}$)	SFO kinetic model after FOCUS Kinetics (2006) Depth of soil layer: 5 cm Depth of tillage layer: 20 cm Soil bulk density 1.5 g cm^{-3}

$PEC_{(s)}$		Multiple application Actual	Multiple application Time weighted average
Initial		0.065	-
Short term	24h	0.065	0.065
	2d	0.064	0.065
	4d	0.064	0.064
Long term	7d	0.064	0.064
	14d	0.064	0.064
	21d	0.064	0.064
	28d	0.063	0.064
	50d	0.062	0.063
	100d	0.060	0.062

PEC_{soil} values for potential accumulation calculations of BF 500-6 after multi-year application of 250 g a.s. ha^{-1} to cereals*

PEC		Value [mg kg^{-1}]	Comment
$PEC_{soil,plateau}$	(over 20 cm)	0.057	
$PEC_{soil,max}$	(over 5 cm)	0.065	see table above
$PEC_{soil,accu}$	(over 5 cm)	0.121	= $PEC_{soil,plateau}$ + $PEC_{soil,max}$

* covering other crop uses/use patterns

BF 500-7

Method of calculation	SFO kinetic model after FOCUS Kinetics (2006) Depth of the soil layer: 5 cm Soil bulk density 1.5 g cm^{-3} <u>Substance parameters:</u> $DT_{50,soil}$ (d): 1000 worst-case default Max. occurrence in soil from lab and field data: 19% (field) Molar mass correction factor: 0.768 (metabolite is a dimer)
Application rate	2 x 250 g a.s. ha^{-1} to cereals (BBCH 25), 21 day interval (worst case scenario) Plant interception: 50% (first application), 70% (second application)
Potential accumulation ($PEC_{soil,accu}$)	SFO kinetic model after FOCUS Kinetics (2006) Depth of soil layer: 5 cm Depth of tillage layer: 20 cm Soil bulk density 1.5 g cm^{-3}

$PEC_{(s)}$		Multiple application Actual	Multiple application Time weighted average
Initial		0.039	-
Short term	24h	0.039	0.039
	2d	0.039	0.039
	4d	0.038	0.039
Long term	7d	0.038	0.038
	14d	0.038	0.038
	21d	0.038	0.038
	28d	0.038	0.038
	50d	0.037	0.038
	100d	0.036	0.037

PEC_{soil} values for potential accumulation calculations of BF 500-7 after multi-year application of 250 g a.s. ha^{-1} to cereals*

PEC		**Value [mg kg^{-1}]**	**Comment**
$PEC_{soil,plateau}$	(over 20 cm)	0.034	
$PEC_{soil,max}$	(over 5 cm)	0.039	see table above
$PEC_{soil,accu}$	(over 5 cm)	0.073	= $PEC_{soil,plateau}$ + $PEC_{soil,max}$

* covering other crop uses/use patterns

Route and rate of degradation in water (SANCO/11802 data point 7.2 and SANCO/11803 data point 9.2)

Hydrolysis of active substance and relevant metabolites > 10% (DT_{50}) (state pH and temperature)	pH 5: at 25°C, stable
	pH 7: at 25°C, stable
	pH 9: at 25°C, very slow hydrolysis
Photolytic degradation of active substance and relevant metabolite above 10%	DT_{50} parent: <2 hours CO_2: after 25 days 22% with chlorophenyl-label, about 4% with tolyl-label 33 minor metabolites (<10%) 5 major metabolites: BF 500-11: max. 45% after 21 days BF 500-13: max. 17% after 6 days BF 500-14: max. 21% after 3 hours BF 500-15: max. 27% after 1 day 500M58: max. 23% after 1 day

Readily biodegradable (yes/no)	no
Water/sediment study Degradation in water/sediment - DT_{50} water - DT_{90} water - DT_{50} whole system - DT_{90} whole system	 0.44 (DFOP) – 2.30 d (DFOP), two labels, two systems (n=4) 6.19 (DFOP)– 28.8 d (FOMC), two labels, two systems (n=4) 23.3 (SFO) – 26.8 d (SFO), two labels, one system (n=2) 77.4 (SFO) – 89.1 d (SFO), two labels, one system (n=2)
Mineralisation	about 5% after 100 days
Non-extractable residues	pond system 62%, river system 54% after 100 days
Distribution in water / sediment systems (active substance)	pond system: sediment max. 53% after 14 days, decreasing to 7% after 100 days river system: sediment max. 62% after 2 days, decreasing to 10% after 100 days
Distribution in water / sediment systems (metabolites)	<u>in water:</u> BF 500-3: max. 2% <u>in sediment:</u> BF 500-3: max. 12% (pond) after 100 days; max. 66% (river) after 14 days, decreasing to 29% after 100 days BF 500-6: max. 7% after 61 days (only in pond) BF 500-7: max. 6% after 61 days (only in pond)
Irradiated water/sediment study (two studies) Degradation in water/sediment - DT_{50} water - DT_{90} water - DT_{50} whole system - DT_{90} whole system	 0.3 (DFOP) – 4.47 d (SFO), two labels, two systems (n=2) 3.9 (DFOP) – 14.84 d (SFO), two labels, two systems (n=2) 7.22 (SFO) – 13.5 d (HS), two labels, two systems (n=2) 23.98 (SFO) – 54.4 d (HS), two labels, two systems (n=2)
Mineralisation	<u>Study #1 (calculated from material balance difference):</u> about 23% after 62 days (chlorophenyl-label) about 7% after 62 days (tolyl-label) <u>Study #2 (measured):</u> max. 4.2% after 42 days (chlorophenyl-label) max. 3.4% after 42 days (tolyl-label)
Non-extractable residues	<u>Study #1:</u> 28% after 62 days (chlorophenyl-label) 26% after 62 days (tolyl-label) <u>Study #2:</u> max. 62.9% after 42 days (chlorophenyl-label) max. 65.9% after 42 days (tolyl-label)

Distribution in water / sediment systems (active substance)	Study #1: water: <1% after 62 days sediment: max. 18% after 7 days, decreasing to 0.3% after 62 days Study #2: water: <1% after 42 days sediment: max. 63.0% after 3 days (chlorophenyl-label) max. 54.2% after 3 days (tolyl-label)
Distribution in water / sediment systems (metabolites)	Study #1: water: BF 500-11: max. 11% after 21 days, DT_{50} 20 days BF 500-13: max. 16% after 62 days, DT_{50} see aqueous photolysis study BF 500-14: max. 11% after 10 days, DT_{50} 14 days sediment: BF 500-3: max. 16-17% after 30 days, DT_{50} 99 days Study #2: same metabolites detected as in study #1, but in amounts < 5%

PEC (surface water and sediment) (SANCO/11803 data point 9.2.5)

Parent

Parameters used in $FOCUS_{SW}$ Step 1 and Step 2	Molar mass (g mol^{-1}): 387.8 Water solubility (mg L^{-1}): 1.9 (20°C) $K_{f,oc}$ (L kg^{-1}): 9304, arithmetic mean (n=6) $DT_{50,soil}$ (d): 28.3, bias-corrected geometric mean of field studies (n=8, normalized to 20°C, pF2) Tier 1 (endpoints derived from dark w/s study) $DT_{50,whole\ system}$ (d): 25, geometric mean $DegT_{50}$, two labels, one dark w/s system $DT_{50,water}$ (d): 1000, worst-case default $DT_{50,sediment}$ (d): 25 = $DegT_{50,whole\ system}$ Tier 2 (endpoints derived from irradiated w/s study) $DT_{50,whole\ system}$ (d): 7.2, $DegT_{50}$ from level P-I analysis, two labels $DT_{50,water}$ (d): 7.5, $DegT_{50}$ from level P-II analysis, two labels $DT_{50,sediment}$ (d): 6.5, $DegT_{50}$ from level P-II analysis, two labels

Parameters used in $FOCUS_{SW}$ Step 3 and Step 4	Molar mass (g/mol): 387.8 Water solubility (mg/L): 1.9 (20°C) $K_{f,oc}$ (L kg^{-1}): 9304, arithmetic mean (n=6) 1/n: 0.95, arithmetic mean (n=6) $DT_{50,soil}$ (d): 28.3, bias-corrected geometric mean of field studies (n=8, normalized to 20°C, pF2) Foliar DT_{50}: 10 d, default Wash-off coefficient: 0.5 cm^{-1} PRZM, 0.05 mm^{-1} MACRO, default Crop uptake factor: 0 <u>Tier 1 (endpoints derived from dark w/s study)</u> $DT_{50,whole\ system}$ (d): 25, geometric mean $DegT_{50}$, two labels, one dark w/s system $DT_{50,water}$ (d): 1000, worst-case default $DT_{50,sediment}$ (d): 25 = $DegT_{50,whole\ system}$ <u>Tier 2 (endpoints derived from irradiated w/s study)</u> $DT_{50,whole\ system}$ (d): 7.2, $DegT_{50}$ from level P-I analysis, two labels $DT_{50,water}$ (d): 7.5, $DegT_{50}$ from level P-II analysis, two labels $DT_{50,sediment}$ (d): 6.5, $DegT_{50}$ from level P-II analysis, two labels
Application rate	Single and twofold application of 250 g a.s. ha^{-1} to winter and spring cereals (BBCH 25, 21 days interval) Single application of 200 g a.s. ha^{-1} to maize (BBCH 30) Single and fourfold application of 17 g a.s. ha^{-1} to potatoes (BBCH 51, 10 days interval)
Main routes of entry	Spray drift, runoff, drainage

Tier 1 (parameters from dark water/sediment study)

Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 250 g a.s. ha^{-1} to winter cereals (Tier 1)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	17.031	-[a]	-[a]	1160
Step 2	-	2.299	-[a]	-[a]	187.230
Step 3	-	1.589 D2 d, single	1.509 D2 d, single	1.437 D2 d, single	10.583 R4 s, twofold
Step 4	50N	0.794 D2 d, single	0.754 D2 d, single	0.718 D2 d, single	(not reported)
	75N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	90N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	5mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.467 D2 s, single	
	5mD50N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	5mD75N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD50N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD+R	0.283 R3 s, single	0.260 D2 s, single	0.247 D2 s, single	
	15mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	20mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	20mD+R	0.147 R3 s, single	0.135 D2 s, single	0.129 D2 s, single	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application, twofold = twofold application
[a] only maximum values for Step 1 and 2 are reported

Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 250 g a.s. ha^{-1} to spring cereals (Tier 1)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	17.031	-[a]	-[a]	1160
Step 2	-	2.299	-[a]	-[a]	187.230
Step 3	-	1.759 D1 d, twofold	1.680 D1 d, twofold	1.611 D1 d, twofold	9.836 D1 d, twofold
Step 4	50N	0.877 D1 d, twofold	0.837 D1 d, twofold	0.803 D1 d, twofold	(not reported)
	75N	0.437 D1 d, twofold	0.417 D1 d, twofold	0.400 D1 d, twofold	
	90N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	5mD	0.507 D1 s, single	0.433 D1 d, twofold	0.415 D1 d, twofold	
	5mD50N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	5mD75N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	10mD	0.269 D1 s, single	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	10mD50N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	10mD+R	0.269 D1 s, single	0.224 D1 d, twofold	0.215 D1 d, twofold	
	15mD	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	20mD	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, single + R4 s, twofold	
	20mD+R	0.140 D1 s, single	0.113 D1 d, twofold	0.109 D1 d, twofold	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application, twofold = twofold application
[a] only maximum values for Step 1 and 2 are reported

Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 200 g a.s. ha^{-1} to <u>maize</u> (Tier 1)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	6.813	-[a]	-[a]	462.701
Step 2	-	1.839	-[a]	-[a]	95.014
Step 3	-	1.037 D3 d, single	0.797 D3 d, single	0.508 D3 d, single	6.572 R4 s, single
Step 4	50N	0.519 D3 d, single	0.398 D3 d, single	0.254 D3 d, single	(not reported)
	75N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	90N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	5mD	0.425 R3 s, single	0.264 R4 s, single	0.219 R4 s, single	
	5mD50N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	10mD	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	10mD+R	0.225 R3 s, single	0.138 D3 d, single	0.100 R4 s, single	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application
[a] only maximum values for Step 1 and 2 are reported

Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 17 g a.s. ha^{-1} to <u>potatoes</u> (Tier 1)

FOCUS level	SURFACE WATER			SEDIMENT
	Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	2.316	-[a]	-[a]	157.319
Step 2	0.164	-[a]	-[a]	14.142
Step 3	0.088 D3 d + D6 d, single	0.065 D6 d, single	0.043 D6 d, multiple	1.550 R1 s, multiple

s = stream, d = ditch
single = single application, multiple = multiple application
[a] only maximum values for Step 1 and 2 are reported

Tier 2 (parameters from irradiated water/sediment study)

Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 250 g a.s. ha^{-1} to winter cereals (Tier 2)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	17.031	-[a]	-[a]	1160
Step 2	-	2.299	-[a]	-[a]	177.447
Step 3	-	1.589 D2 d, single	1.491 D2 d, single	1.406 D2 d, single	9.724 R4 s, twofold
Step 4	90N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	(not reported)
	5mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.456 D2 s, single	
	5mD50N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	5mD75N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD50N	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	10mD+R	0.283 R3 s, single	0.257 D2 s, single	0.242 D2 s, single	
	15mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	20mD	0.543 R4 s, twofold	0.532 R4 s, twofold	0.394 R4 s, twofold	
	20mD+R	0.147 R3 s, single	0.133 D2 s, single	0.126 D2 s, single	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application, twofold = twofold application
[a] only maximum values for Step 1 and 2 are reported

Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 250 g a.s. ha^{-1} to spring cereals (Tier 2)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	17.031	-[a]	-[a]	1160
Step 2	-	2.299	-[a]	-[a]	177.447
Step 3	-	1.588 D1 d, single	1.474 D1 d, single	1.375 D1 d, single	5.314 R4 s, single
Step 4	50N	0.794 D1 d, single	0.737 D1 d, single	0.687 D1 d, single	(not reported)
	75N	0.397 D1 d, single	0.368 D1 d, single	0.344 D1 d, single	
	90N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	5mD	0.507 D1 s, single	0.399 D1 d, single	0.373 D1 d, single	
	5mD50N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	5mD75N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	10mD	0.269 D1 s, single	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	10m50N	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	10mD+R	0.269 D1 s, single	0.212 D1 d, single	0.198 D1 d, single	
	15mD	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	20mD	0.267 R4 s, single + R4 s, twofold	0.256 R4 s, single + R4 s, twofold	0.218 R4 s, twofold	
	20mD+R	0.140 D1 s, single	0.110 D1 d, single	0.103 D1 d, single	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application, twofold = twofold application
[a] only maximum values for Step 1 and 2 are reported

Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 200 g a.s. ha^{-1} to <u>maize</u> (Tier 2)

FOCUS level	Mitigation	SURFACE WATER			SEDIMENT
		Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	-	6.813	-[a]	-[a]	462.701
Step 2	-	1.839	-[a]	-[a]	91.939
Step 3	-	1.037 D3 d, single	0.776 D3 d, single	0.489 D3 d, single	6.010 R4 s, single
Step 4	50N	0.519 D3 d, single	0.388 D3 d, single	0.244 D3 d, single	(not reported)
	75N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	90N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	5mD	0.425 R3 s, single	0.264 R4 s, single	0.219 R4 s, single	
	5mD50N	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	10mD	0.301 R4 s, single	0.264 R4 s, single	0.219 R4 s, single	
	10mD+R	0.225 R3 s, single	0.135 D3 d, single	0.100 R4 s, single	

N = Drift mitigation by nozzle reduction
D = Drift mitigation by no-spray buffer zones
R = Runoff mitigation by vegetated filter strips
s = stream, d = ditch
single = single application
[a] only maximum values for Step 1 and 2 are reported

Summary of highest global PEC_{sw} and PEC_{sed} values of pyraclostrobin following application of 17 g a.s. ha^{-1} to <u>potatoes</u> (Tier 2)

FOCUS level	SURFACE WATER			SEDIMENT
	Max. PEC_{sw} [µg L^{-1}]	1-d TWA [µg L^{-1}]	2-d TWA [µg L^{-1}]	Max. PEC_{sed} [µg kg^{-1}]
Step 1	2.316	-[a]	-[a]	157.319
Step 2	0.156	-[a]	-[a]	13.003
Step 3	0.088 D3 d, single	0.062 D6 d, single	0.040 D6 d, multiple	1.327 R1 s, multiple

s = stream, d = ditch
single = single application, multiple = multiple application
[a] only maximum values for Step 1 and 2 are reported

BF 500-3

Parameters used in $FOCUS_{SW}$ step 1 and 2	BF 500-3 Molar mass (g mol^{-1}): 357.8 Water solubility (mg L^{-1}): 0.03 (20°C) $K_{f,oc}$ (L kg^{-1}): 9315, arithmetic mean (n=6) $DT_{50,soil}$ (d): 1, worst-case default (transient metabolite) Max. occurrence in soil (%): 0.01, worst-case default (no occurrence in aerobic soil) Endpoints derived from dark w/s study $DT_{50,whole\ system}$ (d): 1000, worst-case default $DT_{50,water}$ (d): 1000, worst-case default $DT_{50,sediment}$ (d): 1000, worst-case default Max. occurrence in water/sediment (%): 67.7 Endpoints derived from irradiated w/s study $DT_{50,whole\ system}$ (d): 92.5, level M-I analysis, two labels $DT_{50,water}$ (d): 92.5, level M-I analysis, two labels $DT_{50,sediment}$ (d): 92.5, level M-I analysis, two labels Max. occurrence in water/sediment (%): 21.9
Application rate	Metabolite is not applied but formed from parent in sediment only according to max. occurrence
Main routes of entry	Spray drift, runoff, drainage (of parent)

BF 500-5

Parameters used in $FOCUS_{SW}$ step 1 and 2	BF 500-5 Molar mass (g mol^{-1}): 194.6 Water solubility (mg L^{-1}): 21.8 (20°C) $K_{f,oc}$ (L kg^{-1}): 705 (arithmetic mean, n=5) $DT_{50,soil}$ (d): 1, worst-case of lab data (n=3, normalized to 20°C, pF2) Max. occurrence in soil (%): 0.01, worst-case default (no occurrence in aerobic soil) $DT_{50,whole\ system}$ (d): 1000, worst-case default $DT_{50,water}$ (d): 1000, worst-case default $DT_{50,sediment}$ (d): 1000, worst-case default Max. occurrence in water/sediment (%): 10.9 (from OECD 309 study)

Application rate	Metabolite is not applied but formed from parent in water only according to max. occurrence
Main routes of entry	Spray drift, runoff, drainage (of parent)

