Dimoxystrobin

Document M-CA, Section 1

IDENTITY OF THE ACTIVE SUBSTANCE

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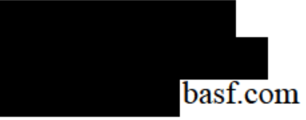

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CA 1 IDENTITY OF THE ACTIVE SUBSTANCE

CA 1.1 Applicant

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CA 1.2 Producer

Producer of dimoxystrobin

BASF SE
67056 Ludwigshafen
Germany

Contact person: Please refer to CA 1.1 Applicant.

Location of manufacturing site for dimoxystrobin

CONFIDENTIAL information - data provided separately (Document J)

CA 1.3 Common Name Proposed or ISO-accepted and synonyms

ISO common name: Dimoxystrobin (ISO)

CA 1.4 Chemical Name (IUPAC and CA nomenclature)

IUPAC name: (E)-o-(2,5-dimethylphenoxymethyl)-2-methoxyimino-N-methylphenylacetamide

CA nomenclature: Benzeneacetamide, 2-[(2,5-dimethylphenoxy)methyl]-α-(methoxyimino)-N-methyl-,αE)-

CA 1.5 Producer's Development Code Numbers

BASF Number: BAS 505 F
BASF Registry Number: Reg.No. 285028

CA 1.6 CAS, EC and CIPAC Numbers

CAS No.: 149961-52-4
CIPAC No.: 739
EC No.: not assigned

CA 1.7 Molecular and Structural Formula, Molar Mass

Molecular formula: $C_{19}H_{22}N_2O_3$
Molar mass: 326.394 g/mol

Structural formula:

CA 1.8 Method of Manufacture (synthesis pathway) of the active substance

CONFIDENTIAL information - data provided separately (Document J)

CA 1.9 Specification of Purity of the Active Substance in g/kg

Minimum purity: 980 g/kg

CA 1.10 Identity and Content of Additives (such as Stabilisers) and impurities

CA 1.10.1 Additives

CONFIDENTIAL information - data provided separately (Document J)

CA 1.10.2 Significant impurities

CONFIDENTIAL information - data provided separately (Document J)

CA 1.10.3 Relevant impurities

Dimoxystrobin does not contain impurities of toxicological, eco-toxicological or environmental relevance.

CA 1.11 Analytical Profile of Batches

CONFIDENTIAL information - data provided separately (Document J)

Dimoxystrobin

Document M-CA, Section 10

CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

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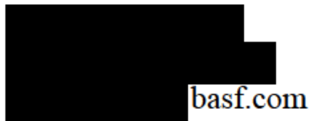
E-mail  basf.com

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CA 10 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

The following harmonized classification and labelling was adopted for Dimoxystrobin:

Table 10-1: Harmonised classification of dimoxystrobin according to (EC) No 1272/2008

Legislation	Classification	Labelling	Concentration limits
Regulation (EC) No 1272/2008	Hazard class and category code: Carc. 2 Repr. 2 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1 Hazard statement code: H332, H351, H361d, H400, H410	Pictogram signal word code: GHS08 GHS07 GHS09 Wng Hazard statement code: H351, H361d, H332, H410	M = 10
Directive 67/548/EEC	Carc. Cat. 3; R40 Repr. Cat. 3; R63 Xn; R20 N; R50-53	Xn; N R: 20-40-63-50/53 S: (2-)36/37-46-60-61	N; R50-53: C ≥ 2,5 % N; R51-53: 0,25 % ≤ C < 2,5 % R52-53: 0,025 % ≤ C < 0,25 %

Since the last evaluation of dimoxystrobin new mechanistic data on reproductive toxicity and the assumed susceptibility of young vs. older rats were generated. The new data have been presented in the context of the results of the generation toxicity studies and the mechanistic investigations showing that the classification with Repr. Cat. 2 (H361d) is not justified. A justification document ("Proposal for the re-classification of Dimoxystrobin") has been prepared and is submitted with this dossier (BASF DocID 2015/1152529). All relevant (new) data and assessments are also included in the respective chapters of this dossier (M-CA 5.6, 5.8 and Doc N1).

The following changes to classification and labelling of the active substance dimoxystrobin are proposed. Thus, the submission of a classification and labelling dossier to the European Chemicals Agency (ECHA) is required.

Table 10-2: Proposed changes in Hazard and Precautionary Statements

Legislation	Classification	Labelling	Concentration limits
Regulation (EC) No 1272/2008	Hazard class and category code: Carc. 2 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1 Hazard statement code: H332, H351, H400, H410	Pictogram signal word code: GHS08 GHS07 GHS09 Wng Hazard statement code: H351, H332, H410	

Dimoxystrobin

Document M-CA, Section 3

FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

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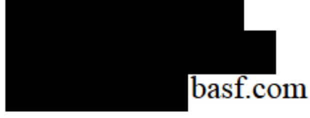

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CA 3 FURTHER INFORMATION ON THE ACTIVE SUBSTANCE

CA 3.1 Use of the Active Substance

Dimoxystrobin is a member of the strobilurin group of fungicidal compounds, which are analogues of strobilurin A, a natural metabolite of the fungus *Strobilurus tenacellus*.