BF 500-6

Parameters used in $FOCUS_{SW}$ step 1 and 2	<u>BF 500-6</u> Molar mass (g mol^{-1}): 305.8 (0.5 of molar mass as metabolite is a dimer) Water solubility (mg L^{-1}): 0.003 (20°C) K_{oc} (L kg^{-1}): 107301, median (n=11) $DT_{50,soil}$ (d): 321, bias-corrected geometric mean of lab and field data (n=11, normalized to 20°C, pF2) Max. occurrence in soil (%): 31 (laboratory data) $DT_{50,whole\ system}$ (d): 1000, worst-case default $DT_{50,water}$ (d): 1000, worst-case default $DT_{50,sediment}$ (d): 1000, worst-case default Max. occurrence in water/sediment (%): 6.5 (from dark w/s study)
Application rate	Metabolite is not applied but formed from parent in soil and in sediment according to max. occurrence
Main routes of entry	Spray drift, runoff, drainage (of parent), runoff, drainage (of metabolite)

BF 500-7

Parameters used in $FOCUS_{SW}$ step 1 and 2	BF 500-7 Molar mass (g mol^{-1}): 297.8 (0.5 of molar mass as metabolite is a dimer) Water solubility (mg L^{-1}): 0.005 (20°C) K_{oc} (L kg^{-1}): 149900, median (n=11) $DT_{50,soil}$ (d): 312, bias-corrected geometric mean of lab and field data (n=9, normalized to 20°C, pF2) Max. occurrence in soil (%): 19 (field soil degradation study) $DT_{50,whole\ system}$ (d): 1000, worst-case default $DT_{50,water}$ (d): 1000, worst-case default $DT_{50,sediment}$ (d): 1000, worst-case default Max. occurrence water/sediment (%): 6.3 (from dark w/s study)
Application rate	Metabolite is not applied but formed from parent in soil and in sediment according to max. occurrence
Main routes of entry	Spray drift, runoff, drainage (of parent), runoff, drainage (of metabolite)

BF 500-11

Parameters used in $FOCUS_{SW}$ step 1 and 2	BF 500-11 Molar mass (g mol^{-1}): 277.3 Water solubility (mg L^{-1}): 1000, worst-case default K_{oc} (L kg^{-1}): $1x10^{-10}$, worst-case default $DT_{50,soil}$ (d): 1, worst-case default Max. occurrence in soil (%): 0.01, worst-case default (no occurrence in soil) $DT_{50,whole\ system}$ (d): 22.6 (level M-I analysis, two labels, irradiated w/s study) $DT_{50,water}$ (d): 22.6 (level M-I analysis, two labels, irradiated w/s study) $DT_{50,sediment}$ (d): 22.6 (level M-I analysis, two labels, irradiated w/s study) Max. occurrence in water/sediment (%): 12 (from irradiated w/s study)
Application rate	Metabolite is not applied but formed from parent in water only according to max. occurrence
Main routes of entry	Spray drift, runoff, drainage (of parent)

BF 500-13

Parameters used in $FOCUS_{SW}$ step 1 and 2	BF 500-13 Molar mass (g mol^{-1}): 247.3 Water solubility (mg L^{-1}): 1000, worst-case default K_{oc} (L kg^{-1}): $1x10^{-10}$, worst-case default Max. occurrence in soil (%): 0.01, worst-case default (no occurrence in soil) $DT_{50,whole\ system}$ (d): 1000, worst-case default $DT_{50,water}$ (d): 61.3 (aqueous photolysis study) $DT_{50,sediment}$ (d): 1000, worst-case default Max. occurrence in water/sediment (%): 17.8 (from irradiated w/s study)
Application rate	Metabolite is not applied but formed from parent in water only according to max. occurrence
Main routes of entry	Spray drift, runoff, drainage (of parent)

BF 500-14

Parameters used in $FOCUS_{SW}$ step 1 and 2	BF 500-14 Molar mass (g mol^{-1}): 387.8 Water solubility (mg L^{-1}): 1000, worst-case default K_{oc} (L kg^{-1}): $1x10^{-10}$, worst-case default Max. occurrence in soil (%): 0.01, worst-case default (no occurrence in soil) $DT_{50,whole\ system}$ (d): 17.3 (level M-I analysis, two labels, irradiated w/s study) $DT_{50,water}$ (d): 17.3 (level M-I analysis, two labels, irradiated w/s study) $DT_{50,sediment}$ (d): 17.3 (level M-I analysis, two labels, irradiated w/s study) Max. occurrence in water/sediment (%): 12.1 (from irradiated w/s study)
Application rate	Metabolite is not applied but formed from parent in water only according to max. occurrence
Main routes of entry	Spray drift, runoff, drainage (of parent)

Metabolites observed in dark water/sediment study: BF 500-3, BF 500-5, BF 500-6, BF 500-7

Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 250 g a.s. ha^{-1} to cereals (dark water/sediment study)

Cereals				
Compound	SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]		SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]	
	Step 1	Step 2	Step 1	Step 2
BF 500-3	not relevant		20.030	17.430
BF 500-6			304.937	60.344
BF 500-7			182.976	36.588
BF 500-5	0.256	0.179	not relevant	

Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 200 g a.s. ha^{-1} to maize (dark water/sediment study)

Maize				
Compound	SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]		SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]	
	Step 1	Step 2	Step 1	Step 2
BF 500-3	not relevant		8.012	7.948
BF 500-6			121.975	24.752
BF 500-7			73.190	15.040
BF 500-5	0.102	0.101	not relevant	

Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 17 g a.s. ha^{-1} to potatoes (dark water/sediment study)

Potatoes				
Compound	SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]		SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]	
	Step 1	Step 2	Step 1	Step 2
BF 500-3	not relevant		2.724	1.805
BF 500-6			41.471	4.912
BF 500-7			24.885	2.989
BF 500-5	0.035	0.016	not relevant	

Metabolites observed in irradiated water/sediment study:
BF 500-3, BF 500-11, BF 500-13, BF 500-14

Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 250 g a.s. ha^{-1} to cereals (irradiated water/sediment study)

Cereals				
Compound	SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]		SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]	
	Step 1	Step 2	Step 1	Step 2
BF 500-3	not relevant		6.507	5.090
BF 500-11	0.407	0.266	not relevant	
BF 500-13	0.533	0.413		
BF 500-14	0.573	0.352		

Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 200 g a.s. ha^{-1} to maize (irradiated water/sediment study)

Maize				
Compound	SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]		SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]	
	Step 1	Step 2	Step 1	Step 2
BF 500-3	not relevant		2.603	2.485
BF 500-11	0.163	0.158	not relevant	
BF 500-13	0.213	0.209		
BF 500-14	0.229	0.223		

Summary of highest global maximum PEC_{sw} and PEC_{sed} values of the metabolites of pyraclostrobin following application of 17 g a.s. ha^{-1} to potatoes (irradiated water/sediment study)

Potatoes				
Compound	SURFACE WATER - highest global max. PEC_{sw} [µg L^{-1}]		SEDIMENT - highest global max. PEC_{sed} [µg kg^{-1}]	
	Step 1	Step 2	Step 1	Step 2
BF 500-3	not relevant		0.885	0.511
BF 500-11	0.055	0.024	not relevant	
BF 500-13	0.072	0.041		
BF 500-14	0.078	0.031		

PEC (ground water) (SANCO/11803 data point 9.2.4)

Method of calculation and type of study (*eg.* modelling, monitoring, lysimeter)	Calculations according to *FOCUS (2000), FOCUS groundwater scenarios in the EU plant protection product review process, Sanco/321/2000; FOCUS (2009), Assessing Potential for Movement of Active Substances and their Metabolites to Groundwater in the EU Final Report of the Groundwater Workgroup of FOCUS, Sanco/13144/2010* and *FOCUS (2012), Generic guidance for Tier 1 FOCUS Ground Water Assessments, v2.1* Model/scenario: FOCUS-PEARL 4.4.4, FOCUS-PELMO 5.5.3. with all scenarios parameterized for winter and spring cereals, maize and potatoes Application every year (calculation for 26 years)
	Substance parameters: Pyraclostrobin Molar mass (g mol^{-1}): 387.8 Water solubility (mg L^{-1}): 1.9 (20°C) Vapor pressure (Pa): 2.6 x 10^{-8} (20°C) $K_{f,oc}$ (L kg^{-1}): 9304 ($K_{f,om}$: 5397), arithmetic mean (n=6) 1/n: 0.95, arithmetic mean (n=6) $DT_{50,soil}$ (d): 28.3, bias-corrected geometric mean of field studies (n=8, normalized to 20°C, pF2) TSCF/PUF (-): 0
	BF 500-3 Molar mass (g mol^{-1}): 357.8 Water solubility (mg L^{-1}): 0.03 (20°C) Vapor pressure (Pa): 1 x 10^{-10}, worst-case default $K_{f,oc}$ (L kg^{-1}): 9315 ($K_{f,om}$: 5403) (arithmetic mean, n=6) 1/n: 1, worst-case default $DT_{50,soil}$ (d): 1, worst-case default (transient metabolite) formation fraction: 1 (from parent), worst-case default (transient metabolite) TSCF/PUF (-): 0

BF 500-4

Molar mass (g mol^{-1}): 299.8

Water solubility (mg L^{-1}): 1000, worst-case default

Vapor pressure (Pa): 1 x 10^{-10}, worst-case default

K_{oc} (L kg^{-1}): 9819 ($K_{f,om}$: 5695), arithmetic mean (n=5)

1/n: 1, worst-case default

$DT_{50,soil}$ (d): 2.82, geometric mean of lab data (n=3, normalized to 20°C, pF2)

formation fraction: 1 (from BF 500-3), worst-case default (transient metabolite)

TSCF/PUF (-): 0

BF 500-5

Molar mass (g mol^{-1}): 194.6

Water solubility (mg L^{-1}): 21.8 (20°C)

Vapor pressure (Pa): 1 x 10^{-10}, worst-case default

$K_{f,oc}$ (L kg^{-1}): 705 ($K_{f,om}$: 409)

1/n: 0.85, arithmetic mean (n=5)

$DT_{50,soil}$ (d): 1, worst-case of lab data (n=3, normalized to 20°C, pF2)

formation fraction: 0.409 (from BF 500-4), from anaerobic degradation study (n=1)

TSCF/PUF (-): 0

BF 500-6

Molar mass (g mol^{-1}): 305.8 (0.5 of molar mass as metabolite is a dimer)

Water solubility (mg L^{-1}): 0.003 (20°C)

Vapor pressure (Pa): 1 x 10^{-10}, worst-case default

$K_{f,oc}$ (L kg^{-1}): 107301 ($K_{f,om}$: 62240), median (n=11)

1/n: 1, worst-case default

$DT_{50,soil}$ (d): 321, bias-corrected geometric mean of lab and field data (n=11, normalized to 20°C, pF2)

formation fraction: 0.293 (from BF 500-4), arithmetic mean of lab and field data (n=9)

TSCF/PUF (-): 0

	BF 500-7 Molar mass (g mol^{-1}): 297.8 (0.5 of molar mass as metabolite is a dimer) Water solubility (mg L^{-1}): 0.005 (20°C) Vapor pressure (Pa): 1 x 10^{-10}, worst-case default $K_{f,oc}$ (L kg^{-1}): 149900 ($K_{f,om}$: 86949), median (n=11) 1/n: 1, worst-case default $DT_{50,soil}$ (d): 312, bias-corrected geometric mean of lab and field data (n=9, normalized to 20°C, pF2) formation fraction: 0.127 (from BF 500-4), arithmetic mean of lab and field data (n=6) TSCF/PUF (-): 0
Application rate	2 x 250 g a.s. ha^{-1} to winter and spring cereals (BBCH 25, 21 days interval) Plant interception: 50% (first application), 70% (second application) 1 x 200 g a.s. ha^{-1} to maize (BBCH 30) Plant interception: 50% 4 x 17 g a.s. ha^{-1} to potatoes (BBCH 51, 10 days interval) Plant interception: 80% for all applications

$PEC_{(gw)}$

80th percentile annual concentration of pyraclostrobin, BF 500-3, BF 500-4, BF 500-5, BF 500-6 and BF 500-7	PEC_{gw} < 0.001 µg L^{-1} for all compounds

Fate and behaviour in air (SANCO/11802 data point 7.3 and SANCO/11803 data point 9.3)

Direct photolysis in air	see photochemical oxidative degradation in air
Quantum yield of direct phototransformation	2.17 x 10^{-1}
Photochemical oxidative degradation in air	DT_{50}: 0.62 hours (Atkinson)
Volatilization	from plant surfaces: about 3% in 24 hours from soil: <1% in 24 hours
Metabolites	no volatile metabolites

PEC (air)

Method of calculation	not necessary due to low volatility and rapid photochemical oxidative degradation

PEC$_{(a)}$

Maximum concentration	not calculated

Definition of the Residue (SANCO/11802 data point 7.4)

Soil (risk assessment & monitoring):	risk assessment: parent, BF 500-6, BF 500-7 monitoring: parent
Surface water (risk assessment & monitoring):	risk assessment: parent, BF 500-6, BF 500-7, BF 500-11, BF 500-13, BF 500-14, BF 500-5 monitoring: parent
Groundwater (risk assessment & monitoring):	risk assessment: parent, BF 500-3, BF 500-4, BF 500-5, BF 500-6, BF 500-7 monitoring: parent
Air (risk assessment & monitoring):	risk assessment: parent monitoring: parent

Monitoring data, if available (SANCO/11802 data point 7.5)

Soil (indicate location and type of study)	none
Surface water (indicate location and type of study)	none
Ground water (indicate location and type of study)	none
Air (indicate location and type of study)	none

Chapter 6: Effects on Non-target Species

Effects on terrestrial vertebrates (SANCO/11802 data point 8.1, SANCO/11803 data point 10.1)

The Annex I Renewal of pyraclostrobin is supported by data from two representative formulations, BAS 500 06 F and BAS 516 07 F. BAS 500 06 F is the representative solo formulation for applications in cereals and maize. BAS 516 07 F (for use in potatoes) contains next to pyraclostrobin as second active substance boscalid. No separate risk assessment for boscalid will be presented in the following chapter, as the active substance in focus of the evaluation is pyraclostrobin. Boscalid is solely addressed in the context of the assessment of combined effects of the formulation.

Acute toxicity to birds	Pyraclostrobin *Colinus virginianus*, *Serinus canaria* $LD_{50\ (geometric\ mean)}$ = 1701 mg a.s./kg b.w. BAS 500 06 F *Colinus virginianus* LD_{50} > 2000 mg/kg b.w. BAS 516 07 F *Colinus virginianus* LD_{50} > 3200 mg/kg b.w.
Dietary toxicity to birds	Pyraclostrobin The two available studies were already evaluated during the previous Annex 1 process and are no longer part of the core data package (EFSA/2009/1438).
Reproductive toxicity to birds	Pyraclostrobin *Colinus virginianus* NOEL = 105 mg a.s./kg b.w./d
Acute toxicity to mammals	Pyraclostrobin Rat LD_{50} > 5 000 mg a.s./kg b.w. BAS 500 06 F Rat LD_{50} ~ 500 mg/kg b.w. BAS 516 07 F Rat LD_{50} > 2000 mg /kg b.w.
Reproductive/long term toxicity to mammals	Pyraclostrobin Rat Tier 1: NOEL = 3 mg a.s./kg b.w./d Higher tier: NOAEL = 8.2 mg a.s./kg b.w./d

Toxicity/exposure ratios for terrestrial vertebrates (SANCO/11803 data point 10.1)

Birds

BAS 500 06 F

***BAS 500 06 F*: Acute risk assessment – Tier 1**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD [1] (mg/kg bw/day)	TER	TER risk assessment trigger
Cereals						
Pyraclostrobin	Cereals Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	$LD_{50\ (geometric\ mean)}$ = 1701 mg a.s./kg b.w.	--	202.8	10
Pyraclostrobin	Cereals BBCH 10 - 29	Small omnivorous bird "lark"		--	257.7	10
Pyraclostrobin	Cereals BBCH 30 -39	Small omnivorous bird "lark"		--	515.5	10
Pyraclostrobin	Cereals BBCH ≥ 40	Small omnivorous bird "lark"		--	859.1	10
Maize						
Pyraclostrobin	Maize BBCH ≥ 20	Small insectivorous bird "wagtail"	$LD_{50\ (geometric\ mean)}$ = 1701 mg a.s./kg b.w.	--	675.0	10
Pyraclostrobin	Maize BBCH 30 - 39	Medium granivorous bird "gamebird"		--	2577.3	10
Pyraclostrobin	Maize BBCH 30 - 39	medium herbivorous/granivorous bird "pigeon"		--	305.9	10
Pyraclostrobin	Maize BBCH 30 - 39	Small omnivorous bird "lark"		--	708.8	10
Pyraclostrobin	Maize BBCH ≥ 40	Medium granivorous bird "gamebird"		--	5315.6	10
Pyraclostrobin	Maize BBCH ≥ 40	medium herbivorous/granivorous bird "pigeon"		--	611.9	10
Pyraclostrobin	Maize BBCH ≥ 40	Small omnivorous bird "lark"		--	1417.5	10
Cereals						
Formulation	Cereals Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	$LD_{50\ (formulation)}$ >2000 mg a.s./kg b.w.	--	>45.9	10
Formulation	Cereals BBCH 10 - 29	Small omnivorous bird "lark"		--	>58.3	10
Formulation	Cereals BBCH 30 -39	Small omnivorous bird "lark"		--	>116.6	10
Formulation	Cereals BBCH ≥ 40	Small omnivorous bird "lark"		--	>194.3	10

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD [1] (mg/kg bw/day)	TER	TER risk assessment trigger
Maize						
Formulation	Maize BBCH ≥ 20	Small insectivorous bird "wagtail"	LD_{50} (formulation) >2000 mg a.s./kg b.w.	--	>152.6	10
Formulation	Maize BBCH 30 - 39	Medium granivorous bird "gamebird"		--	>582.8	10
Formulation	Maize BBCH 30 - 39	medium herbivorous/granivorous bird "pigeon"		--	>69.2	10
Formulation	Maize BBCH 30 - 39	Small omnivorous bird "lark"		--	>160.3	10
Formulation	Maize BBCH ≥ 40	Medium granivorous bird "gamebird"		--	>1201.9	10
Formulation	Maize BBCH ≥ 40	medium herbivorous/granivorous bird "pigeon"		--	>138.4	10
Formulation	Maize BBCH ≥ 40	Small omnivorous bird "lark"		--	1417.5	10

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.

BAS 500 06 F: **Reproductive risk assessment – Tier 1**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD [1] (mg/kg bw/day)	TER	TER risk assessment trigger
Cereals						
Pyraclostrobin	Cereals Early (shoots) autumn-winter BBCH 10-29	Large herbivorous bird "goose"	NOEL = 105 mg a.s./kg b.w./d	--	40.8	5
Pyraclostrobin	Cereals BBCH 10 - 29	Small omnivorous bird "lark"		--	60.6	5
Pyraclostrobin	Cereals BBCH 30 -39	Small omnivorous bird "lark"		--	122.3	5
Pyraclostrobin	Cereals BBCH ≥ 40	Small omnivorous bird "lark"		--	200.1	5
Maize						
Pyraclostrobin	Maize BBCH ≥ 20	Small insectivorous bird "wagtail"	NOEL = 105 mg a.s./kg b.w./d	--	206.4	5
Pyraclostrobin	Maize BBCH 30 - 39	Medium granivorous bird "gamebird"		--	660.4	5
Pyraclostrobin	Maize BBCH 30 - 39	medium herbivorous/granivorous bird "pigeon"		--	86.9	5
Pyraclostrobin	Maize BBCH 30 - 39	Small omnivorous bird "lark"		--	183.4	5
Pyraclostrobin	Maize BBCH ≥ 40	Medium granivorous bird "gamebird"		--	1238.2	5
Pyraclostrobin	Maize BBCH ≥ 40	medium herbivorous/granivorous bird "pigeon"		--	173.8	5
Pyraclostrobin	Maize BBCH ≥ 40	Small omnivorous bird "lark"		--	366.9	5

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.

***BAS 500 06 F:* Secondary poisoning / Drinking water**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD [1] (mg/kg bw/day)	TER	TER risk assessment trigger
Secondary poisoning						
Pyraclostrobin	Cereals [2]	Earthworm-eating bird	NOEL = 105 mg a.s./kg b.w./d	--	783.86	5
		Fish-eating bird		--	578.13	5
Drinking water - puddle scenario (screening step)						
Pyraclostrobin	Cereals [3]	Small granivorous bird	$LD_{50\ (geometric\ mean)}$ = 1701 mg a.s./kg b.w.	--	0.2 [4]	3000 [5]
		Small granivorous bird	NOEL = 105 mg a.s./kg b.w./d	--	3.5 [4]	3000 [5]

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.
2) Highest PEC_{sw} (twa, 21 days) from FOCUS Step 2 (South Europe) calculation for a multiple application scenario of BAS 500 06 F in cereals with parameters from dark water/sediment study as worst-case.
3) Worst-case crop scenario with regard to highest application rate and number of applications for use in cereals
4) Value calculated as ratio of AR_{eff} and relevant toxicity endpoint according to EFSA/2009/1438
5) Drinking water risk assessment is not necessary when trigger value is not exceeded. Trigger according to EFSA/2009/1438

BAS 516 07 F

***BAS 516 07 F*: Acute dietary risk assessment – Tier 1**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD [1] (mg/kg bw/day)	TER	TER risk assessment trigger
Pyraclostrobin	Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail"	$LD_{50\ (geometric\ mean)}$ = 1701 mg a.s./kg b.w.	--	2647.1	10
	Potatoes BBCH ≥ 40	Small omnivorous bird "lark"		--	9264.7	10
Virtual compound	Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail"	$LD_{50\ (mix)}$ = 1931.9 mg a.s./kg b.w.	--	608.4	10
	Potatoes BBCH ≥ 40	Small omnivorous bird "lark"		--	2129.5	10
Formulation	Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail"	$LD_{50\ (formulation)}$ > 3200 mg a.s./kg b.w.	--	> 338.6	10
	Potatoes BBCH ≥ 40	Small omnivorous bird "lark"		--	> 1185.2	10

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.

***BAS 516 07 F*: Reproductive dietary risk assessment – Tier 1**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD [1] (mg/kg bw/day)	TER	TER risk assessment trigger
Pyraclostrobin	Potatoes BBCH ≥ 20	Small insectivorous bird "wagtail"	NOEL = 105 mg a.s./kg b.w./d	--	632.3	5
	Potatoes BBCH ≥ 40	Small omnivorous bird "lark"		--	1858.6	5

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.

BAS 516 07 F: **Secondary poisoning / Drinking water**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD [1] (mg/kg bw/day)	TER	TER risk assessment trigger
Secondary poisoning						
Pyraclostrobin	Potatoes	Earthworm-eating bird	NOEL = 105 mg a.s./kg b.w./d	--	12119.70	5
		Fish-eating bird		--	7415.30	5
Drinking water - puddle scenario (screening step)						
Pyraclostrobin	Potatoes	Small granivorous bird	$LD_{50\ (geometric\ mean)}$ = 1701 mg a.s./kg b.w.	--	0.4 [2]	3000 [3]
		Small granivorous bird	NOEL = 105 mg a.s./kg b.w./d	--	5.9 [2]	3000 [3]

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.
2) Value calculated as ratio of AR_{eff} and relevant toxicity endpoint according to EFSA/2009/1438
3) Drinking water risk assessment is not necessary when trigger value is not exceeded. Trigger according to EFSA/2009/1438

Other terrestrial vertebrates

BAS 500 06 F

BAS 500 06 F: **Acute risk assessment – Tier 1**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD [1] (mg/kg bw/day)	TER	TER risk assessment trigger
Cereals						
Pyraclostrobin	Cereals Early (shoots)	Large herbivorous mammal "lagomorph"	LD_{50} > 5000 mg a.s./kg b.w.	--	>431.9	10
Pyraclostrobin	Cereals BBCH 10-29	Small omnivorous mammal "mouse"		--	>1057.1	10
Pyraclostrobin	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"		--	>3367.0	10
Pyraclostrobin	Cereals BBCH 30 - 39	Small omnivorous mammal "mouse"		--	>2114.2	10
Pyraclostrobin	Cereals BBCH ≥ 40	Small herbivorous mammal "vole"		--	>444.5	10
Pyraclostrobin	Cereals BBCH ≥ 40	Small omnivorous mammal "mouse"		--	>3496.5	10
Maize						
Pyraclostrobin	Maize BBCH ≥ 20	Small insectivorous mammal "shrew"	LD_{50} > 5000 mg a.s./kg b.w.	--	>4629.6	10
Pyraclostrobin	Maize BBCH 30 - 39	Small herbivorous mammal "vole"		--	>366.6	10
Pyraclostrobin	Maize BBCH 30 - 39	Small omnivorous mammal "mouse"		--	>2907.0	10
Pyraclostrobin	Maize BBCH ≥ 40	Small herbivorous mammal "vole"		--	>733.1	10
Pyraclostrobin	Maize BBCH ≥ 40	Small omnivorous mammal "mouse"		--	>5814.0	10
Cereals						
Formulation	Cereals Early (shoots)	Large herbivorous mammal "lagomorph"	LD_{50} (formulation) around 500 mg a.s./kg b.w.	--	**8.3**	10
Formulation	Cereals BBCH 10-29	Small omnivorous mammal "mouse"		--	20.3	10
Formulation	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"		--	64.8	10
Formulation	Cereals BBCH 30 - 39	Small omnivorous mammal "mouse"		--	40.7	10
Formulation	Cereals BBCH ≥ 40	Small herbivorous mammal "vole"		--	**8.5**	10
Formulation	Cereals BBCH ≥ 40	Small omnivorous mammal "mouse"		--	67.2	10
Maize						
Formulation	Maize BBCH ≥ 20	Small insectivorous mammal "shrew"	LD_{50} (formulation) around 500 mg a.s./kg b.w.	--	89.0	10
Formulation	Maize BBCH 30 - 39	Small herbivorous mammal "vole"		--	**7.0**	10
Formulation	Maize BBCH 30 - 39	Small omnivorous mammal "mouse"		--	55.9	10
Formulation	Maize BBCH ≥ 40	Small herbivorous mammal "vole"		--	14.1	10
Formulation	Maize BBCH ≥ 40	Small omnivorous mammal "mouse"		--	111.8	10

TER values in **bold** are below the respective trigger value and will be refined. See table below for results from acute higher tier risk assessment

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.

BAS 500 06 F: **Acute risk assessment – Higher tier[1)]**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD (mg/kg bw/day)	TER	TER risk assessment trigger
Cereals						
Formulation	Cereals Early (shoots)	Large herbivorous mammal "lagomorph" scenario: Hare	LD_{50} (formulation) 'around' 500 mg a.s./kg b.w.	42.37	11.80[1)]	10
Formulation	Cereals BBCH ≥ 40	Small herbivorous mammal "vole"	LD_{50} (formulation) 'around' 500 mg a.s./kg b.w.	15.63	31.99[2)3)]	10
Maize						
Formulation	Maize BBCH 30 - 39	Small herbivorous mammal "vole"	LD_{50} (formulation) 'around' 500 mg a.s./kg b.w.	62.53	7.99[2)3)]	10

n.a. = not applicable

1) The refined acute risk assessment for the large herbivorous mammal "lagomorph" scenario is based on the refinement of **residue decline of pyraclostrobin in plants** for refinement of MAF_{90} and the **identification of representative mammal species** including ecological data (**PD values and FIR**).

2) The refined acute risk assessment for the small herbivorous mammal "vole" scenario is based on the refinement of **residue decline of pyraclostrobin in plants** for refinement of MAF_{90}, the **identification of representative mammal species** including ecological data (**PD values and FIR**), and considers **interception** by cereals and maize plants.

3) **Further refinements for the small herbivorous mammal scenario are based on three lines of evidence** which take into account the results from a body burden modelling approach in small mammals, dietary toxicity studies under field-relevant exposure conditions, and results from two field effect studies with BAS 500 06 F **indicating a low and acceptable risk to small mammals from the use of BAS 500 06 F**. For detailed discussion please refer to chapter M-CP 10.1.2 of BAS 500 06 F.

BAS 500 06 F: **Reproductive risk assessment – Tier 1**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD [1] (mg/kg bw/day)	TER	TER risk assessment trigger
Cereals						
Pyraclostrobin	Cereals Early (shoots)	Large herbivorous mammal "lagomorph"	NOEL = 3 mg a.s./kg b.w./d	--	**0.8**	5
Pyraclostrobin	Cereals BBCH 10-29	Small omnivorous mammal "mouse"		--	**2.4**	5
Pyraclostrobin	Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"		--	9.9	5
Pyraclostrobin	Cereals BBCH 30 - 39	Small omnivorous mammal "mouse"		--	**4.8**	5
Pyraclostrobin	Cereals BBCH ≥ 40	Small herbivorous mammal "vole"		--	**0.9**	5
Pyraclostrobin	Cereals BBCH ≥ 40	Small omnivorous mammal "mouse"		--	8.2	5
Maize						
Pyraclostrobin	Maize BBCH ≥ 20	Small insectivorous mammal "shrew"	NOEL = 3 mg a.s./kg b.w./d	--	14.9	5
Pyraclostrobin	Maize BBCH 30 - 39	Small herbivorous mammal "vole"		--	**0.8**	5
Pyraclostrobin	Maize BBCH 30 - 39	Small omnivorous mammal "mouse"		--	7.3	5
Pyraclostrobin	Maize BBCH ≥ 40	Small herbivorous mammal "vole"		--	**1.6**	5
Pyraclostrobin	Maize BBCH ≥ 40	Small omnivorous mammal "mouse"		--	14.9	5

TER values in **bold** are below the respective trigger value and will be refined. See table below for results from reproductive higher tier risk assessment

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.

BAS 500 06 F: Reproductive risk assessment – Higher tier

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD[1)] (mg/kg bw/day)	TER	TER risk assessment trigger
Cereals						
Pyraclostrobin	Cereals Early (shoots)	Large herbivorous mammal "lagomorph" scenario: Hare	NOEL = 8.2 mg a.s./kg b.w./d	0.782	10.49	5
Pyraclostrobin	Cereals BBCH 10-29	Small omnivorous mammal "mouse" scenario: Wood mouse		0.59	13.9	5
Pyraclostrobin	Cereals BBCH 30 - 39					
Pyraclostrobin	Cereals BBCH ≥ 40	Small herbivorous mammal "vole"[3)]		0.280	29.26[4)]	5
Maize						
Pyraclostrobin	Maize BBCH 30 - 39	Small herbivorous mammal "vole"[3)]	NOEL = 8.2 mg a.s./kg b.w./d	1.121	7.32[4)]	5
Pyraclostrobin	Maize BBCH ≥ 40	Small herbivorous mammal "vole"[3)]		n.a.[5)]	n.a.	n.a

n.a. = not applicable

1) The refined reproductive risk assessment for the large herbivorous mammal "lagomorph" scenario is based on the refinement of the **ecologically relevant reproductive toxicity endpoint** for pyraclostrobin, the **residue decline of pyraclostrobin in plants** for refinement of MAF x twa using the "moving time window approach", and the **identification of representative mammal species** including ecological data (**PT and PD values and FIR**).
2) The refinement for the small omnivorous mammal "mouse" scenarios is based on the refinement of the **ecologically relevant reproductive toxicity endpoint** for pyraclostrobin, the **residue decline of pyraclostrobin in plants** for refinement of MAF x twa using the "moving time window approach", the **identification of representative mammal species** including ecological data (**PD values and FIR**), and considers **interception** by cereal plants.
3) The "vole" scenario is regarded as not relevant for arable crops such as cereals and maize. Nevertheless, a refined reproductive risk assessment is carried out. For detailed discussion please refer to chapter M-CP 10.1.2.
4) The refinement for the small herbivorous mammal "vole" scenarios is based on the refinement of the **ecologically relevant reproductive toxicity endpoint** for pyraclostrobin, the **residue decline of pyraclostrobin in plants** for refinement of MAF x twa using the "moving time window approach", the **identification of representative mammal species** including ecological data (**PD values and FIR**), and considers **interception** by cereal and maize plants.
5) The maize BBCH ≥ 40 scenario is covered by the maize BBCH 30 – 39 scenario.

BAS 500 06 F: Secondary poisoning / Drinking water

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD [1)] (mg/kg bw/day)	TER	TER risk assessment trigger
Secondary poisoning						
Pyraclostrobin	Cereals [2)]	Earthworm-eating mammal	NOEL = 3 mg a.s./kg b.w./d	--	18.37	5
		Fish-eating mammal		--	18.50	5
Drinking water - puddle scenario (screening step)						
Pyraclostrobin	Cereals [3)]	Small granivorous mammal	LD_{50} > 5000 mg a.s./kg b.w.	--	<0.1 [4)]	3000 [5)]
		Small granivorous mammal	NOEL = 3 mg a.s./kg b.w./d	--	120.8 [4)]	3000 [5)]

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.
2) Highest PEC_{sw} (twa, 21 days) from FOCUS Step 2 (South Europe) calculation for a multiple application scenario of BAS 500 06 F in cereals with parameters from dark water/sediment study as worst-case.
3) Worst-case crop scenario with regard to highest application rate and number of applications for use in cereals.
4) Value calculated as ratio of AR_{eff} and relevant toxicity endpoint according to EFSA/2009/1438.
5) Drinking water risk assessment is not necessary when trigger value is not exceeded. Trigger according to EFSA/2009/1438.

BAS 516 07 F

***BAS 516 07 F*: Acute dietary risk assessment – Tier 1**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD[1)] (mg/kg bw/day)	TER	TER risk assessment trigger
Pyraclostrobin	Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew"	LD_{50} > 5000 mg a.s./kg b.w.	--	>36310.8	10
	Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph"		--	>18674.1	10
	Potatoes BBCH ≥ 40	Small herbivorous mammal "vole"		--	>4794.1	10
	Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse"		--	>37707.4	10
Virtual compound	Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew"	$LD_{50\ (mix)}$ = 5000 mg a.s./kg b.w.	--	7348.6	10
	Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph"		--	3779.3	10
	Potatoes BBCH ≥ 40	Small herbivorous mammal "vole"		--	970.2	10
	Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse"		--	7631.3	10
Formulation	Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew"	$LD_{50\ (formulation)}$ > 2000 mg a.s./kg b.w.	--	>987.7	10
	Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph"		--	>507.9	10
	Potatoes BBCH ≥ 40	Small herbivorous mammal "vole"		--	>130.4	10
	Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse"		--	>1025.6	10

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.

***BAS 516 07 F*: Reproductive dietary risk assessment – Tier 1**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD[1)] (mg/kg bw/day)	TER	TER risk assessment trigger
Pyraclostrobin	Potatoes BBCH ≥ 20	Small insectivorous mammal "shrew"	NOEL = 3 mg a.s./kg b.w./d	--	92.2	5
	Potatoes BBCH ≥ 40	Large herbivorous mammal "lagomorph"		--	40.8	5
	Potatoes BBCH ≥ 40	Small herbivorous mammal "vole"		--	8.1	5
	Potatoes BBCH ≥ 40	Small omnivorous mammal "mouse"		--	76.2	5

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.

***BAS 516 07 F:* Secondary poisoning / Drinking water**

Test substance	Crop, use pattern	Crop scenario, indicator species	Toxicity endpoint	DDD[1)] (mg/kg bw/day)	TER	TER risk assessment trigger
Secondary poisoning						
Pyraclostrobin	Potatoes	Earthworm-eating mammal	NOEL = 3 mg a.s./kg b.w./d	--	284.06	5
		Fish-eating mammal		--	237.23	5
Drinking water - puddle scenario (screening step)						
Pyraclostrobin	Potatoes	Small granivorous mammal	LD_{50} > 5000 mg a.s./kg b.w.	--	<0.1[2)]	3000[3)]
		Small granivorous mammal	NOEL = 3 mg a.s./kg b.w./d	--	205[2)]	3000[3)]

1) Note that DDD is not explicitly indicated in the EFSA calculator tool (version of 9 July 2010) and thus cannot be provided.
2) Value calculated as ratio of AR_{eff} and relevant toxicity endpoint according to EFSA/2009/1438
3) Drinking water risk assessment is not necessary when trigger value is not exceeded. Trigger according to EFSA/2009/1438

Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

Publications are available in the scientific literature on the effect of pyraclostrobin, respectively of pyraclostrobin containing formulations on amphibia (Belden et al., 2010; Brühl et al., 2013). In addition, BASF has performed GLP studies to address the risk to amphibians. The results are summarized below.

Laboratory studies on metamorph amphibians under worst-case conditions

Study	Test species	Test substance	Applic. rate [g a.s./ha]	Effect (% mortality)
Belden et al. (2010)	*Bufo cognatus*	BAS 500 00 F	22	0
			220	70
			2200	100
Brühl et al. (2013)	*Rana temporaria*	BAS 500 00 F	22	0
			220	100
		BAS 500 18 F	2200	No significant effect
BASF (2013)	*Rana temporaria*	BAS 500 06 F	40	0
			63	0 (sublethal symptoms)
			99	70
BASF (2014)	*Bufo bufo*	BAS 500 06 F	155 / 15 min 219 / 15 min 310 / 15 min 310 / 60 min 310 / 4 h[1)]	0 0 (20% sublethal) 20 (60% sublethal) 20 (40% sublethal) (20)[2)] (0% sublethal)

1) Animals were exposed to the respective rates either immediately (within 15 minutes) or 1 h or 4 h after spraying.
2) The observed toxicity in this case is not considered treatment-related.