Dimoxystrobin is active against fungal development stages both on the plant surface and within the plant tissues. The preferred method of application is spraying, upon which the compound is absorbed by the leaves with a certain mobility in the transpiration stream and low phloem mobility. Therefore, a systemic and translaminar activity can be observed.

CA 3.2 Function

Dimoxystrobin is a selective fungicide used on dicotyledonous and monocotyledonous crop species. After foliar application (spraying), dimoxystrobin is absorbed by the plant tissue and acropetally translocated.

CA 3.3 Effects on Harmful Organisms

Dimoxystrobin is active against fungal development stages on both the plant surface and in the plant tissues. When applied protectively, dimoxystrobin prevents the germination of fungal spores landing on the plant surface as well as the re-infection since during these extremely energy-consuming phases fungi react very sensitively to disturbances of their mitochondrial respiratory chain. Dimoxystrobin is suitable mainly for preventative treatments.

CA 3.4 Field of Use Envisaged

Agriculture

CA 3.5 Harmful Organisms Controlled and Crops or Products Protected or Treated

Dimoxystrobin is used to control a range of fungal diseases on the following crops:

Oilseed rape:
Plenodomus lingam / Phoma lingam / Leptosphaeria maculans (LEPTMA)
Sclerotinia sclerotiorum (SCLESC)
Alternaria brassicae (ALTEBR)

Sunflower:
Plenodomus lindquistii / Phoma macdonaldi / Leptosphaeria lindqustii (LEPTLI)
Botrytis cinerea (BOTRCI)
Alternaria sp. (ALTESP)
Sclerotinia sclerotiorum (SCLESC)
Diaporthe helianthi / Phomopsis helianthi (DIAPHE)

CA 3.6 Mode of Action

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

In general, strobilurins can inhibit electron transport in mitochondria from a wide range of organisms. The selectivity of dimoxystrobin is given by the very limited capacity of fungal organisms to metabolically inactivate the compound.

Protective application prevents new infections predominantly by inhibiting spore germination. This stage of fungal development requires a very high-energy consumption, which renders it particularly sensitive to an inhibition of the mitochondrial electron transport chain. Hence, dimoxystrobin is mainly suitable for protective applications.

CA 3.7 Information on Occurrence or Possible Occurrence of the Development of Resistance and Appropriate Management Strategies

QoI resistant strains of some plant pathogenic fungal species were identified in some areas after intensive use and high selection pressure. Information which species are affected and to what extent can be followed in the annual minutes of the QoI Working Group of the Fungicide Resistance Action Committee (FRAC) on the FRAC web page (www.frac.info). Most important resistance mechanisms to QoIs are target site mutations, in particular the G143A in the cytochrome *b* (cyt *b*). G143A leads to strong resistance and reduced field efficacy. Two other mutations, F129L and G137R have been described, which lead to lower resistance factors. All QoI fungicides are in the same cross-resistance group and should be managed accordingly.

However, until now several fungal species did not develop field resistance to QoI fungicides. These include different rust species, *Pyrenophora teres* and others. *Puccinia triticina* experienced a comparable selection pressure as *Blumeria graminis* (a species which developed QoI resistance 1-2 years after market launch), but so far no sensitivity changes have been detected. Also, no adaptation has been found for the soybean rust pathogen *Phakopsora pachyrhizi*, despite intensive use of QoIs in large areas. The reason for this was elucidated by Grasso *et al*. (2006), who showed that the structure of the target gene is responsible: If an intron sequence follows directly after codon 143, the G143A does not occur, because the glycine codon 143 is part of the signal sequence which is essential for the intron recognition and plays an essential role in splicing during mRNA maturation. If this codon is altered, no functional complex III can be formed. Therefore it can be concluded, that for a species with an intron sequence directly after codon 143, the G143A is unlikely to occur. However, mutations F129L or G137R are possible. F129L occurs frequently in some European populations of *P. teres*, but due to low resistance factors caused by F129L, field efficacy of strobilurins (as representatively tested with pyraclostrobin) remains good (Semar *et al.* 2007). In other species like *P. triticina* or *P. pachyrhizi*, no mutations in the cyt *b* gene have been reported so far. The gene structure around codon 143 seems to be highly conserved within a species, even for isolates with a diverse geographic, host plant or historic (year of isolation) background. *C*yt *b* of thousands of *P. teres* isolates was analyzed in our laboratory in the last decade and all of them were identical. No G143A mutated isolates showed up in *P. triticina* or *P. pachyrhizi* after many years of QoI selection pressure which confirms the high intraspecific conservation of this gene sequence. Therefore, it is likely that the whole population of a species contains the intron at the same position when it has been detected for some isolates. However, *Botrytis cinerea* seems to be an exception. Two types of cyt *b* gene, (with and without intron, Jiang *et al.* 2009) exist in *B. cinerea* and the genotype without intron developed G143A in various crops and regions. So far published “intron pathogens” are listed in Table 3.7-1.

Table 3.7-1: Plant pathogenic fungi with intron after codon 143 in the cytochrome *b* gene

Pathogen	Disease	Host	Reference
Alternaria dauci	Leaf blight	Carrots	Stammler 2012
Alternaria grandis	Early blight	Tomatoes, potatoes	Stammler 2012
Alternaria solani	Early blight	Tomatoes, potatoes	Grasso *et al*. 2006
Alternaria tomatophila	Early blight	Tomatoes, potatoes	Stammler 2012
Bipolaris maydis	Southern leaf blight	Corn	Stammler 2012
Cercospora zea-maydis	Gray leaf spot	Corn	Stammler 2012
Cochliobolus carbonum	Northern leaf spot	Corn	Stammler 2012
Guignardia bidwellii	Black rot	Grapes	Miessner *et al.* 2011
Hemileia vastatrix	Rust	Coffee	Grasso *et al*. 2006
Monilinia fructicola	Blossom blight, brown rot	Stonefruits	Miessner & Stammler 2010
Monilinia laxa	Blossom blight, brown rot	Stonefruits	Miessner & Stammler 2010
Phakopsora pachyrhizi	Rust	Soy beans	Grasso *et al.* 2006
Phyllosticta citricarpa	Citrus black spot	Citrus species	Stammler *et al.* 2012
Puccinia spp.	Rust	Cereals, corn, others	Grasso *et al.* 2006
Pyrenophora teres	Net blotch	Barley	Grasso *et al.* 2006
Setosphaeria turcica	Northern leaf blight	Corn	Stammler 2012
Uromyces appendiculatus	Rust	Beans	Grasso *et al.* 2006

Sclerotinia sclerotiorum: All isolates used for monitoring SDHI sensitivity in BASF were also used for monitoring of the sensitivity to dimoxystrobin. All isolates were sensitive, there is no report available which indicates QoI resistance in *Sclerotinia sclerotiorum*.

Resistance management strategies including modifiers such as limiting the number of QoI-applications, alternation and combination with other modes of action have been worked out by the FRAC QoI Working Group per crop and pathogen. These recommendations are annually reviewed and actual yearly sensitivity monitoring data are considered for adaptating the resistance management strategies in order to maintain the activity against various plant pathogens in different crops and regions. The current recommendations are available on the FRAC webpage (www.frac.info).

References

Grasso V., Palermo S., Sierotzki H., Garibaldi A. and Gisi U. (2006) Cytochrome *b* gene structure and consequences for resistance to Qo inhibitor fungicides in plant pathogens. *Pest Management Science* **62**, 465-472

Jiang J., Ding L., Michaelidis T.J., Hongye L. and Ma Z. (2009) Molecular characterization of field azoxystrobin-resistant isolates of *Botrytis cinerea*. *Pesticide Biochemistry and Physiology* **93,** 72-76

Miessner S. and Stammler G. (2010) *Monilinia laxa, M. fructigena* and *M. fructicola*: Risk estimation of resistance to QoI fungicides and identification of species with cytochrome b gene sequences. *Journal of Plant Diseases and Protection* **117**, 162-167

Miessner S., Mann W., and Stammler G. (2011) *Guignardia bidwellii*, the causal agent of black rot of grapevine has a low risk for QoI resistance. *Journal of Plant Diseases and Protection* **118**, 51-53

Semar M., Strobel D., Koch A., Klappach K. and Stammler G. (2007) Field efficacy of pyraclostrobin against populations of *Pyrenophora teres* containing the F129L mutation in the cytochrome *b* gene. *Journal of Plant Diseases and Protection* **114**, 117-119

Stammler G. (2012) Resistance risk of corn pathogens to QoI fungicides. *Outlooks on Pest Management* **23**, 211-214

Dimoxystrobin is intended to be re-registered only in mixture with other active ingredients of a different mode of action. For a sound and full Resistance Risk Analysis the final formulation needs to be considered.

CA 3.8 Methods and Precautions Concerning Handling, Storage, Transport or Fire

Exposure Controls / Personal Protection

Control parameters

Occupational exposure limits are defined for dimoxystrobin in some EU member states in accordance to national legislation.

Exposure controls

Personal protective equipment

Respiratory protection:
Suitable respiratory protection for higher concentrations or long-term effect: Particle filter with medium efficiency for solid and liquid particles (e.g. EN 143 or 149, Type P2 or FFP2)

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

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

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

General safety and hygiene measures

Handle in accordance with good industrial hygiene and safety practice. Wearing of closed work clothing is recommended. Store work clothing separately. Keep away from food, drink and animal feeding stuffs.

Handling and Storage

Precautions for safe handling

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

Protection against fire and explosion:
Avoid dust formation. Dust can form an explosive mixture with air. Prevent electrostatic charge - sources of ignition should be kept well clear - fire extinguishers should be kept handy.