The laboratory studies indicate that BAS 500 06 F can be toxic to amphibians at the full field rate under worst case conditions:
LR_0 = 63 g a.s./ha < 250 g a.s./ha (max field rate for BAS 500 06 F applications in cereals)

No toxicity is to be expected from applications of BAS 516 07 F.
LR_0 = 63 g a.s./ha > 17 g a.s./ha (max field rate for BAS 516 07 F applications in potatoes)

For animals exposed to soil residues of BAS 500 06 F the LR_0 was > 155 g a.s./ha if exposed immediately after spraying, while the LR_{50} was > 310 g a.s./ha under these circumstances. No significant substance related effects occurred at the highest rate, if animals were introduced 4 hours after spraying, simulating spraying during the day and the nocturnal amphibian activity (NOEL ≥ 310 g a.s./ha).

In order to address the risk of BAS 500 06 F to amphibians under more realistic conditions, two semi-field studies have been conducted with *R. temporaria* and *Bufo bufo* under realistic worst case conditions in cereals at BBCH 25-30 with application rates of 0.94 L/ha, 1.25 L/ha and 1.56 L/ha (according to 188, 250 and 313 g a.s./ha) for R. temporaria and application rates of 1.25 L/ha, 1.5 L/ha and 12.5 L/ha (according to 250 g a.s./ha, 375 g a.s./ha and 2 500 g a.s./ha).

Higher tier semi-field study with juvenile Rana temporaria under realistic worst-case conditions

Test item	Application rate	Effect
BAS 500 06 F	0.75 x max field rate (0.94 L/ha)	0%
	1.0 x max field rate (1.25 L/ha)	0%
	1.25 x max field rate (1.56 L/ha)	0% [#]

[#] One single individual (out of 16) showed a short term, transient behavioural impact

Higher tier semi-field study with juvenile Bufo bufo under realistic worst-case conditions

Test item	Application rate	Effect
BAS 500 06 F	1.0 x max field rate (1.25 L/ha)	0%
	1.5 x max field rate (1.875 L/ha)	0%
	10 x max field rate (12.5 L/ha)	50 (75)% [#]

[#] One animal was moribund (if counted as dead mortality results in 75%)

The semi-field studies demonstrate that there is low risk of BAS 500 06 F use in cereals to cause unacceptable impact to amphibians; NOER > 1.25 L BAS 500 06 F/ha (max field rate)

Toxicity data for aquatic species (most sensitive species of each group) (SANCO data point IIA 8.2 and SANCO/11803 data point IIIA 10.2)

	Group	Test substance	Time-scale	End-point	Toxicity [mg/L]
Acute toxicity to fish	*Oncorhynchus mykiss*,	Pyraclostrobin	96 h	LC_{50}	0.00616
	O. mykiss [1)]	Pyraclostrobin	96 h	LC_{50}	0.00620
	Cyprinodon variegatus [1), 2)]	Pyraclostrobin	96 h	LC_{50}	0.0769
	Cyprinus carpio	Pyraclostrobin	96 h	LC_{50}	0.0177
	Danio rerio [4)]	Pyraclostrobin	96 h	LC_{50}	0.0619
	Lepomis macrochirus	Pyraclostrobin	96 h	LC_{50}	0.0254
	L. macrochirus [3)]	Pyraclostrobin	96 h	LC_{50}	0.0114
	Leuciscus idus melanotus [3)]	Pyraclostrobin	96 h	LC_{50}	0.0191
	Oryzias latipes [3)]	Pyraclostrobin	96 h	LC_{50}	0.0533
	Pimephales promelas [3)]	Pyraclostrobin	96 h	LC_{50}	0.0161
	O. mykiss [1)]	BF 500-3	96 h	LC_{50}	> 0.0948
	O. mykiss [1)]	BF 500-5	96 h	LC_{50}	11.3
	O. mykiss	BF 500-11	96 h	LC_{50}	> 100
	O. mykiss	BF 500-13	96 h	LC_{50}	> 50 < 100
	O. mykiss	BF 500-14	96 h	LC_{50}	> 39.4 < 82.6
	O. mykiss [1)]	BAS 500 06 F	96 h	LC_{50}	0.0360
	O. mykiss	BAS 516 07 F *	96 h	LC_{50}	0.088
Chronic toxicity to fish	*O. mykiss* (ELS study)	Pyraclostrobin	98 d	NOEC	0.00235
	C. variegatus [1), 2)] (ELS study)	Pyraclostrobin	36 d	NOEC	0.0108
	P. promelas [1)] (ELS study)	Pyraclostrobin	36 d	NOEC	0.00414
Acute toxicity to aquatic invertebrates	*Daphnia magna*	Pyraclostrobin	48 h	EC_{50}	0.0157
	D. magna [1)]	BF 500-3	48 h	EC_{50}	> 0.100
	D. magna [1)]	BF 500-5	48 h	EC_{50}	> 10.0
	D. magna	BF 500-11	48 h	EC_{50}	> 100
	D. magna	BF 500-13	48 h	EC_{50}	> 100
	D. magna	BF 500-14	48 h	EC_{50}	> 60.9
	D. magna [1)]	BAS 500 06 F	48 h	EC_{50}	0.065
	D. magna	BAS 516 07 F	48 h	EC_{50}	0.210
Chronic toxicity to aquatic invertebrates	*D. magna*	Pyraclostrobin	21 d	NOEC	0.004
Acute toxicity to sediment dwelling aquatic invertebrates	*Leptocheirus plumulosus* [1), 2)] (spiked sediment)	Pyraclostrobin	10 d	LC_{50}	4.41 mg/kg dry sediment

	Group	Test substance	Time-scale	End-point	Toxicity [mg/L]
Chronic toxicity to sediment dwelling aquatic invertebrates	*Chironomus riparius* (spiked water)	Pyraclostrobin	28 d	NOEC	0.040
	C. riparius [1)] (spiked sediment)	Pyraclostrobin	28 d	NOEC	1.37 mg/kg dry sediment
	C. riparius [1)] (spiked sediment)	BF 500-3	28 d	NOEC	≥ 16.0 mg/kg dry sediment
	C. riparius [1)] (spiked sediment)	BF 500-6	28 d	NOEC	1.2 mg/kg dry sediment
	C. riparius [1)] (spiked sediment)	BF 500-7	28 d	NOEC	≥ 123.5 mg/kg dry sediment
Toxicity to algae	*Pseudokirchneriella subcapitata*	Pyraclostrobin	72 h	E_rC_{50}	> 0.843 [4)]
	P. subcapitata [1)]	BF 500-3	72 h	E_rC_{50}	> 1.17 [5)]
	P. subcapitata [1)]	BF 500-5	72 h	E_rC_{50}	5.33
	Scenedesmus subspicatus	BF 500-11	72 h	E_rC_{50}	> 100
	S. subspicatus	BF 500-13	72 h	E_rC_{50}	> 100
	S. subspicatus	BF 500-14	72 h	E_rC_{50}	> 100
	P. subcapitata	BAS 500 06 F	72 h	E_rC_{50}	14.2
	P. subcapitata	BAS 516 07 F	72 h	E_rC_{50}	10.8
Toxicity to aquatic macrophytes	*Lemna gibba* [1)]	Pyraclostrobin	14 d	E_bC_{50}	1.72
Higher tier studies / calculations	*O. mykiss* (TTE study; different exposure durations) [1)]	Pyraclostrobin	96 h	LC_{50}	> 0.027 (0.5 h exposure) [6)]
			96 h	LC_{50}	0.022 (2 h) [6)]
			96 h	LC_{50}	0.015 (8 h) [6)]
	SSD (based on 96 h NOECs for 8 fish species)	Pyraclostrobin	--	HC_5 (NOEC)	0.00338
	O. mykiss (ELS study with multiple exposure)	Pyraclostrobin	97 d	NOEC	0.005
	Geomean based on chronic ELS with three fish species	Pyraclostrobin	--	$NOEC_{geomean}$	0.00472
	outdoor mesocosm (multiple spray application) [3)]	Pyraclostrobin	6 mo	NOEC	0.008
			6 mo	NOEAEC	> 0.008 < 0.024

ELS = early life stage; NO(E)AEC = No observed (ecologically) adverse effect concentration; LC = life cycle; TTE = Time-To-Effect/Event; SSD = Species Sensitivity Distribution; HC_5 = 5% hazardous concentration

***** Study was conducted with the minor change formulation BAS 516 00 F (reference is made to Document JCP, CP 1.4.1 of this dossier).

1) Study was not submitted during the Annex I inclusion process of pyraclostrobin.

2) Marine / saltwater species

3) Study was performed with a previous representative solo-formulation BAS 500 00 F; however, results are given in mg a.s./L

4) In accordance to recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints of the EU agreed study on the green alga have been (re-)calculated from original data. The re-calculated values are presented here and are used in the aquatic risk assessment; for details on these calculations please refer to the supplement. A summary of this study and the re-calculations is provided in chapter 8.2 of the MCA dossier part for Annex I renewal.

5) In accordance to recent guidelines (EFSA, 2013; OECD, 2011), the 72 h endpoints obtained in the 96 h / 120 h alga studies are considered as relevant endpoint and are presented here.

6) Endpoint obtained in the 96 hour TTE study after exposure over respective duration, *i.e.* the value in brackets (0.5, 2 or 8 h exposure time).

TER-calculations

For the sake of clarity, it has been abstained from showing the TER-calculations for each individual species that has been tested; instead TER calculations are performed with a representative of each group and with the relevant higher tier endpoint (e.g. only one representative fish species plus the HC_5 for all fishes and only *Daphnia* and *Chironomus* data for water and sediment dwelling aquatic invertebrates plus the higher tier mesocosm).

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
BAS 500 06 F								
Pyraclostrobin	winter cereals, 1 - 2 x 250 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} = 6.2	D1 ditch	20 m D + R	0.118	**53**	100
				D1 stream	20 m D + R	0.135	**46**	100
				D2 ditch	20 m D + R	0.119	**52**	100
				D2 stream	20 m D + R	0.142	**44**	100
				D3 ditch	20 m D + R	0.117	**53**	100
				D4 pond	50% N	0.033	188	100
					5 m D	0.056	111	
				D4 stream	20 m D + R	0.119	**52**	100
				D5 pond	50% N	0.033	188	100
					5 m D	0.057	109	
				D5 stream	20 m D + R	0.127	**49**	100
				D6 ditch	20 m D + R	0.115	**54**	100
				R1 pond	50% N	0.042	148	100
					5 m D	0.056	111	
				R1 stream	20 m D + R	0.104	**60**	100
				R3 stream	20 m D + R	0.147	**42**	100
				R4 stream	20 m D + R	0.130	**48**	100
	spring cereals 1 - 2 x 250 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} = 6.2	D1 ditch	20 m D + R	0.119	**52**	100
				D1 stream	20 m D + R	0.140	**44**	100
				D3 ditch	20 m D + R	0.117	**53**	100
				D4 pond	50% N	0.033	188	100
					5 m D	0.056	111	
				D4 stream	20 m D + R	0.131	**47**	100
				D5 pond	50% N	0.034	182	100
					5 m D	0.058	107	
				D5 stream	20 m D + R	0.133	**47**	100
				R4 stream	20 m D + R	0.104	**60**	100
	maize, 1 x 200 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} = 6.2	D3 ditch	10 m D +R	0.180	**34**	100
				D4 pond	--	0.042	148	100
				D4 stream	10 m D +R	0.200	**31**	100
				D5 pond	--	0.042	148	100
				D5 stream	10 m D +R	0.198	**31**	100
				D6 ditch	10 m D +R	0.179	**35**	100
				R1 pond	--	0.042	148	100
				R1 stream	10 m D +R	0.161	**39**	100
				R2 stream	10 m D +R	0.215	**29**	100
				R3 stream	10 m D +R	0.225	**28**	100
				R4 stream	10 m D +R	0.157	**39**	100

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
Pyraclostrobin	winter cereals, 1 - 2 x 250 g a.s./ha	fish HC_5 based on acute NOECs of 8 species	HC_5 = 3.38	D1 ditch	50% N	0.792	4.3	3 *
					5 m D	0.429	7.9	
				D1 stream	50% N	0.673	5.0	3 *
					5 m D	0.491	6.9	
				D2 ditch	50% N	0.794	4.3	3 *
					5 m D	0.431	7.8	
				D2 stream	50% N	0.707	4.8	3 *
					5 m D	0.516	6.6	
				D3 ditch	50% N	0.783	4.3	3 *
					5 m D	0.424	8.0	
				D4 pond	--	0.065	52	3 *
				D4 stream	50% N	0.590	5.7	3 *
					5 m D	0.431	7.8	
				D5 pond	--	0.066	51	3 *
				D5 stream	50% N	0.631	5.4	3 *
					5 m D	0.461	7.3	
				D6 ditch	50% N	0.773	4.4	3 *
					5 m D	0.419	8.1	
				R1 pond	--	0.065	52	3 *
				R1 stream	--	1.037	3.3	3 *
				R3 stream	50% N	0.731	4.6	3 *
					5 m D	0.534	6.3	
				R4 stream	--	1.038	3.3	3 *
	spring cereals 1 - 2 x 250 g a.s./ha	fish HC_5 based on acute NOECs of 8 species	HC_5 = 3.38	D1 ditch	50% N	0.877	3.9	3 *
					5 m D	0.453	7.5	
				D1 stream	50% N	0.694	4.9	3 *
					5 m D	0.507	6.7	
				D3 ditch	50% N	0.784	4.3	3 *
					5 m D	0.425	8.0	
				D4 pond	--	0.065	52	3 *
				D4 stream	50% N	0.650	5.2	3 *
					5 m D	0.474	7.1	
				D5 pond	--	0.068	50	3 *
				D5 stream	50% N	0.659	5.1	3 *
					5 m D	0.481	7.0	
				R4 stream	--	1.038	3.3	3 *
	maize, 1 x 200 g a.s./ha	fish HC_5 based on acute NOECs of 8 species	HC_5 = 3.38	D3 ditch	--	1.037	3.3	3 *
				D4 pond	--	0.042	80	3 *
				D4 stream	--	0.895	3.8	3 *
				D5 pond	--	0.042	80	3 *
				D5 stream	--	0.888	3.8	3 *
				D6 ditch	--	1.029	3.3	3 *
				R1 pond	--	0.042	80	3 *

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
				R1 stream	--	0.720	4.7	3 *
				R2 stream	--	0.963	3.5	3 *
				R3 stream	--	1.011	3.3	3 *
				R4 stream	--	0.703	4.8	3 *
					5 m D	0.301	7.8	
Pyraclostrobin	winter cereals, 1 - 2 x 250 g a.s./ha	Fish, chronic, geomean of 3 ELS studies	Geomean = 4.72	D1 ditch	75% N	0.396	12	10
					5 m D	0.429	11	
				D1 stream	75% N	0.336	14	10
					5 m D + 50% N	0.246	19	
					10 m D	0.260	18	
				D2 ditch	75% N	0.397	12	10
					5 m D	0.431	11	
				D2 stream	75% N	0.353	13	10
					5 m D + 50% N	0.258	18	
					10 m D	0.274	17	
				D3 ditch	75% N	0.392	12	10
					5 m D	0.424	11	
				D4 pond	--	0.065	73	10
				D4 stream	75% N	0.295	16	10
					5 m D	0.431	11	
				D5 pond	--	0.066	72	10
				D5 stream	75% N	0.316	15	10
					5 m D	0.461	10	
				D6 ditch	75% N	0.387	12	10
					5 m D	0.419	11	
				R1 pond	--	0.065	73	10
				R1 stream	75% N	0.259	18	10
					5 m D	0.379	12	
				R3 stream	75% N	0.365	13	10
					5 m D + 50% N	0.267	18	
					10 m D	0.283	17	
				R4 stream	10 m D + R	0.248	19	10
	spring cereals 1 - 2 x 250 g a.s./ha	Fish, chronic, geomean of 3 ELS studies	Geomean = 4.72	D1 ditch	75% N	0.437	11	10
					5 m D	0.453	10	
				D1 stream	75% N	0.347	14	10
					5 m D + 50% N	0.254	19	
					10 m D	0.269	18	
				D3 ditch	75% N	0.392	12	10
					5 m D	0.425	11	
				D4 pond	--	0.065	73	10
				D4 stream	75% N	0.325	15	10
					5 m D +50% N	0.237	20	
					10 m D	0.252	19	

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
				D5 pond	--	0.068	69	10
				D5 stream	75% N	0.329	14	10
					5 m D + 50% N	0.241	20	
					10 m D	0.255	19	
				R4 stream	75% N	0.267	18	10
					5 m D	0.379	12	
	maize, 1 x 200 g a.s./ha	Fish, chronic, geomean of 3 ELS studies	Geomean = 4.72	D3 ditch	75% N	0.259	18	10
					5 m D	0.340	14	
				D4 pond	--	0.042	112	10
				D4 stream	50% N	0.448	11	10
					5 m D	0.377	13	
				D5 pond	--	0.042	112	10
				D5 stream	50% N	0.444	11	10
					5 m D	0.374	13	
				D6 ditch	75% N	0.257	18	10
					5 m D	0.337	14	
				R1 pond	--	0.042	112	10
				R1 stream	50% N	0.360	13	10
					5 m D	0.303	16	
				R2 stream	75% N	0.241	20	10
					5 m D	0.405	12	
				R3 stream	75% N	0.253	19	10
					5 m D	0.425	11	
				R4 stream	50% N	0.351	13	10
					5 m D	0.301	16	
Pyraclostrobin	winter cereals, 1 - 2 x 250 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} = 15.7	D1 ditch	5 m D + 75% N	0.107	147	100
					10 m D + 50% N	0.114	138	100
					15 m D	0.155	101	100
				D1 stream	90% N	0.134	117	100
					5 m D + 75% N	0.123	128	100
					10 m D + 50% N	0.130	121	100
					20 m D	0.135	116	100
				D2 ditch	5 m D + 75% N	0.108	145	100
					10 m D + 50% N	0.114	138	100
					15 m D	0.156	101	100
				D2 stream	90% N	0.141	111	100
					5 m D + 75% N	0.129	122	100
					10 m D + 50% N	0.137	115	100
					20 m D	0.142	111	100
				D3 ditch	90% N	0.157	100	100