Conditions for safe storage, including any incompatibilities

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

Storage stability:
Storage duration: 24 Months

Protect from temperatures above: 40 °C
Changes in the properties of the product may occur if substance/product is stored above indicated temperature for extended periods of time.

First-Aid Measures

Description of first aid measures

Remove contaminated clothing.

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

On skin contact:
Wash thoroughly with soap and water.

On contact with eyes:
Wash affected eyes for at least 15 minutes under running water with eyelids held open.

On ingestion:
Immediately rinse mouth and then drink 200-300 ml of water, seek medical attention.

Most important symptoms and effects, both acute and delayed

Symptoms: The most important known symptoms and effects are described in the labelling. Further important symptoms and effects are so far not known.

Indication of any immediate medical attention and special treatment needed

Treatment: Treat according to symptoms (decontamination, vital functions), no known specific antidote.

Transport Information

Land transport

ADR

UN number	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains DIMOXYSTROBINE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	Tunnel code: E

RID

UN number	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains DIMOXYSTROBINE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known

Inland waterway transport

ADN

UN number	UN3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains DIMOXYSTROBINE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known
Transport in inland waterway vessel:	Not evaluated

Sea transport

IMDG

UN number:	UN 3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains DIMOXYSTROBINE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes Marine pollutant: YES
Special precautions for user:	None known

Air transport
IATA/ICAO

UN number:	UN 3077
UN proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (contains DIMOXYSTROBINE)
Transport hazard class(es):	9, EHSM
Packing group:	III
Environmental hazards:	yes
Special precautions for user:	None known

Fire-Fighting Measures

Extinguishing media

Suitable extinguishing media:
water spray, dry powder, foam

Unsuitable extinguishing media for safety reasons:
carbon dioxide

Special hazards arising from the substance or mixture

carbon monoxide, nitrogen oxides
The substances/groups of substances mentioned can be released in case of fire.

Advice for fire-fighters

Special protective equipment:
Wear self-contained breathing apparatus and chemical-protective clothing.

Further information

Collect contaminated extinguishing water separately, do not allow to reach sewage or effluent systems. Dispose of fire debris and contaminated extinguishing water in accordance with official regulations. In case of fire and/or explosion do not breathe fumes. Keep containers cool by spraying with water if exposed to fire.

CA 3.9 Procedures for Destruction or Decontamination

Waste treatment methods

For purposes of disposal, combustion of Dimoxystrobin or its pesticide products in a licensed incinerator is recommended. This method of disposal applies also to contaminated packages, which cannot be cleaned or reused.

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

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

CA 3.10 Emergency Measures in Case of an Accident

Personal precautions, protective equipment and emergency procedures

Avoid dust formation. Use personal protective clothing. Avoid contact with the skin, eyes and clothing.

Environmental precautions

Do not discharge into drains/surface waters/groundwater. Do not discharge into the subsoil/soil.

Methods and material for containment and cleaning up

For small amounts: Contain with dust binding material and dispose of.
For large amounts: Sweep/shovel up.
Avoid raising dust. Cleaning operations should be carried out only while wearing breathing apparatus. Dispose of absorbed material in accordance with regulations. Collect waste in suitable containers, which can be labeled and sealed. Clean contaminated floors and objects thoroughly with water and detergents, observing environmental regulations.

Dimoxystrobin

Document M-CA, Section 4

ANALYTICAL METHODS

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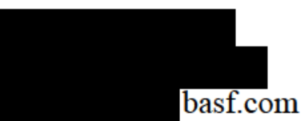

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CA 4 ANALYTICAL METHODS

CA 4.1 Methods used for the generation of pre-approval data

CA 4.1.1 Methods for the analysis of the active substance as manufactured

(a)Determination of the pure active substance in the active substance as manufactured and specified in the dossier submitted in support of approval under Regulation (EC) No 1107/2009

Method APL0694/01 (equivalent to CIPAC MT 739 = CIPAC 4710/m)
This method and its validation were not mentioned in the application. Due to the fact that it was decided to submit a newer 5 batch analysis (see JCA 1.11 in the confidential section), where this method was applied, the studies need to be submitted.

Report:	CA 4.1.1/1 Sonnenschein L., 2014a Analytical method APL694/01 - Determination of the active ingredient Dimoxystrobin (Reg. No. 285028) in technical grade active ingredient BAS 505 F (TGAI) by means of gas chromatography 2014/1141719
Guidelines:	none
GLP:	no

Report:	CA 4.1.1/2 Fries J., 2015a Dimoxystrobin 739 - CIPAC collaborative trial, GC method CIPAC 4710/m 2015/1050784
Guidelines:	none
GLP:	no

The validation study was moved to document JCA because of confidential information. The results of the validation, however, are reported in this chapter.

Method APL0694/01 is identical to CIPAC MT 739/TC/(M) (preliminary code CIPAC 4710/m). According to CIPAC this “pre-published” method is going to be released in slightly modified and edited form (no changes regarding the analytical method) in the new CIPAC Handbook O presumably in 2015. Due to the fact that the method was tested in a ring-test this method is regarded fully-validated even without submission of a validation study. However, due to the fact that this method was not published during dossier preparation also the validation study is submitted.

Principle of the method
Method APL0694/01 describes the determination of the content of the active ingredient Dimoxystrobin (Reg. No. 285028) in technical grade active ingredient BAS 505 F (TGAI) using gas chromatography with internal standard calibration and FID detection.

Method parameters (according to DocID 2014/1141719)

Column	Optima-1701, 60 m length; 0.32 mm i. D.; 0.25 µm film thickness, or equivalent
Injector temperature	280°C
Detector temperature	280°C
Oven temperature	280°C
Carrier gas	Helium 4.6
Detector gas	Hydrogen
Split ratio	Split ratio 1:20, split gas flow 40 mL/min
Column flow	2.0 mL/min, constant flow
Injection volume	1 µL
Analysis time	15 min
RT	Reg. No. 258028 – 10.8 min

Identity
The identity of the active ingredient dimoxystrobin in technical grade active ingredient BAS 505 F were confirmed by the injection of the test item and the authentic reference item (Reg. No. 285028) in GC and in GC-MS. The identity of the active ingredient in technical grade dimoxystrobin was confirmed by coincidence of retention times in the test item, spiked solutions of the test item and the authentic reference standards dimoxystrobin. GC mass spectra were recorded both in a standard solution as well as in a test item solution.

Specificity
The specificity of the method was examined by comparing the GC chromatograms of blank injections (pure Acetone, mixture of Acetone and THF), injections of the internal standard, the pure active ingredient dimoxystrobin, the test item, the impurities and injections of the reference item dimoxystrobin spiked with approximately 0.24 % (w/w) of the potential process related impurities. The chromatograms do not show any interference of the impurity signals and the signal of dimoxystrobin. Therefore the specificity of the GC method is given. The retention times of the peak of dimoxystrobin in the reference samples and in the test item samples were checked and found to be identical.

Linearity

The linear range for the determination of dimoxystrobin was determined by injection of seven individually weighted solutions at different concentration levels containing dimoxystrobin (Reg. No. 285028) in the range of 3.95 g/L to 6.29 g/L. These concentrations correspond to dimoxystrobin contents in the technical grade active ingredient in the range of 789.2 g/kg to 1257.8 g/kg. Each solution was injected twice. The amount of internal standard that was added to each calibration solution was 49.73 mg (in 50 mL).

y-axis intercept (b) : 0 (linear through zero)
slope (m): 0.5840
correlation factor: 0.999945
concentration range: 3.95 g/L to 6.29 g/L corresponding to 789.2 g/kg to 1257.8 g/kg

Precision/Repeatability

Precision is calculated as the relative standard deviation of seven individual sample weights of the test item. Each sample solution was injected twice. The acceptability of the % RSD values (relative standard deviation) for precision was proved by the Horwitz equation, an exponential relationship between the inter laboratory relative standard deviation (RSDR) and concentration C (expressed as decimal fraction):

$$\%RSDR = 2^{(1-0.5 \log C)}$$

which is modified for the estimation of repeatabilities (RSDr internal laboratory) to:

%RSDr = % RSDR x 0.67

Horwitz results for the repeatability test with BAS 505 F TGAI:

Item	nominal conc. [%]	corresp. conc. \`C\`	%RSDR Horwitz (Inter Lab. RSD)	%RSDr Horwitz (Intra Lab. RSD)	%RSD analyzed	%RSD accepted
BAS 505 F	99.594	0.99594	2.001	1.341	0.224	yes

No outliers or stragglers were determined.

Conclusion

The present study has shown that the conditions employed in the analytical GC method APL0694/01 are suitable for the quantification of dimoxystrobin (Reg. No. 285028) in technical grade active ingredient BAS 505 F (TGAI).

Method CP 272

In various analyses of dimoxystrobin TGAI the content of the active substance dimoxystrobin was also carried out with the formerly used HPLC method CP 272 (present number APL0256/01). This method was submitted and peer-reviewed in the Annex I Registration process. Hence this method and its validation is not submitted and described again in this dossier.

Water

(b) Determination of significant and relevant impurities and additives (such as stabilisers) in the active substance as manufactured

Dimoxystrobin as manufactured does not contain impurities of toxicological, eco-toxicological or environmental concern.

Information on significant impurities and additives is provided in the confidential part (see Document JCA).

CA 4.1.2 Methods for risk assessment

Note: The order of the study summaries is differing compared to the information given in the application submitted for renewal of approval. In case references are summarized that were not contained in the application or in case references listed in the application are not contained in this chapter, comments are made where appropriate.