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
					5 m D + 75% N	0.106	148	100
					10 m D + 50% N	0.112	140	100
					15 m D	0.154	102	100
				D4 pond	--	0.065	242	100
				D4 stream	90% N	0.118	133	100
					5 m D + 75% N	0.108	145	100
					10 m D + 50% N	0.114	138	100
					15 m D	0.156	101	100
				D5 pond	--	0.066	238	100
				D5 stream	90% N	0.126	125	100
					5 m D + 75% N	0.115	137	100
					10 m D + 50% N	0.122	129	100
					20 m D	0.127	124	100
				D6 ditch	90% N	0.155	101	100
					5 m D + 75% N	0.105	150	100
					10 m D + 50% N	0.111	141	100
					15 m D	0.152	103	100
				R1 pond	--	0.065	242	100
				R1 stream	20 m D + R	0.104	151	100
				R3 stream	20 m D + R	0.147	107	100
				R4 stream	20 m D + R	0.130	121	100
	spring cereals 1 - 2 x 250 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} = 15.7	D1 ditch	5 m D + 75% N	0.113	139	100
					10 m D + 50% N	0.117	134	
					20 m D	0.119	132	
				D1 stream	90% N	0.139	113	100
					5 m D + 75% N	0.127	124	
					10 m D + 50% N	0.134	117	
					20 m D	0.140	112	
				D3 ditch	90% N	0.157	100	100
					5 m D + 75% N	0.106	148	
					10 m D + 50% N	0.113	139	
					15 m D	0.154	102	
				D4 pond	--	0.065	242	100
				D4 stream	90% N	0.130	121	100
					5 m D + 75% N	0.119	132	
					10 m D + 50% N	0.126	125	
					20 m D	0.131	120	

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
				D5 pond	--	0.068	231	100
				D5 stream	90% N	0.132	119	100
					5 m D + 75% N	0.120	131	
					10 m D + 50% N	0.128	123	
					20 m D	0.133	180	
				R4 stream	20 m D + R	0.104	151	100
	maize, 1 x 200 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} = 15.7	D3 ditch	90% N	0.104	151	100
					10 m D (+ R)	0.180	**87**	
				D4 pond	--	0.042	374	100
				D4 stream	90% N	0.089	176	100
					10 m D (+ R)	0.200	**79**	
				D5 pond	--	0.042	374	100
				D5 stream	90% N	0.089	176	100
					10 m D (+ R)	0.198	**79**	
				D6 ditch	90% N	0.103	152	100
					10 m D (+ R)	0.179	**88**	
				R1 pond	--	0.042	374	100
				R1 stream	90% N	0.153	103	100
					5 m D + 50% N	0.153	103	
					10 m D (+ R)	0.161	**98**	
				R2 stream	90% N	0.096	164	100
					10 m D (+ R)	0.215	**73**	
				R3 stream	10 m D (+ R)	0.225	**70**	100
				R4 stream	10 m D + R	0.157	100	100
Pyraclostrobin	winter cereals, 1 - 2 x 250 g a.s./ha	Aquatic invertebrates, chronic (*D. magna*)	NOEC = 4.0	D1 ditch	75% N	0.396	10	10
					5 m D + 50% N	0.214	19	
					10 m D	0.228	18	
				D1 stream	75% N	0.336	12	10
					5 m D + 50% N	0.246	16	
					10 m D	0.260	15	
				D2 ditch	75% N	0.397	10	10
					5 m D + 50% N	0.215	19	
					10 m D	0.228	18	
				D2 stream	75% N	0.353	11	10
					5 m D + 50% N	0.258	16	
					10 m D	0.274	15	
				D3 ditch	75% N	0.392	10	10
					5 m D + 50% N	0.212	19	
					10 m D	0.225	18	
				D4 pond	--	0.065	62	10
				D4 stream	75% N	0.295	14	10
					5 m D + 50% N	0.215	19	

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
					10 m D	0.228	18	
				D5 pond	--	0.066	61	10
				D5 stream	75% N	0.316	13	10
					5 m D + 50% N	0.231	17	
					10 m D	0.244	16	
				D6 ditch	75% N	0.387	10	10
					5 m D + 50% N	0.210	19	
					10 m D	0.222	18	
				R1 pond	--	0.065	62	10
				R1 stream	75% N	0.259	15	10
					5 m D	0.379	11	
				R3 stream	75% N	0.365	11	10
					5 m D + 50% N	0.267	15	
					10 m D	0.283	14	
				R4 stream	10 m D + R	0.248	16	10
	spring cereals 1 - 2 x 250 g a.s./ha	Aquatic invertebrates, chronic (*D. magna*)	NOEC = 4.0	D1 ditch	90% N	0.174	23	10
					5 m D + 50% N	0.226	18	
					10 m D	0.235	17	
				D1 stream	75% N	0.347	12	10
					5 m D + 50% N	0.254	16	
					10 m D	0.269	15	
				D3 ditch	75% N	0.392	10	10
					5 m D + 50% N	0.212	19	
					10 m D	0.225	18	
				D4 pond	--	0.065	62	10
				D4 stream	75% N	0.325	12	10
					5 m D + 50% N	0.237	17	
					10 m D	0.252	16	
				D5 pond	--	0.068	59	10
				D5 stream	75% N	0.329	12	10
					5 m D + 50% N	0.241	17	
					10 m D	0.255	16	
				R4 stream	75% N	0.267	15	10
					5 m D	0.379	11	
	maize, 1 x 200 g a.s./ha	Aquatic invertebrates, chronic (*D. magna*)	NOEC = 4.0	D3 ditch	75% N	0.259	15	10
					5 m D	0.340	12	
				D4 pond	--	0.042	95	10
				D4 stream	75% N	0.224	18	10
					5 m D	0.377	11	
				D5 pond	--	0.042	95	10
				D5 stream	75% N	0.222	18	10
					5 m D	0.374	11	

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
				D6 ditch	75% N	0.257	16	10
					5 m D	0.337	12	
				R1 pond	--	0.042	95	10
				R1 stream	50% N	0.360	11	10
					5 m D	0.303	13	
				R2 stream	75% N	0.241	17	10
					5 m D + 50% N	0.203	20	
					10 m D	0.215	19	
				R3 stream	75% N	0.253	16	10
					5 m D + 50% N	0.213	19	
					10 m D	0.225	18	
				R4 stream	50% N	0.351	11	10
					5 m D	0.301	13	
Pyraclostrobin	cereals 1 - 2 x 250 g a.s./ha	outdoor mesocosm (multiple spray application) [3]	NOEC = 8.0	2	--	2.299	3.5	2
Pyraclostrobin	maize, 1 x 200 g a.s./ha	outdoor mesocosm (multiple spray application) [3]	NOEC = 8.0	2	--	1.839	4.4	2
Pyraclostrobin	cereals, 1 - 2 x 250 g a.s./ha	Aquatic insects, chronic (*C. riparius*), water exposure	NOEC = 40	Step 2	--	2.299	17	10
Pyraclostrobin	maize, 1 x 200 g a.s./ha	Aquatic insects, chronic (*C. riparius*), water exposure	NOEC = 40	Step 2	--	1.839	22	10
Pyraclostrobin	winter cereals, 1 - 2 x 250 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC = 1370 µg/kg dry sediment [1]	D1 ditch	--	8.901 [2]	154	10
				D1 stream	--	0.836 [2]	1639	10
				D2 ditch	--	5.032 [2]	272	10
				D2 stream	--	4.212 [2]	325	10
				D3 ditch	--	1.289 [2]	1063	10
				D4 pond	--	0.698 [2]	1963	10
				D4 stream	--	0.065 [2]	21077	10

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
				D5 pond	--	0.649 2)	2111	10
				D5 stream	--	0.242 2)	5661	10
				D6 ditch	--	3.807 2)	360	10
				R1 pond	--	0.897 2)	1527	10
				R1 stream	--	4.063 2)	337	10
				R3 stream	--	2.011 2)	681	10
				R4 stream	--	10.583 2)	129	10
	spring cereals 1 - 2 x 250 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC = 1370 µg/kg dry sediment 1)	D1 ditch	--	9.836 2)	139	10
				D1 stream	--	1.015 2)	1350	10
				D3 ditch	--	1.496 2)	916	10
				D4 pond	--	0.586 2)	2338	10
				D4 stream	--	0.257 2)	5331	10
				D5 pond	--	0.646 2)	2121	10
				D5 stream	--	0.093 2)	14731	10
				R4 stream	--	5.553 2)	247	10
	maize, 1 x 200 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC = 1370 µg/kg dry sediment 1)	Step 2	--	95.014 2)	14	10
Pyraclostrobin	cereals, 1 - 2 x 250 g a.s./ha	Algae, chronic (*P. subcapitata*)	E_rC_{50} > 843	Step 1	--	17.031	> 49	10
	maize, 1 x 200 g a.s./ha	Algae, chronic (*P. subcapitata*)	E_rC_{50} > 843	Step 1	--	6.813	> 124	10
Pyraclostrobin	cereals, 1 - 2 x 250 g a.s./ha	Higher aquatic plant (*L. gibba*)	E_rC_{50} = 1720	Step 1	--	17.031	101	10
	maize, 1 x 200 g a.s./ha	Higher aquatic plant (*L. gibba*)	E_rC_{50} = 1720	Step 1	--	6.813	252	10
BF 500-3	cereals, 1 - 2 x 250 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC ≥ 16000 µg/kg dry sediment 1)	1	--	20.030 2)	≥ 799	10
	maize, 1 x 200 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC ≥ 16000 µg/kg dry sediment 1)	1	--	8.012 2)	≥ 1997	10

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
BF 500-5	cereals, 1 - 2 x 250 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} = 11300	1	--	0.256	44141	100
	maize, 1 x 200 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} = 11300	1	--	0.102	110784	100
	cereals, 1 - 2 x 250 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 10000	1	--	0.256	> 39063	100
	maize, 1 x 200 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 10000	1	--	0.102	> 98039	100
	cereals, 1 - 2 x 250 g a.s./ha	Algae, chronic (*P. subcapitata)*	E_rC_{50} = 5330	1	--	0.256	20820	10
	maize, 1 x 200 g a.s./ha	Algae, chronic (*P. subcapitata)*	E_rC_{50} = 5330	1	--	0.102	52255	10
BF 500-6	cereals, 1 - 2 x 250 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC = 1200 µg/kg dry sediment [1)]	2	--	60.344 [2)]	20	10
	maize, 1 x 200 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC = 1200 µg/kg dry sediment [1)]	2	--	24.752 [2)]	48	10
BF 500-7	cereals, 1 - 2 x 250 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC ≥ 123500 µg/kg dry sediment [1)]	1	--	182.976 [2)]	≥ 675	10
	maize, 1 x 200 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC ≥ 123500 µg/kg dry sediment [1)]	1	--	73.190 [2)]	≥ 1687	10
BF 500-11 [§]	cereals, 1 - 2 x 250 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} > 100000	1	--	0.407	> 245700	100
	maize, 1 x 200 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} > 100000	1	--	0.163	> 613497	100
	cereals, 1 - 2 x 250 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 100000	1	--	0.407	> 245700	100
	maize, 1 x 200 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 100000	1	--	0.163	> 613497	100

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
	cereals, 1 - 2 x 250 g a.s./ha	Algae, chronic (*S. subspicatus)*	E_rC_{50} > 100000	1	--	0.407	> 245700	10
	maize, 1 x 200 g a.s./ha	Algae, chronic (*S. subspicatus)*	E_rC_{50} > 100000	1	--	0.163	> 613497	10
BF 500-13 §	cereals, 1 - 2 x 250 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} > 50000 < 100000	1	--	0.533	> 93809 < 187617	100
	maize, 1 x 200 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} > 50000 < 100000	1	--	0.213	> 234742 < 469484	100
	cereals, 1 - 2 x 250 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 100000	1	--	0.533	> 187617	100
	maize, 1 x 200 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 100000	1	--	0.213	> 469484	100
	cereals, 1 - 2 x 250 g a.s./ha	Algae, chronic (*S. subspicatus)*	E_rC_{50} > 100000	1	--	0.533	> 187617	10
	maize, 1 x 200 g a.s./ha	Algae, chronic (*S. subspicatus)*	E_rC_{50} > 100000	1	--	0.213	> 469484	10
BF 500-14 §	cereals, 1 - 2 x 250 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} > 39400 < 82600	1	--	0.573	> 68761 < 144154	100
	maize, 1 x 200 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} > 39400 < 82600	1	--	0.229	> 172052 < 360699	100
	cereals, 1 - 2 x 250 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 60900	1	--	0.573	> 106283	100
	maize, 1 x 200 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 60900	1	--	0.229	> 265939	100
	cereals, 1 - 2 x 250 g a.s./ha	Algae, chronic (*S. subspicatus)*	E_rC_{50} > 100000	1	--	0.573	> 174520	10
	maize, 1 x 200 g a.s./ha	Algae, chronic (*S. subspicatus)*	E_rC_{50} > 100000	1	--	0.229	> 436681	10
BAS 516 07 F								
Pyraclostrobin	potato, 1 - 4 x 17 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} = 6.2	D3 ditch	--	0.088	**70**	100
				D4 pond	--	0.005	1240	100
				D4 stream	--	0.069	**90**	100
				D6 (1st) ditch	--	0.088	**70**	100
				D6 (2nd) ditch	--	0.087	**71**	100
				R1 pond	--	0.010	620	100

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
				R1 stream	--	0.061	102	100
				R2 stream	--	0.081	**77**	100
				R3 stream	--	0.086	**72**	100
Pyraclostrobin	potato, 1 - 4 x 17 g a.s./ha	fish HC_5 based on acute NOECs of 8 species	HC_5 = 3.38	D3 ditch	--	0.088	38	3 *
				D4 pond	--	0.005	676	3 *
				D4 stream	--	0.069	49	3 *
				D6 (1^{st}) ditch	--	0.088	38	3 *
				D6 (2^{nd}) ditch	--	0.087	39	3 *
				R1 pond	--	0.010	338	3 *
				R1 stream	--	0.061	55	3 *
				R2 stream	--	0.081	42	3 *
				R3 stream	--	0.086	39	3 *
Pyraclostrobin	potato, 1 - 4 x 17 g a.s./ha	Fish, chronic, ELS (*O. mykiss*)	NOAEC = 2.35	D3 ditch	--	0.088	27	10
				D4 pond	--	0.005	470	10
				D4 stream	--	0.069	34	10
				D6 (1^{st}) ditch	--	0.088	27	10
				D6 (2^{nd}) ditch	--	0.087	27	10
				R1 pond	--	0.010	235	10
				R1 stream	--	0.061	39	10
				R2 stream	--	0.081	29	10
				R3 stream	--	0.086	27	10
Pyraclostrobin	potato, 1 - 4 x 17 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} = 15.7	D3 ditch	--	0.088	178	100
				D4 pond	--	0.005	3140	100
				D4 stream	--	0.069	228	100
				D6 (1^{st}) ditch	--	0.088	178	100
				D6 (2^{nd}) ditch	--	0.087	180	100
				R1 pond	--	0.010	1570	100
				R1 stream	--	0.061	257	100
				R2 stream	--	0.081	194	100
				R3 stream	--	0.086	183	100
Pyraclostrobin	potato, 1 - 4 x 17 g a.s./ha	Aquatic invertebrates, chronic (*D. magna*)	NOEC = 4.0	2	--	0.164	24	10

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
Pyraclostrobin	potato, 1 - 4 x 17 g a.s./ha	outdoor mesocosm (multiple spray application) 3)	NOEC = 8.0	1	--	2.316	3.5	2
Pyraclostrobin	potato, 1 - 4 x 17 g a.s./ha	Aquatic insects, chronic (*C. riparius*), water exposure	NOEC = 40	1	--	2.316	17	10
Pyraclostrobin	potato, 1 - 4 x 17 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC = 1370 µg/kg dry sediment 1)	2	--	14.142 2)	97	10
Pyraclostrobin	potato, 1 - 4 x 17 g a.s./ha	Algae, chronic (*P. subcapitata*)	E_rC_{50} > 843	1	--	2.316	> 364	10
Pyraclostrobin	1 - 4 x 17 g a.s./ha	Higher aquatic plant (*L. gibba*)	E_rC_{50} = 1720	1	--	2.316	743	10
BF 500-3	potato, 1 - 4 x 17 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC ≥ 16000 µg/kg dry sediment 1)	1	--	2.724 2)	≥ 5874	10
BF 500-5	potato, 1 - 4 x 17 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} = 11300	1	--	0.035	322857	100
	potato, 1 - 4 x 17 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 10000	1	--	0.035	> 285714	100
	potato, 1 - 4 x 17 g a.s./ha	Algae, chronic (*P. subcapitata)*	E_rC_{50} = 5330	1	--	0.035	152286	10
BF 500-6	potato, 1 - 4 x 17 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC = 1200 µg/kg dry sediment 1)	1	--	41.471 2)	29	10
BF 500-7	potato, 1 - 4 x 17 g a.s./ha	Aquatic insects, chronic (*C. riparius*), sediment exposure	NOEC ≥ 123500 µg/kg dry sediment 1)	1	--	24.885 2)	≥ 4963	10

Test substance	Use pattern	Organism	Toxicity endpoint [µg/L]	FOCUS step/ scenario	Mitigation measures	PEC [µg/L]	TER	TER risk assessment trigger
BF 500-11 §	potato, 1 - 4 x 17 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} > 100000	1	--	0.055	> 1818182	100
	potato, 1 - 4 x 17 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 100000	1	--	0.055	> 1818182	100
	potato, 1 - 4 x 17 g a.s./ha	Algae, chronic (*S. subspicatus)*	E_rC_{50} > 100000	1	--	0.055	> 1818182	10
BF 500-13 §	potato, 1 - 4 x 17 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} > 50000 < 100000	1	--	0.072	> 694444 < 1388889	100
	potato, 1 - 4 x 17 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 100000	1	--	0.072	> 1388889	100
	potato, 1 - 4 x 17 g a.s./ha	Algae, chronic (*S. subspicatus*	E_rC_{50} > 100000	1	--	0.072	> 1388889	10
BF 500-14 §	potato, 1 - 4 x 17 g a.s./ha	Fish, acute (*O. mykiss*)	LC_{50} > 39400 < 82600	1	--	0.078	> 505128 < 1058974	100
	potato, 1 - 4 x 17 g a.s./ha	Aquatic invertebrates, acute (*D. magna*)	EC_{50} > 60900	1	--	0.078	> 780769	100
	potato, 1 - 4 x 17 g a.s./ha	Algae, chronic (*S. subspicatus*	E_rC_{50} > 100000	1	--	0.078	> 1282051	10

TERs shown in **bold** fall below the relevant trigger.
D = Drift mitigation using no-spray buffer zones; D + R = Drift and runoff mitigation using no-sprayed vegetated filter strips
SSD = Species Sensitivity Distribution

§ Metabolites were mainly observed in irradiated water/sediment studies; accordingly only PEC values derived from irradiated water/sediment studies are reported.