(a)Methods in soil, water, sediment, air and any additional matrices used in support of environmental fate studies

Since the analytical methods for determination of residues of dimoxystrobin in soil, water and air presented in the previous dossier are rather old, new analytical methods were developed for environmental matrices according to newest guidelines and considering the current state of art techniques used in analytical laboratories.

Soil

Report:	CA 4.1.2/1 Penning H. et al., 2013a Validation of analytical method L0189/01 for the determination of BAS 505 F, 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No. 354562) and 505M09 (Reg.No. 354563) in soil and sediment by LC-MS/MS 2012/1287158
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the methods

Method Number L0189/01:

The BASF Method allows the determination of BAS 505 F (Dimoxystrobin) and its metabolites 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No 354562) and 505M09 (Reg.No. 354563) in soil and sediment. Soil/sediment samples (5 g) are extracted with 50 ml methanol/water (80/20, v/v) by mechanical shaking for 30 min at 225 rpm. A 5 ml aliquot of the extract is centrifuged for 5 min at 4000 rpm (20°C). The extract is taken directly or diluted with methanol/water (80/20, v/v) to the appropriate final volume. The concentration of each analyte in the extract is measured by HPLC-MS/MS. The limit of quantification (LOQ) of the method is 0.002 mg/kg for each analyte.

Recovery findings

Method L0189/01 was proved to be suitable to determine residues of BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in soil and sediment to a limit of quantification (LOQ) of 0.002 mg/kg. The mean recovery values of the validation experiments were between 70% and 110%. Detailed results of recoveries for each mass transition and matrix are given in Table 4.1.2-1.

Results of stability investigations of calibration and fortification solutions as well as stability investigations of the extracts showed that the fortification and calibration solutions of each analyte were stable (less than 10% decline) for at least 4 weeks refrigerate and that BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) are stable in the extracts of the two soils and the sediment over the tested time period of 7 days.

Table 4.1.2-1: Recoveries for BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in both soils and sediment

Matrix	Test Item	Mass transition	No. of replicates	Fortification level [mg/kg]	Mean Recovery [%]	RSD [%]
LUFA Soil 2.2	BAS 505 F	327 → 205	5	0.002	102.5	1.5
			5	0.02	101.0	3.1
		327 → 116	5	0.002	103.4	1.7
			5	0.02	101.5	2.3
	505M98	327 → 205	5	0.002	113.9	0.7
			5	0.02	112.4	3.0
		327 → 116	5	0.002	115.2	1.2
			5	0.02	111.5	2.6
	505M01	223 → 176	5	0.002	96.8	0.9
			5	0.02	96.1	1.3
		223 → 116	5	0.002	98.4	3.7
			5	0.02	95.6	2.8
	505M08	357 → 205	5	0.002	101.9	2.1
			5	0.02	99.3	1.6
		357 → 116	5	0.002	102.5	2.4
			5	0.02	100.4	1.2
	505M09	357 → 205	5	0.002	99.0	2.6
			5	0.02	97.8	1.8
		357 →116	5	0.002	95.0	1.9
			5	0.02	94.6	1.6

Table 4.1.2-1: Recoveries for BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in both soils and sediment

Matrix	Test Item	Mass transition	No. of replicates	Fortification level [mg/kg]	Mean Recovery [%]	RSD [%]
LUFA Soil 5M	BAS 505 F	327 → 205	5 5	0.002 0.02	98.3 96.2	6.0 2.5
		327 → 116	5 5	0.002 0.02	98.5 96.0	6.9 3.3
	505M98	327 → 205	5 5	0.002 0.02	108.0 105.8	5.7 2.8
		327 → 116	5 5	0.002 0.02	111.3 105.4	6.5 4.1
	505M01	223 → 176	5 5	0.002 0.02	92.7 89.1	4.3 4.0
		223 → 116	5 5	0.002 0.02	93.9 86.7	5.9 6.7
	505M08	357 → 205	5 5	0.002 0.02	97.0 93.9	3.8 1.5
		357 → 116	5 5	0.002 0.02	97.4 94.3	3.3 1.7
	505M09	357 → 205	5 5	0.002 0.02	95.3 92.8	5.5 1.1
		357 →116	5 5	0.002 0.02	96.5 92.6	3.6 1.5
Berghäuser Altrhein Sediment	BAS 505 F	327 → 205	5 5	0.002 0.02	97.5 97.6	1.0 2.8
		327 → 116	5 5	0.002 0.02	97.8 98.1	1.5 2.8
	505M98	327 → 205	5 5	0.002 0.02	108.1 107.9	2.3 2.5
		327 → 116	5 5	0.002 0.02	107.5 107.7	2.9 5.1
	505M01	223 → 176	5 5	0.002 0.02	92.6 92.2	1.2 1.1
		223 → 116	5 5	0.002 0.02	89.0 89.7	1.4 2.3
	505M08	357 → 205	5 5	0.002 0.02	97.6 97.7	0.8 3.2
		357 → 116	5 5	0.002 0.02	98.1 97.8	1.6 2.8
	505M09	357 → 205	5 5	0.002 0.02	94.4 95.3	1.1 2.9
		357 →116	5 5	0.002 0.02	94.2 95.8	1.4 4.2

Linearity

Good linearity (regression coefficients ≥ 0.99) was observed in the range of 0.04 ng/mL to 2.0 ng/mL for the two mass transitions of BAS 505 F, 505M98, 505M01, 505M08 and 505M09.