* Reduced assessment factor for acute risk assessment based on the HC_5 value from the fish SSD (for details see "Refined Risk Assessment" presented in chapter 10.2 of the MCP dossier part for Annex I renewal).

1) Based on sediment concentration in spiked water study.

2) $PEC_{sed, max}$ [µg/kg dry sediment]

3) Study was performed with the solo-formulation BAS 500 00 F (containing 250 g pyraclostrobin/L, nominally)

Bioconcentration

Log Pow	3.99
Bioconcentration factor (BCF)	379 – 507 (whole fish) 178 - 191 (edibles)
Risk assessment trigger for the bioconcentration factor	100 BAS 500 F is not stable in water and is rapidly metabolised and excreted by fish (CT_{50} < 1 day), thus there is low risk for bioaccumulation
Clearance time (CT_{50}) (CT_{90})	CT_{50} < 1 day
Level of residues (%) in organisms after the 14 day depuration phase	Not applicable

Effects on honeybees (SANCO data point 8.3.1 and SANCO/11803 data point 10.3.1)

Acute oral toxicity	LD_{50} (48 h) > 73.1 µg a.s./bee LD_{50} (48 h) > 110.0 µg a.s./bee LD_{50} (96 h) > 97.2 µg a.s./bumblebee LD_{50} (48 h) > 381.84 µg BAS 500 06 F/bee LD_{50} (48 h) > 258.7 µg BAS 516 00 F/bee
Acute contact toxicity	LD_{50} (48 h) > 100.0 µg a.s./bee LD_{50} (48 h) > 100.0 µg a.s./bee LD_{50} (96 h) > 100.0 µg a.s./bumblebee LD_{50} (72 h) > 368.20 µg BAS 500 06 F/bee LD_{50} (48 h) > 299.4 µg BAS 516 00 F/bee

No unacceptable lethal or sublethal effects were found in honeybee colonies exposed to 1.25 L product/ha in a semi-field tunnel test (*Phacelia tanacetifolia*).
Two studies were conducted with pyraclostrobin containing products in sunflower and oilseed rape (OSR) to determine the residues in the bee relevant matrices pollen and nectar. The highest exposure was found in pollen, i.e. 16.6 ppm (sunflower) and 6.7 ppm (OSR).
Additionally, no unacceptable effects in queen development, queen survival and adult worker bee health at 400 ppm Pristine treated pollen (based on analysis equivalent to 22 ppm pyraclostrobin treated pollen) were observed.

Hazard quotients for honey bees (SANCO/11803 data point 10.3.1)

BAS 516 07 F:

Test substance	Use pattern	Exposure route	Endpoint [µg/bee]	Maximum single application rate	Hazard quotient	TER risk assessment trigger
pyraclostrobin	potatoes	acute oral	$LD_{50} > 73.1$	17 g a.s./ha	< 0.23	50
		acute contact	$LD_{50} > 100$		< 0.17	
pyraclostrobin		acute oral	$LD_{50} > 110$	17 g a.s./ha	< 0.15	
		acute contact	$LD_{50} > 100$		< 0.17	
BAS 516 00 F		acute oral	$LD_{50} > 258.7$	250 g/ha	< 0.97	
		acute contact	$LD_{50} > 299.4$		< 0.84	

BAS 500 06 F:

Test substance	Use pattern	Exposure route	Endpoint [µg/bee]	Maximum single application rate	Hazard quotient	TER risk assessment trigger
pyraclostrobin	cereals, maize	acute oral	$LD_{50} > 73.1$	250 g a.s./ha	< 3.4	50
		acute contact	$LD_{50} > 100$		< 2.5	
pyraclostrobin		acute oral	$LD_{50} > 110$	250 g a.s./ha	< 2.3	
		acute contact	$LD_{50} > 100$		< 2.5	
BAS 500 06 F		acute oral	$LD_{50} > 381.84$	1305 g/ha *	< 3.4	
		acute contact	$LD_{50} > 368.20$		< 3.5	

* Taking into account the density of BAS 500 06 F of 1.044 g/cm^3.

Effects on other arthropod species (SANCO data point 8.3.2 and SANCO/11803 data point 10.3.2)

BAS 516 07 F:

Test substance	Use pattern	Species	Test type	Endpoint [kg/ha]	PER in-field [kg/ha]	PER off-field [kg/ha]	HQ in-field	HQ off-field
Tier I								
BAS 516 07 F	4 x 0.25 kg/ha	*T. pyri* protonymphs	Laboratory test, artificial substrate, 2D	LR_{50} > 5.4	0.675	0.0012	< 0.13	< 0.002
		A. rhopalosiphi adults		LR_{50} > 5.4	0.675	0.0012	< 0.13	< 0.002
Tier II								
BAS 516 07 F	4 x 0.25 kg/ha	*C. carnea* larvae	Laboratory test, artificial substrate, 2D	LR_{50} > 3.6	0.675	0.0012 [2)]	Endpoint ≥ PER → acceptable risk	
		C. carnea larvae	Extended laboratory test, natural substrate, 2D	LR_{50} > 2 x 1.8 ER_{50} > 2 x 1.8	0.675	0.0012 [2)]	Endpoint ≥ PER → acceptable risk	
		P. cupreus adults	Laboratory test, artificial substrate,3D	LR_{50} > 3.6 ER_{50} > 3.6	0.675	0.012 [2)]	Endpoint ≥ PER → acceptable risk	
		A. bilineata adults	Laboratory test, artificial substrate, 2D	ER_{50} > 3.6	0.675	0.0012 [2)]	Endpoint ≥ PER → acceptable risk	
		Pardosa spec. adults	Laboratory test, artificial substrate, 3D	LR_{50} > 3.6 ER_{50} > 3.6	0.675	0.012 [2)]	Endpoint ≥ PER → acceptable risk	
Higher Tier								
BAS 516 07 F	4 x 0.25 kg/ha	Predatory mites all live stages	Field tests, orchards, 3D (3 studies)	No unacceptable effects up to 5 x 0.75 kg/ha	Max. intended use = 4 x 0.25 kg/ha		Endpoint ≥ intended application rate → acceptable risk	

BAS 500 06 F:

Test substance	Use pattern	Species	Test type	Endpoint [L/ha]	PER in-field [L/ha]	PER off-field [L/ha] [1)]	HQ in-field	HQ off-field
Tier I								
BAS 500 06 F	2 x 1.25 L/ha	*T. pyri* protonymphs	Laboratory test, artificial substrate, 2D	LR_{50} = 0.87	2.125	0.005	**2.4**	0.057
		A. rhopalosiphi adults		LR_{50} = 0.04	2.125	0.005	**53**	0.125
Tier II								
BAS 500 06 F	2 x 1.25 L/ha	*T. pyri* protonymphs	Extended laboratory test, natural substrate, 2D	LR_{50} = 2.45 L/ha ER_{50} > 2.5 L/ha	2.125	0.005	Endpoint ≥ PER → acceptable risk	
		A. rhopalosiphi adults	Extended laboratory test, natural substrate, 3D	LR_{50} > 2.5 L/ha ER_{50} > 2.5 L/ha	2.125	0.051	Endpoint ≥ PER → acceptable risk	
		C. carnea larvae	Extended laboratory test, natural substrate, 2D	LR_{50} = 0.72 ER_{50} > 0.63	2.125	0.005	Endpoint < PER [2)] → further testing necessary	
		C. carnea larvae	Extended laboratory test, natural substrate, 2D	No unacceptable effects on survival and reproduction on DAT 7 at up to 2.5 L/ha	2.125	0.005	Endpoint ≥ PER → acceptable risk	
		A. bilineata adults	Aged-residue design using, natural substrate, 2D	ER_{50} > 3.75	2.125	0.051	Endpoint ≥ PER → acceptable risk	

HQ values in **bold** are above the trigger.

1) In the Tier 2 off-field risk assessment, the standard 5-fold uncertainty (correction) factor should be included to the calculation to cover the inter-species variability in sensitivity of off-field non-target arthropod species. However, as additional species are tested, the uncertainty is reduced and no additional safety factor was applied.

2) Endpoint < $PER_{in\text{-}field}$, $PER_{off\text{-}field}$ is covered.

Effects on earthworms (SANCO/11802 data point 8.4.2 and SANCO/11803 data point 10.4.1)

Acute toxicity	$LC_{50\ CORR}$ = 283 mg a.s./kg dry soil $LC_{50\ CORR}$ > 500 mg BF 500-6/kg dry soil $LC_{50\ CORR}$ > 500 mg BF 500-7/kg dry soil $LC_{50\ CORR}$ > 500 mg BAS 516 07 F/kg dry soil $LC_{50\ CORR}$ = 193 mg BAS 500 06 F/kg dry soil
Reproductive toxicity	$NOEC_{CORR}$ = 11.6 mg/kg dry soil $NOEC_{CORR} \geq$ 160 mg BF 500-6/kg dry soil $NOEC_{CORR} \geq$ 160 mg BF 500-7/kg dry soil NOEC = 40 mg BAS 516 07 F/kg dry soil NOEC = 30 mg BAS 500 06 F/kg dry soil

Toxicity/exposure ratios for earthworms (SANCO/11803 data point 10.4.1)

BAS 516 07 F:

Test substance	Use pattern [g/ha]	Species	Test type	Endpoint [mg/kg dry soil]	PEC [mg/kg dry soil]	TER	Trigger
pyraclostrobin	4 x 17	*Eisenia fetida*	56-d reproduction test	$NOEC_{CORR}$ = 11.6	0.015	773	5
BF 500-6	--		56-d reproduction test	$NOEC_{CORR} \geq$ 160	0.008	≥ 20000	
BF 500-7	--		56-d reproduction test	$NOEC_{CORR} \geq$ 160	0.005	≥ 32000	
BAS 516 07 F	pyraclostrobin: 4 x 17		56-d reproduction test	NOEC = 2.7	0.015	180	
	total a.s.: 4 x 84			NOEC = 13.4	0.260	52	

BAS 500 06 F:

Test substance	Use pattern	Species	Test type	Endpoint [mg/kg dry soil]	PEC [mg/kg dry soil]	TER	Trigger
pyraclostrobin	2 x 250 g a.s./ha	*Eisenia fetida*	56-d reproduction test	$NOEC_{CORR}$ = 11.6	0.228	51	5
BF 500-6	--		56-d reproduction test	$NOEC_{CORR} \geq$ 160	0.121	≥ 1322	
BF 500-7	--		56-d reproduction test	$NOEC_{CORR} \geq$ 160	0.073	≥ 2192	
pyraclostrobin in BAS 500 06 F	2 x 250 g a.s./ha		56-d reproduction test	NOEC = 5.75	0.228	25	

Effects on non-target soil meso- and macrofauna (other than earthworms) (SANCO/11802 data point 8.4.1 and SANCO/11803 data point 10.4.2)

Species level testing	*Folsomia candida*: NOEC ≥ 1000 mg BF 500-6/kg dry soil NOEC ≥ 800 mg BF 500-7/kg dry soil NOEC = 250 mg BAS 516 07 F/kg dry soil NOEC = 125 mg BAS 500 06 F/kg dry soil *Hypoaspis aculeifer*: NOEC ≥ 500 mg BAS 516 07 F/kg dry soil NOEC ≥ 90.0 mg BAS 500 06 /kg dry soil
Higher tier testing	--

Effects on soil nitrogen transformation (SANCO/11802 data point 8.5 and SANCO/11803 data point 10.5)

Nitrogen mineralization	**BAS 516 07 F:** No unacceptable effects (i.e. < 25%) up to 24 mg/kg dry soil, corresponding to 1.6 mg pyraclostrobin and 8.0 mg total a.s./kg dry soil. These concentrations represent more than the maximum PEC values of 0.015 mg pyraclostrobin and 0.260 mg total a.s./kg dry soil. **BAS 500 06 F:** No unacceptable effects (i.e. < 25%) up to 17.3 mg/kg dry soil, corresponding to 3.33 mg pyraclostrobin/kg dry soil. This concentration represents more than the maximum PEC value of 0.228 mg pyraclostrobin/kg dry soil. **BF 500-6:** No unacceptable effects (i.e. < 25%) up to 1.0 mg/kg dry soil. This concentration represents more than the maximum PEC values of 0.121 and 0.008 mg/kg dry soil, respectively. **BF 500-7:** No unacceptable effects (i.e. < 25%) up to 0.5 mg/kg dry soil. This concentration represents more than the maximum PEC values of 0.073 and 0.005 mg/kg dry soil, respectively.

Effects on terrestrial non-target higher plants (SANCO/11802 data point 8.6 and SANCO/11803 data point 10.6)

Most sensitive crop species	Test substance	ER_{50} vegetative vigour	ER_{50} emergence	Exposure	TER	Trigger
Onion, oat, pea, cabbage, carrot, sunflower	BAS 516 00 F	> 5.4 kg/ha	--	0.007 kg/ha	> 771	5
Carrot, lettuce, oilseed rape, cabbage, soybean, tomato, onion, ryegrass, wheat and corn	BAS 516 07 F	> 8.0 kg/ha	> 8.0 kg/ha 0	0.007 kg/ha	> 1143	
Carrot, lettuce, oilseed rape, cabbage, soy bean, tomato, onion, rye grass, oat, corn	BAS 500 06 F	> 1.25 L/ha	> 1.25 L/ha	0.0346 L/ha	> 36	

Effects on biological methods for sewage treatment (SANCO/11802 data point 8.8)

Test type/organism	Endpoint
Respiration inhibition test (activated sludge)	EC_{20} > 1000 mg a.s./L

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT N3

SUBSTANCES AND METABOLITES; STRUCTURES, CODES, SYNONYMS

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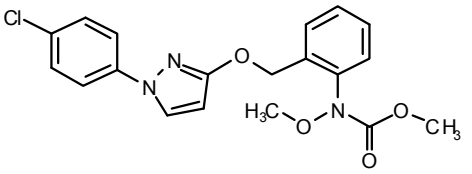
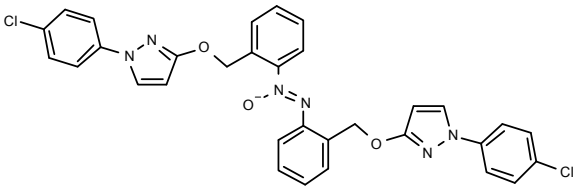
Version history[1]

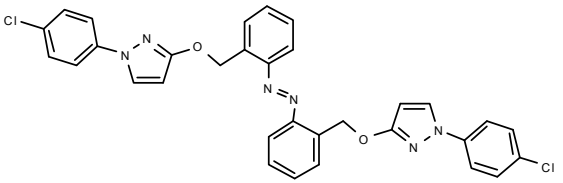
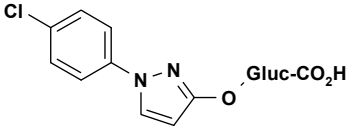
Date	Data points containing amendments or additions and brief description	Document identifier and version number
18-Jul-2014		BASF DocID 2014/1165143 (version 1)
27-Feb-2017	The document was amended in order to add the metabolites identified in a rat metabolism study after dosing metabolite 500M106. New or changed text is marked in yellow.	BASF DocID 2017/1032929 (version 2)

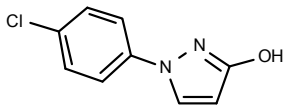
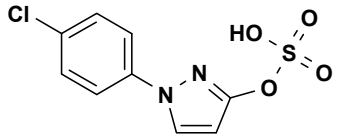
[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

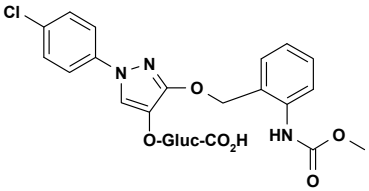
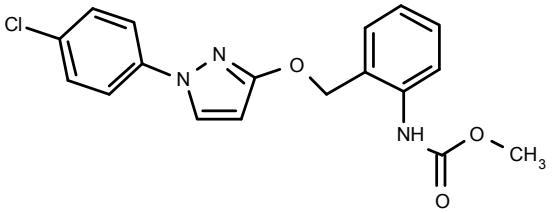
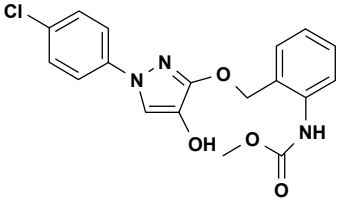
Substances and metabolites; structures, codes, synonyms

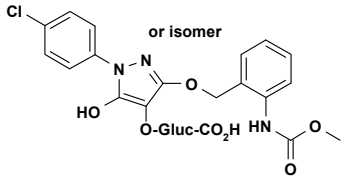
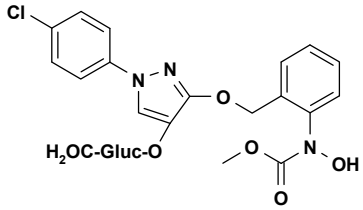
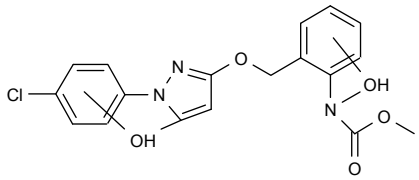
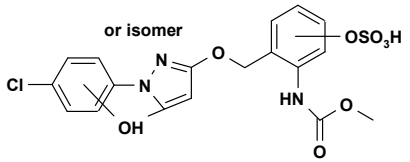
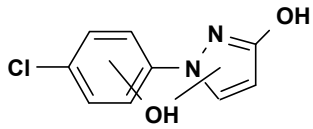
The table below is summarizing all metabolites addressed within this dossier. For some of the compounds, structural elucidation is based on mass spectroscopic experiments. MS does typically not allow a clear assignment of e.g. the position of hydroxyl groups in aromatic ring systems. Same applies to information on stereochemistry of e.g. carbohydrate conjugates (glucosides / glucuronic acid conjugates). Hydroxylation followed by subsequent conjugate reactions result in the formation of isomers. The structures shown below are depicted in a way indicating this fact. For such structures no exact IUPAC chemical name can be provided. If not stated otherwise in the "occurrence column", the metabolites indicated for rat and goat have been found in the corresponding *in-vivo* studies.