Specificity

The method L0189/01 determines residues of BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in soil and sediment. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of each analyte.

Limit of Quantification

The limit of quantification defined by the lowest fortification level successfully tested was 0.002 mg/kg for all analytes (corresponding to 0.2 ng/mL in extract). The limit of detection was an estimated 20% hereof, equivalent to 0.0004 mg/kg for all analytes.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in the table above.

Reproducibility

Reproducibility of the method was not determined within this validation study.

Extractability

The extractability of dimoxystrobin and its metabolites 505M01, 505M98, 505M08), 505M09 in soil was investigated in the BASF Study DocID 2015/1105484. Selected samples from a terrestrial field dissipation study were extracted using the extraction solvent used for method L0189/01. Recoveries between 93% and 100% were obtained for dimoxystrobin and its metabolites.

Conclusion

The method for analysis of dimoxystrobin and its metabolites 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No 354562) and 505M09 (Reg.No. 354563) uses HPLC-MS/MS for quantitative determinations. The limit of quantification was 0.002 mg/kg for each analyte.

It could be demonstrated that method L0189/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of the fungicide BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in both soil and sediment.

Report: CA 4.1.2/2
Keller W., 1998a
Validation of analytical method No. 427: GC/MS-determination of 285028 (BAS 505 F ai) and its metabolites 354562 (BF 505-7), 354563 (BF 505-8) and 358104 (BF 505-4) residues in soil and sediment
1998/11332
Guidelines: EPA 860.1340, BBA IV 2
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Principle of the method:
The analytical method No. 427 was developed for the determination of dimoxystrobin (Reg.No. 285028) and its metabolites 505M08 (BF 505-7, Reg.No. 354562), 505M09 (BF 505-8, Reg.No. 354563) and 505M01 (BF 505-4, Reg.No. 358104) in soil and sediment.
Soil or sediment samples are extracted with 80 % aqueous methanol. An aliquot of the extract is evaporated to the aqueous phase and acidified with HCI. The acidified aqueous phase is partitioned with ethyl acetate. The ethyl acetate extract is evaporated to dryness. The residue is dissolved in methanol and methylated with ethereal diazomethane. After a silica gel column clean up the analytes are dissolved in an appropriate volume of internal standard solution in toluene and quantified by GC/MS determination (SIM-mode). The limit of quantification (LOQ) of the method is 0.01 mg kg^{-1}.

Recovery findings:
The method proved to be suitable to determine dimoxystrobin and its metabolites in soil and sediment samples.
The study was performed with two soil types and one sediment which were fortified at the limit of quantification and 10 and 100 times higher: 0.01, 0.1 and 1.0 mg kg^{-1}.
Mean recovery values (mean of five replicates per fortification level and analyte) corrected for interferences from matrix compounds of the appropriate unfortified sample were between 69% and 130% for all analytes (see table below).

Table 4.1.2-2: Summary of the recovery data in soil matrices

Soil	Test Item	Fortification Level [mg kg⁻¹]	Number of replicates	Mean Recovery [%]	RSD [%]	Overall Mean Recovery [%]	RSD [%]
Soil 2.2	Dimoxystrobin Reg.No.285028	0.01	5	102	3.6	98	7.4
		0.1	5	91	1.7		
		1.0	5	97	1.8		
US soil		0.01	5	108	0.8		
		0.1	5	91	0.6		
		1.0	5	94	1.6		
Sediment		0.01	5	112	1.6		
		0.1	5	95	0.9		
		1.0	5	95	1.8		
Soil 2.2	505M08 BF 505-7 Reg.No.354562	0.01	5	112	17.6	95	13.8
		0.1	5	97	3.6		
		1.0	5	90	2.1		
US soil		0.01	5	103	9.5		
		0.1	5	87	2.1		
		1.0	5	81	1.8		
Sediment		0.01	5	114	4.6		
		0.1	5	90	1.6		
		1.0	5	83	2.9		
Soil 2.2	505M09 BF 505-8 Reg.No.354563	0.01	5	124	8.1	97	15.0
		0.1	5	99	2.4		
		1.0	5	100	2.6		
US soil		0.01	5	74	2.3		
		0.1	5	89	1.0		
		1.0	5	92	3.0		
Sediment		0.01	5	113	2.8		
		0.1	5	89.7	2.1		
		1.0	5	88.4	2.2		
Soil 2.2	505M01 BF 505-4 Reg.No.358104	0.01	5	90	14.9	92	27.5
		0.1	5	70	11.5		
		1.0	5	73	8.2		
US soil		0.01	5	98	19.4		
		0.1	5	69	14.0		
		1.0	5	75	11.4		
Sediment		0.01	5	130	30.7		
		0.1	5	113	11.1		
		1.0	5	106	4.3		

Linearity

Good linearity was observed in the range of 20 to 1000 ng mL^{-1}. Calibration standards were prepared in internal standard solution (= 0.2 µg internal standard Reg. No. 304554 in 1 mL toluene).