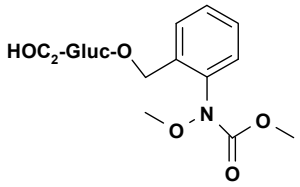
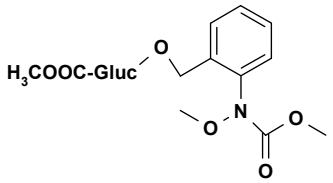
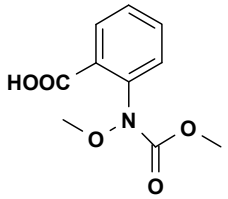
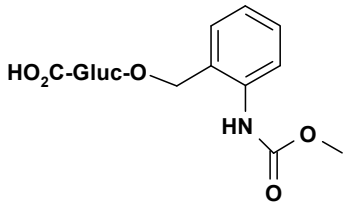
Code Numbers			**Description**		
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
BAS 500 F	304428	500M01	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)-(N-methoxy)carbamate 175013-18-0		
500M01	364380	BF 500-6	N,N'-bis-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)diazene N-oxide not assigned	soil, sediment rat after dosing with metabolite 500M106	

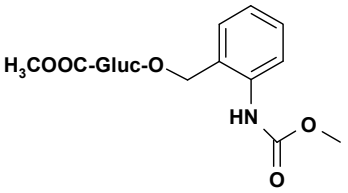
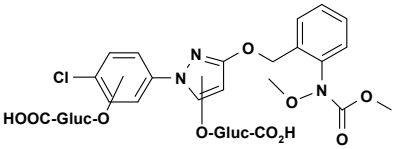
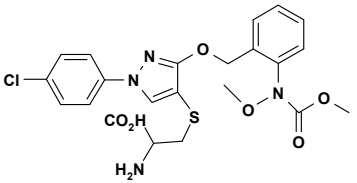
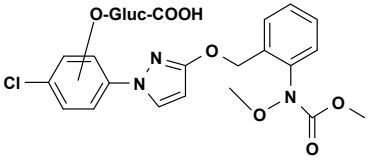
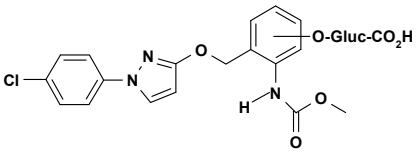
Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**		
500M02	369315	BF 500-7	N,N'-bis-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)diazene not assigned	soil, sediment human, dog, rabbit (*in-vitro*) rat after dosing with metabolite 500M106	
500M03	not assigned		not assigned	rat human, rabbit (*in-vitro*) rat after dosing with metabolite 500M106	

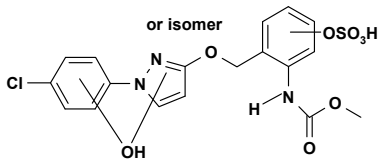
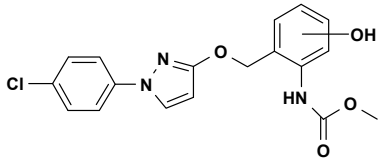
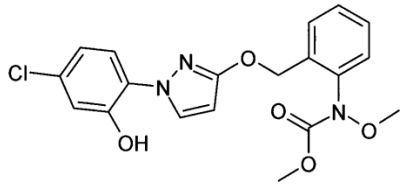
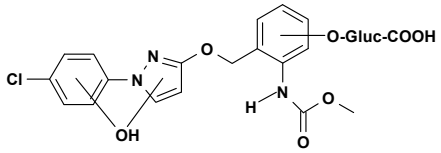
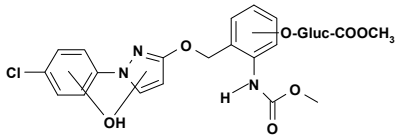
Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**		
500M04	298327	BF 500-5	1-(4-chlorophenyl)-1H-pyrazol-3-ol 76205-19-1	soil, water grapes, Chinese cabbage, wheat, potatoes, hydrolysis (olive oil, high temp.) hen, goat (*in-vivo* and *in-vitro*), cow (*in-vitro*), fish (bioaccumulation) rat human, rabbit, dog, rat (*in-vitro*) rat after dosing with metabolite 500M106	
500M05	not assigned		not assigned	goat rat rat after dosing with metabolite 500M106	

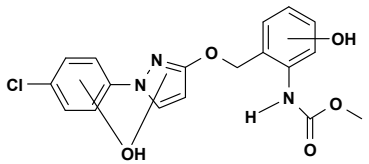
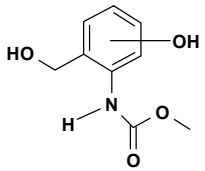
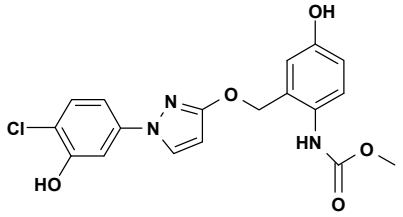
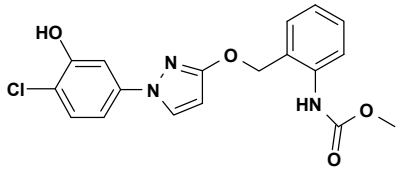
Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M06	not assigned		not assigned	hen rat (*in-vivo*, plasma) human, rabbit, rat (*in-vitro*)	
500M07	340266	BF 500-3	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) carbamate 512165-96-7	soil, sediment grapes, Chinese cabbage, potatoes, wheat, rice, hydro-lysis (olive oil, high temp.) hen, goat rat	
500M08	not assigned		not assigned	goat, fish (bioaccumulation) rat (*in-vivo*, plasma)	

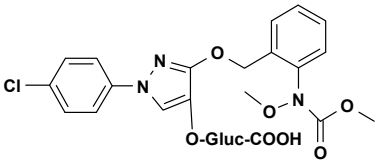
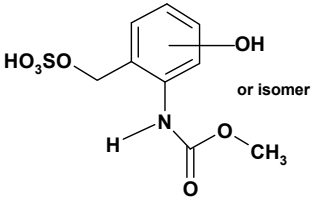
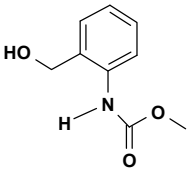
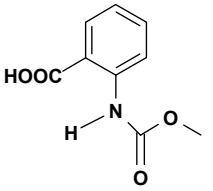
Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**		
500M13	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M15	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M18	not assigned		not assigned	rat	
500M19	not assigned		not assigned	rat	
500M21	not assigned		not assigned	rat rat after dosing with metabolite 500M106	

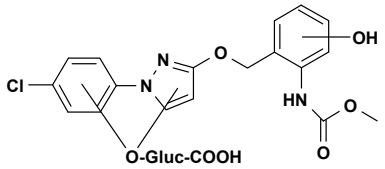
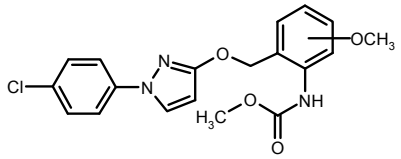
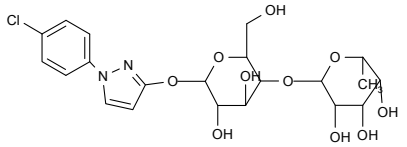
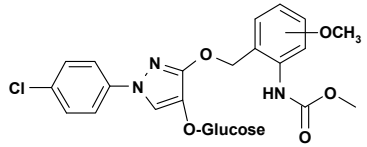
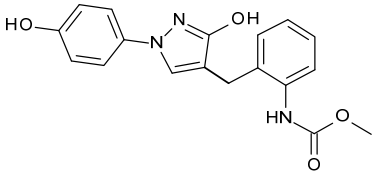
Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M22	not assigned		not assigned	rat	
500M23	not assigned		not assigned	rat	
500M24	5916421		2-[methoxy1 methoxycarbonyl]amino benzoic acid not assigned	wheat rat	
500M25	not assigned		not assigned	rat	

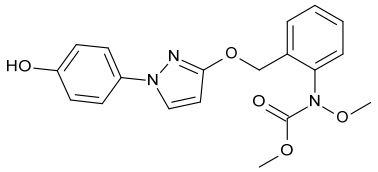
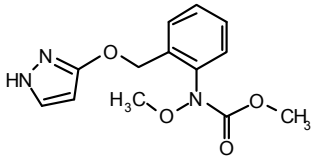
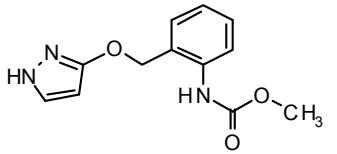
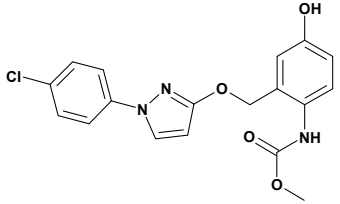
Code Numbers			Description		
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M26	not assigned		not assigned	rat	
500M29	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M30	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M31	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M32	not assigned		not assigned	hen rat (*in-vivo*, plasma)	

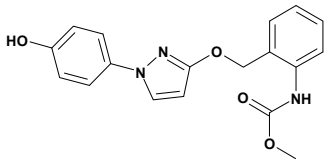
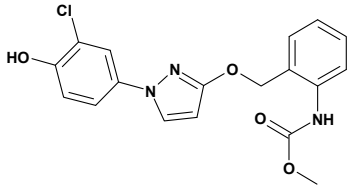
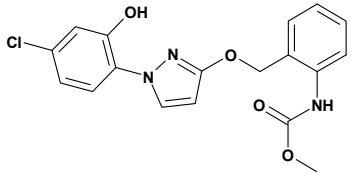
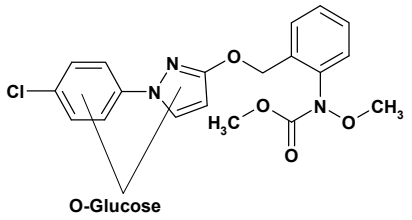
Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M33	not assigned		not assigned	rat	
500M34	not assigned		not assigned	wheat goat, cow (*in-vitro*) rat (*in-vivo*, plasma)	
500M35	412040		methyl N-(2-{[1-(4-chloro-2-hydroxyphenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-methoxy carbamate not assigned	goat, cow (*in-vitro*) rat (*in-vivo*, plasma)	
500M37	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M38	not assigned		not assigned	rat (*in-vivo*, plasma)	

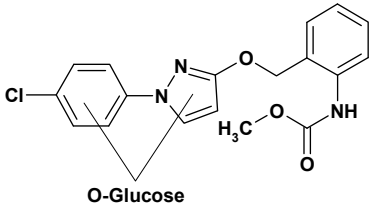
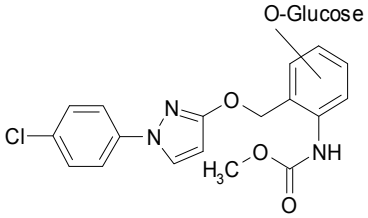
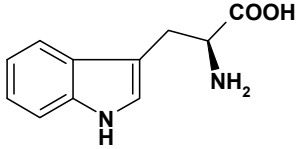
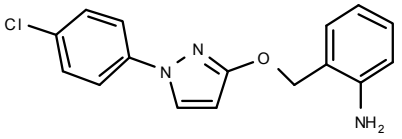
Code Numbers			Description		
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M39	not assigned		not assigned	hen, goat rat	
500M40	not assigned		not assigned	rat	
500M44	not assigned		not assigned	rat	
500M45	not assigned		not assigned	goat, fish (bioaccumulation) rat (*in-vivo*, plasma)	

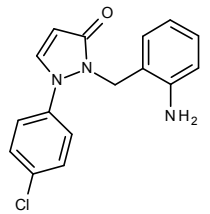
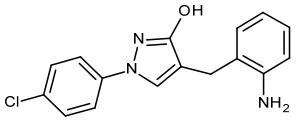
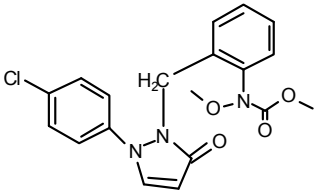
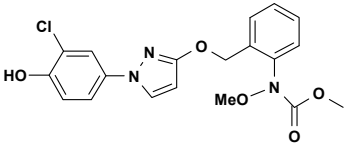
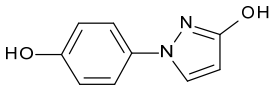
Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**		
500M46	not assigned		not assigned	rat (*in-vivo*, plasma)	
500M48	not assigned		not assigned	rat	
500M49	5916420		(2-hydroxymethyl- phenyl)-carbamic acid methyl ester not assigned	hydrolysis (olive oil, high temp.) hen	
500M51	78810		2-[(methoxycarbonyl) amino]benzoic acid 6268-38-8	goat rat	

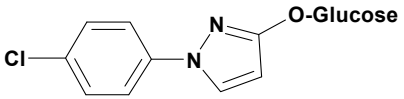
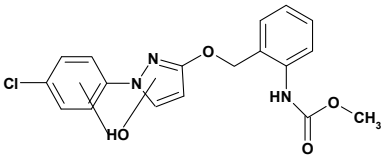
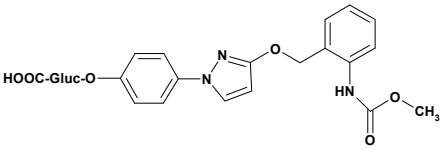
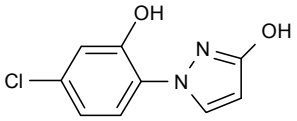
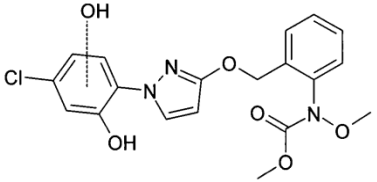
Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**		
500M52	not assigned		not assigned	rat	
500M54	not assigned		not assigned	grapes, potatoes, wheat	
500M55	not assigned		not assigned	grapes	
500M56	not assigned		not assigned	grapes	
500M58	not assigned		not assigned	water (photolysis)	

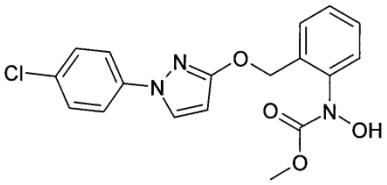
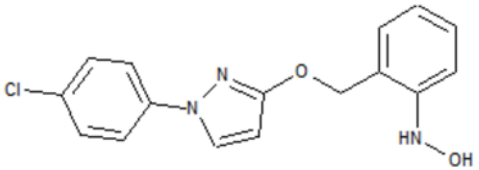
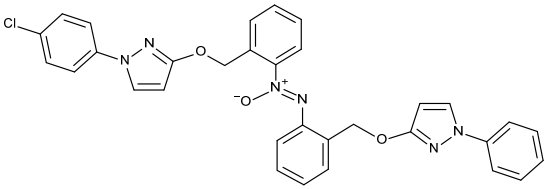
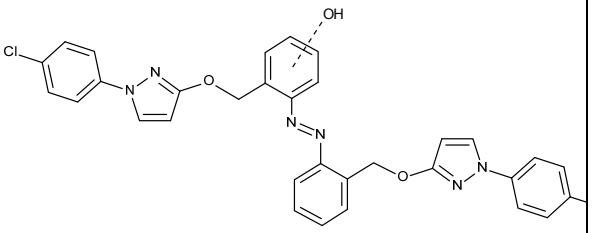
Code Numbers			Description		
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M59	412053	BF-500-12	methyl N-(2-{[1-(4-hydroxyphenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-methoxy carbamate not assigned	water (photolysis)	
500M60	411847	BF 500-11	methyl N-methoxy N-{2-[(1H-pyrazol-3-yl)oxymethyl]phenyl}carbamate 175013-17-9	water (photolysis)	
500M62	412785	BF 500-13	methyl N-[2-(1H-pyrazol-3-yloxymethyl)phenyl]carbamate not assigned	water (photolysis)	
500M64	not assigned		not assigned	hen, goat	

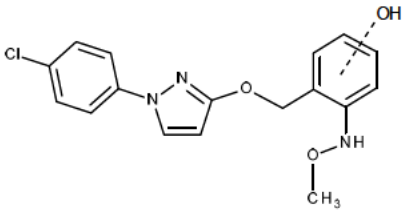
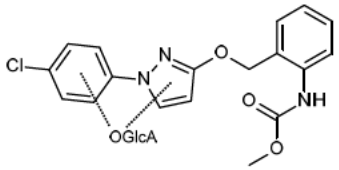
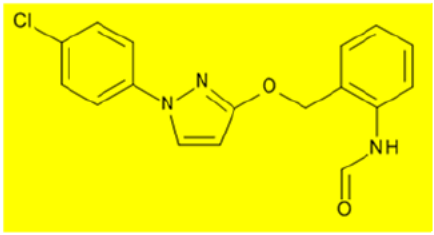
Code Numbers			Description		
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M65	not assigned		not assigned	goat	
500M66	not assigned		not assigned	hen, goat	
500M67	not assigned		not assigned	goat	
500M68	not assigned		not assigned	grapes, potatoes, wheat	

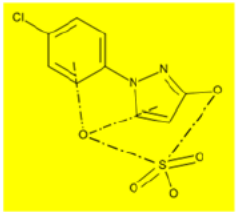
Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M70	not assigned		not assigned	wheat	
500M71	not assigned		not assigned	grapes, wheat	
500M72		L tryptophan	(S)-2-Amino-3-(3-indolyl)propionic acid, L-α-Amino-3-indolepropionic acid 73-22-3	Chinese cabbage, potatoes, wheat	
500M73	358672	BF 500-4	2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenylamine not assigned	soil (anaerobic) human, rabbit, dog, rat (*in-vitro*) rat after dosing with metabolite 500M106	

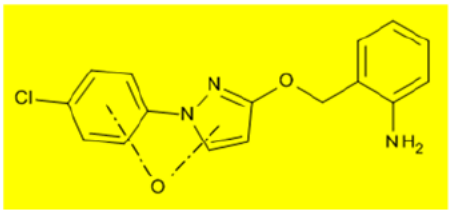
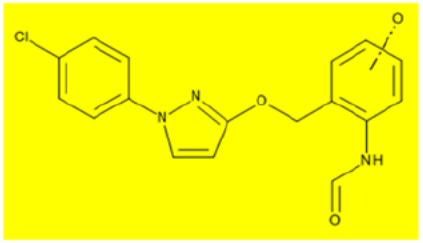
Code Numbers			Description		
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M74	not assigned	isomer of 500M73 (BF 500-4)	not assigned	soil (anaerobic)	
500M75	not assigned	isomer of 500M73 (BF 500-4)	not assigned	soil (anaerobic)	
500M76	413038	BF 500-14	methyl N-{2-[2-(4-chlorophenyl)-5-oxo-2,5-dihydro-pyrazol-1-ylmethyl]-phenyl} N-methoxy carbamate not assigned	water (photolysis) wheat	
500M77	4001763	BF 500-16	methyl-N-(2{[1-(3-chloro 4 hydroxyphenyl) -1H-pyrazol-3-yl] oxymethyl} phenyl) N methoxy Carbamate not assigned	hen	
500M78	377613	BF 500-15	1-(4-hydroxyphenyl)-1H-pyrazol-3-ol not assigned	water (photolysis)	

Code Numbers			Description	Compound found in	Structure
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M79	5937091		1-(4-chlorophenyl) -1H-pyrazol-3-yl glucopyranoside not assigned	grapes, potatoes	
500M80	not assigned		not assigned	hen	
500M83	not assigned		not assigned	hen	
500M85	399530	BF 500-8	1-(4 chloro2-hydroxy phenyl) 1Hpyrazol- 3oyl not assigned	goat	
500M86 / 500M87	not assigned		not assigned	goat, cow (*in-vitro*)	

Code Numbers			Description		
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M88	322410	BF 500-1	methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) N-hydroxy carbamate 220897-76-7	goat, cow (*in-vitro*) human, rabbit, dog, rat (*in-vitro*)	
500M89	334089		N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) hydroxylamine not assigned	fish (dietary)	
500M96	not assigned		not assigned	soil	
500M97	not assigned		not assigned	soil	

Code Numbers			Description		
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M98 / 500M99	not assigned		not assigned	soil	
500M103	not assigned		not assigned	human, rabbit, rat (*in-vitro*)	
500M104	not assigned		not assigned	rat (*in-vivo*, plasma) human, rabbit, rat (*in-vitro*)	
500M105	not assigned		not assigned	rat after dosing with metabolite 500M106	

Code Numbers			Description		
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M106	399379		N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl) O-methylhydroxylamine not assigned	rat (*in-vivo*, plasma) human, rabbit (*in-vitro*)	
500M107	not assigned		not assigned	rat (*in-vivo*, plasma) human, rabbit (*in-vitro*)	
500M108	not assigned		not assigned	rat (*in-vivo*, plasma) human, rabbit, rat (*in-vitro*)	
500M109	not assigned		not assigned	rat after dosing with metabolite 500M106	

Code Numbers			Description		
Substance/ Metabolite Code	**Reg No.**	**Synonyms**	**Chemical Name** **CAS-No.**	**Compound found in**	**Structure**
500M112	not assigned		not assigned	rat after dosing with metabolite 500M106	
500M117	not assigned		not assigned	rat after dosing with metabolite 500M106	

BASF
The Chemical Company

Pyraclostrobin

DOCUMENT N4

RELEVANCE OF METABOLITES IN GROUNDWATER

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Table of Contents

1 INTRODUCTION

2 FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.1 Summary of degradation pathway in soil

The degradation of pyraclostrobin in aerobic soil studies is characterized by a rather low mineralization rate (about 5% of applied radioactivity (AR) within 100 days) and a formation of high amounts of bound residues (about 55% AR within 100 days). The two dimeric metabolites, BF 500-6 and BF 500-7, were regularly found in aerobic soil in amounts > 5% AR.

Under anaerobic conditions, a very fast de-methoxylation of pyraclostrobin takes place, forming the metabolite BF 500-3 in high amounts. BF 500-3 is then further degraded to BF 500-4. The cleavage product BF 500-5 can also be formed in soil in low amounts.

2.2 Summary of identification of metabolites in soil

The following metabolites were considered for groundwater risk assessment:
BF 500-3, BF 500-4, BF 500-5, BF 500-6, and BF 500-7

3 RELEVANCE OF METABOLITES IN GROUNDWATER

3.1 Step 1: Exclusion of degradation products of no concern

None of the metabolites can be considered as "product of no concern".

3.2 Step 2: Quantification of potential groundwater contamination

PECgroundwater calculations were performed for five pyraclostrobin metabolites (BF 500-3, BF 500-4, BF 500-5, BF 500-6, and BF 500-7). None of the metabolites showed any risk of leaching into groundwater due to their strong sorption properties and/or their fast degradation rate in soil. All calculated PECgroundwater values were << 0.1 µg/L.

3.3 Step 3: Hazard assessment: identification of relevant metabolites

A further hazard assessment is not considered necessary since none of the metabolites showed any risk for leaching.

3.3.1 Step 3, Stage 1: screening for biological activity

Considered not necessary.

3.3.2 Step 3, Stage 2: screening for genotoxicity

Considered not necessary.

3.3.3 Step 3, Stage 3: screening for toxicity

Considered not necessary.

3.4 Step 4: Exposure assessment – threshold of concern approach

Considered not necessary.

3.5 Step 5: Refined risk assessment for non-relevance of metabolites

Considered not necessary.

3.6 Overall conclusion

Neither pyraclostrobin nor one of its metabolites poses any risk for groundwater.

4 REFERENCES

Please see PECgroundwater calculations in section M-CP 9.2.4.1.

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Pyraclostrobin

DOCUMENT N5

CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

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1 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

Pyraclostrobin does not consist of any isomers.

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Pyraclostrobin

DOCUMENT OCA

COMPLETENESS CHECK FORM

Compiled by:

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Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

OCA Evaluation Form Active Substance

for use in checking that all test and study reports required in accordance with SANCO/11802 have been provided

Active Substance: Pyraclostrobin Applicant: BASF SE Date: 18/Jul/2014

This evaluation form comprises the information provided in both, the original dossier and the supplementary dossier. Whenever **new information, tests or studies** are part of the supplementary dossier, "SD yes" is stated in the column "Information, test or study provided". A simple "yes" means that basically all the information, tests and/or studies were already part of the original dossier for Annex I inclusion.

SANCO/ 11802 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
1	Identity of the active substance				
1.1	Applicant	yes			☐
1.2	Producer	yes			☐
1.3	Common name proposed or ISO-accepted and synonyms	yes			☐
1.4	Chemical name (IUPAC and CA nomenclature)	yes			☐
1.5	Producer's development code numbers	yes			☐
1.6	CAS, EC and CIPAC numbers	yes			☐
1.7	Molecular and structural formula, molar mass	yes			☐
1.8	Method of manufacture (synthesis pathway) of the active substance	SD yes	Doc J		☐
1.9	Specification of purity of the active substance in g/kg	yes			☐
1.10	Identity and content of additives (such as stabilisers) and impurities				
1.10.1	Additives	yes	Doc J		☐
1.10.2	Significant impurities	SD yes	Doc J		☐
1.10.3	Relevant impurities	yes			☐
1.11	Analytical profile of batches	SD yes	Doc J		☐
2	Physical and chemical properties of the active substance				
2.1	Melting point and boiling point	yes			☐
2.2	Vapour pressure and volatility	yes			☐
2.3	Appearance (physical state, colour)	SD yes			☐
2.4	Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	SD yes			☐
2.5	Solubility in water	yes			☐
2.6	Solubility in organic solvents	yes			☐
2.7	Partition coefficient n-octanol/water	yes			☐
2.8	Dissociation in water	SD yes			☐
2.9	Flammability and self-heating	SD yes			☐
2.10	Flash point	not relevant	M-CA 2.10		☐
2.11	Explosive properties	SD yes			☐
2.12	Surface tension	yes			☐
2.13	Oxidising properties	SD yes			☐
2.14	Other studies	not relevant	M-CA 2.14		☐

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3	Further information on the active substance (function, mode of action, handling)				
3.1	Use of the active substance	yes			☐
3.2	Function	yes			☐
3.3	Effects on harmful organisms	yes			☐
3.4	Fields of use envisaged	yes			☐
3.5	Harmful organisms controlled and crops or products protected or treated	SD yes			☐
3.6	Mode of action	yes			☐
3.7	Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies	SD yes			☐
3.8	Methods and precautions concerning handling, storage transport or fire	yes			☐
3.9	Procedures for destruction or decontamination	yes			☐
3.10	Emergency measures in case of an accident	yes			☐
4	Analytical methods				
4	Introduction				
4.1	Methods used for the generation of pre-approval data	SD yes			☐
4.1.1	Methods of the analysis of the active substance as manufactured	SD yes	partly Doc J		☐
4.1.2	Methods for risk assessment	SD yes			☐
4.2	Methods for post-approval control and monitoring purposes	SD yes			☐
5	Toxicological and toxicokinetic studies on the active substance				
5.1	Studies on absorption, distribution, excretion and metabolism in mammals				
5.1.1	Absorption, distribution, excretion and metabolism by oral route	SD yes			☐
5.1.2	Absorption, distribution, excretion and metabolism by other routes	SD yes			☐
5.2	Acute toxicity				
5.2.1	Oral	yes			☐
5.2.2	Dermal	yes			☐
5.2.3	Inhalation	SD yes			☐
5.2.4	Skin irritation	yes			☐
5.2.5	Eye irritation	yes			☐
5.2.6	Skin sensitization	yes			☐
5.2.7	Phototoxicity	SD yes			☐
5.3	Short-term toxicity				
5.3.1	Oral 28-day study	yes			☐
5.3.2	Oral 90-day study	SD yes			☐
5.3.3	Other routes	SD yes			☐
5.4	Genotoxicity				
5.4.1	*In vitro* studies	yes			☐
5.4.2	*In vivo* studies in somatic cells	yes			☐
5.4.3	*In vivo* studies in germ cells	SD yes			☐

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5.5	Long-term toxicity and carcinogenicity	SD yes			☐
5.6	Reproductive toxicity				
5.6.1	Generational studies	yes			☐
5.6.2	Developmental toxicity studies	yes			☐
5.7	Neurotoxicity				
5.7.1	Neurotoxicity studies in rodents	yes			☐
5.7.2	Delayed polyneuropathy studies	not relevant	M-CA 5.7.2		☐
5.8	Other toxicological studies				
5.8.1	Toxicity studies of metabolites as referred to in the introduction	SD yes			☐
5.8.2	Supplementary studies on the active substance	SD yes			☐
5.8.3	Endocrine disrupting properties	SD yes			☐
5.9	Medical data				
5.9.1	Medical surveillance on manufacturing plant personnel and monitoring studies	SD yes			☐
5.9.2	Data collected on humans	SD yes			☐
5.9.3	Direct observations	SD yes			☐
5.9.4	Epidemiological studies	not relevant	M-CA 5.9.4		☐
5.9.5	Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests	not relevant	M-CA 5.9.5		☐
5.9.6	Proposed treatment: first aid measures, antidotes, medical treatment	SD yes			☐
5.9.7	Expected effects of poisoning	SD yes			☐
6	Metabolism and residues data				
6.1	Storage stability of residues	SD yes			☐
6.2.	Metabolism, distribution and expression of residues				
6.2.1	Plants	SD yes			☐
6.2.2	Poultry	yes			☐
6.2.3	Lactating ruminants	SD yes			☐
6.2.4	Pigs	not relevant	M-CA 6.2.4		☐
6.2.5	Fish	SD yes			☐
6.3	Magnitude of residue trials in plants	SD yes			☐
6.4	Feeding studies				
6.4.1	Poultry	SD yes			☐
6.4.2	Ruminants	yes			☐
6.4.3	Pigs	not relevant	M-CA 6.4.3		☐
6.4.4	Fish	not relevant	M-CA 6.4.4		☐
6.5	Effects of processing				
6.5.1	Nature of the residue	SD yes			☐
6.5.2	Distribution of the residue in inedible peel/pulp	not relevant	M-CA 6.5.2		☐
6.5.3	Magnitude of residues in processed commodities	SD yes			☐
6.6	Residues in rotational crops				
6.6.1	Metabolism in rotational crops	SD yes			☐
6.6.2	Magnitude of residues in rotational crops	not relevant	M-CA 6.6.2		☐

SANCO/ 11802 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
6.7	Proposed residue definition and maximum residue levels				
6.7.1	Proposed residue definitions	SD yes			☐
6.7.2	Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed	SD yes			☐
6.7.3	Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerances)	SD yes			☐
6.8	Proposed safety intervals	SD yes			☐
6.9	Estimation of the potential and actual exposure through diet and other sources	SD yes			☐
6.10	Other studies	not relevant	M-CA 6.10		☐
6.10.1	Effect on the residue level in pollen and bee products	not relevant	M-CA 6.10.1		☐
7	Fate and behaviour in the environment				
7.1.1	Fat and behaviour in soil				
7.1.1.1	Aerobic degradation	SD yes			☐
7.1.1.2	Anaerobic degradation	yes			☐
7.1.1.3	Soil photolysis	SD yes			☐
7.1.2	Rate of degradation in soil				
7.1.2.1	Laboratory studies				
7.1.2.1.1	Aerobic degradation of the active substance	SD yes			☐
7.1.2.1.2	Aerobic degradation metabolites, breakdown and reaction products	SD yes			☐
7.1.2.1.3	Anaerobic degradation of the active substance	SDyes			☐
7.1.2.1.4	Anaerobic degradation of metabolites, breakdown and reaction products	SDyes			☐
7.1.2.2	Field studies				
7.1.2.2.1	Soil dissipation studies	SD yes			☐
7.1.2.2.2	Soil accumulation studies	not relevant	M-CA 7.1.2.2.2		☐
7.1.3	Adsorption and desorption in soil				
7.1.3.1	Adsorption and desorption				
7.1.3.1.1	Adsorption and desorption of the active substance	yes			☐
7.1.3.1.2	Adsorption and desorption of metabolites, breakdown and reaction products	SD yes			☐
7.1.3.2	Aged sorption	not relevant	M-CA 7.1.3.2		☐
7.1.4	Mobility in soil				
7.1.4.1	Column leaching studies				
7.1.4.1.1	Column leaching of the active substance	yes			☐
7.1.4.1.2	Column leaching of metabolites, breakdown and reaction products	yes	M-CA 7.1.4.1.1		☐
7.1.4.2	Lysimeter studies	not relevant	M-CA 7.1.4.2		☐
7.1.4.3	Field leaching studies	not relevant	M-CA 7.1.4.3		☐

SANCO/ 11802 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
7.2	Fate and behaviour in water and sediment				
7.2.1	Route and rate of degradation in aquatic systems (chemical and photochemical degradation)				
7.2.1.1	Hydrolytic degradation	SD yes			☐
7.2.1.2	Direct photochemical degradation	yes			☐
7.2.1.3	Indirect photochemical degradation	not relevant	M-CA 7.2.1.3		☐
7.2.2	Route and rate of biological degradation in aquatic systems				
7.2.2.1	"Ready biodegradability"	yes			☐
7.2.2.2	Aerobic mineralisation in surface water	SD yes			☐
7.2.2.3	Water/sediment study	SD yes			☐
7.2.2.4	Irradiated water/sediment study	SD yes			☐
7.2.3	Degradation in the saturated zone	not relevant	M-CA 7.2.3		☐
7.3	Fate and behaviour in air				
7.3.1	Route and rate of degradation in air	SD yes			☐
7.3.2	Transport via air	not relevant	M-CA 7.3.2		☐
7.3.3	Local and global effects	not relevant	M-CA 7.3.3		☐
7.4	Definition of the residue				
7.4.1	Definition of the residue for risk assessment	SD yes			☐
7.4.2	Definition of the residue for monitoring	SD yes			☐
7.5	Monitoring data	SD yes			☐
8	Ecotoxicological studies				
8.1	Effects on birds and other terrestrial vertebrates				
8.1.1	Effects on birds				
8.1.1.1	Acute oral toxicity to birds	SD yes			☐
8.1.1.2	Short-term dietary toxicity to birds	yes			☐
8.1.1.3	Sub-chronic toxicity and reproductive to birds	yes			☐
8.1.2	Effects on terrestrial vertebrates other than birds				
8.1.2.1	Acute oral toxicity to mammals	SD yes			☐
8.1.2.2	Long-term and reproductive toxicity to mammals	SD yes			☐
8.1.3	Effects of active substance bioconcentration in prey of birds and mammals	not relevant	M-CA 8.1.3		☐
8.1.4	Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)	SD yes			☐
8.1.5	Endocrine disrupting properties	not relevant	M-CA 8.1.5		☐
8.2	Effects on aquatic organisms				
8.2.1	Acute toxicity to fish	SD yes			☐
8.2.2	Long-term and chronic toxicity to fish				
8.2.2.1	Fish early life stage toxicity test	SD yes			☐
8.2.2.2	Fish full life cycle test	yes			☐
8.2.2.3	Bioconcentration in fish	yes			☐
8.2.3	Endocrine disrupting properties	not relevant	M-CA 8.2.3		☐
8.2.4	Acute toxicity to aquatic invertebrates				
8.2.4.1	Acute toxicity to *Daphnia magna*	SD yes			☐
8.2.4.2	Acute toxicity to an additional aquatic invertebrate species	SD yes			☐

SANCO/ 11802 data point	Information, test or study	Information, test or study provided	Justification provided	Undertaking provided	*Data gap*
8.2.5	Long term and chronic toxicity to aquatic invertebrates				
8.2.5.1	Reproductive and development toxicity to *Daphnia magna*	yes			☐
8.2.5.2	Reproductive and development toxicity to an additional aquatic invertebrate species	SD yes			☐
8.2.5.3	Development and emergence in *Chironomus species*	SD yes			☐
8.2.5.4	Sediment dwelling organisms	SD yes			☐
8.2.6	Effects on algal growth				
8.2.6.1	Effects on growth of green algae	SD yes			☐
8.2.6.2	Effects on growth of an additional algal species	SD yes			☐
8.2.7	Effects on aquatic macrophytes	SD yes			☐
8.2.8	Further testing on aquatic organisms	SD yes			☐
8.3	Effects on arthropods				
8.3.1	Effects on bees				
8.3.1.1	Acute toxicity to bees	yes			
8.3.1.1.1	Acute oral toxicity	SD yes			☐
8.3.1.1.2	Acute contact toxicity	SD yes			☐
8.3.1.2	Chronic toxicity to bees	not relevant	M-CA 8.3.1.2		☐
8.3.1.3	Effects on honeybees development and other honeybee life stages	yes	M-CP 10.3.1.5		☐
8.3.1.4	Sub-lethal effects	yes	M-CP 10.3.1.5		☐
8.3.2	Effects on non-target arthropods other than bees	yes	M-CP 10.3.2.1		☐
8.3.2.1	Effects on *Aphidius rhopalosiphi*	yes	M-CP 10.3.2.1		☐
8.3.2.2	Effects on *Typhlodromus pyri*	yes	M-CP 10.3.2.1		☐
8.4	Effects on non-target soil meso- and macrofauna				
8.4.1	Earthworm – sub-lethal effects	SD yes			☐
8.4.2	Effects on non-target soil meso- and macrofauna (other than earthworms)	SD yes			☐
8.4.2.1	Species level testing	not relevant	M-CA 8.4.2.1		☐
8.5	Effects on soil nitrogen transformation	yes			☐
8.6	Effects on terrestrial non-target higher plants				
8.6.1	Summary of screening data	not relevant	M-CA 8.6.1		☐
8.6.2	Testing on non-target plants	SD yes	M-CP 10.6.2		☐
8.7	Effects on other terrestrial organisms (flora and fauna)	not relevant	M-CA 8.7		☐
8.8	Effects on biological methods for sewage treatment	yes			☐
8.9	Monitoring data	SD yes			☐
9	Literature data	SD yes			☐
10	Classification and labelling	SD yes			☐