Specificity

The method allows the specific determination of dimoxystrobin and its metabolites in soil and sediment. No or negligible interfering peaks occurred in untreated blank soil/sediment samples. A further analytical procedure as confirmatory technique is not necessary, because GC/MS is a very specific method. lf unexpected residues are found in the SIM-mode, then one may record the whole MS spectrum or use 2 further characteristic mass fragments for confirmatory of the compound.

Matrix Effects

Matrix effects were quantified with the help of unfortified control samples. The analytical method used an internal standard (Reg. No. 304554).

Limit of Quantification

The limit of quantification (LOQ) was defined by the lowest fortification level successfully tested. The LOQ is 0.01 mg kg^{-1} for all analytes.

Limit of Detection

The limit of detection (LOD) was not determined.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were with one exception below 20% (see table above).

Standard Stability

The solutions should be stored in the refrigerator and are expected to be stable at least for 4 weeks.

Extract Stability

The good recovery values show that the extracts were stable during the course of the study.

Reproducibility

Reproducibility of the method was not determined within this validation study.

Extractability

The extractability of dimoxystrobin and its metabolites 505M01, 505M08, 505M09 in soil was investigated in the BASF Study DocID 2015/1105484. Selected samples from a terrestrial field dissipation study were extracted using the extraction solvent used for method No. 427 (MeOH/Water, 80/20 v/v). Recoveries between 93% and 100% were obtained for dimoxystrobin and its metabolites. This extraction solvent is the same used in the analytical method L0189/01.

Conclusion

The BASF analytical method 427 for analysis of dimoxystrobin (Reg.No. 285028) and its metabolites 505M08 (BF 505-7, Reg.No. 354562), 505M09 (BF 505-8, Reg.No. 354563) and 505M01 (BF 505-4, Reg.No. 358104) in soil and sediment samples uses GC/MS for final determination, with a limit of quantification of 0.01 mg kg^{-1}.

The study results confirm that method 427 is well suited for the determination of residues of dimoxystrobin (BAS 505 F) and its metabolites 505M08 (BF 505-7), 505M09 (BF 505-8) and 505M01 (BF 505-4) in soil and sediment samples.

Report: CA 4.1.2/3
Obermann M., 2015a
Comparative analysis of extraction procedures on Dimoxystrobin – BAS 505 F soil samples originating from a field soil dissipation study
2015/1105484

Guidelines: SANCO/3029/99 rev. 4 (11 July 2000)

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

Executive Summary

The purpose of the study was, to compare the extraction efficiency of analytical method L0189/01 against the extraction procedures from two different metabolism studies for dimoxystrobin (BAS 505 F) and its metabolites 505M98, 505M01, 505M08 and 505M09. Therefore, four soil samples, originating from a terrestrial field dissipation study (BASF DocID 2014/1289336) were used and extracted with the three different procedures. The instrumental analytics of the extracts were done according to analytical method L0189/01 by LC-MS/MS. It could be demonstrated that the three tested extraction procedures are comparable for the analytes dimoxystrobin and its metabolites 505M08 and 505M09. For the metabolites M505M98 and 505M01 no relevant residues were determined, and therefore no comparison can be done. To check the validity of residue analytical method L0189/01, fortified samples with LUFA 2.2 standard soil were prepared and analyzed. The results show good recovery and repeatability in the range of 93 % - 113 % for all analytes. Additionally to the fortification experiments with untreated standard soil, quality control samples were prepared using a treated field sample. This means extracts from the different extraction procedures were fortified with known amount of the reference items and analyzed. Also within this experiment, the recovery values were in the expected concentration range and no relevant matrix effect from the field soil matrix could be determined.

I. MATERIAL AND METHODS

A. TEST SYSTEMS

The following test systems were considered in the study:

Test Matrix 1 (for comparison of extraction methods): 4 Field soil samples originated from soil dissipation study

- L1501790001
- L1501790002
- L1501790003
- L1501790004

Test Matrix 2 (for concurrent method validation): standard soil

- LUFA 2.2: loamy sand (DIN 4220)

B. STUDY DESIGN

Extraction Procedures

Extraction procedure 1 (based on L0189/01)
A 5 g soil sample (wet soil) is extracted with methanol/water (80/20, v/v) on a mechanical shaker (30 min at 225 rpm). Approx. 5 mL of the suspension is centrifuged. The extract is diluted with an appropriate amount of methanol/water (80/20, v/v) for LC MS/MS measurement.

Extraction procedure 2 (MeOH + MeOH/Water)
A 10 g soil sample (wet soil) is extracted three times with methanol and three times with methanol/water (50/50, v/v) on a mechanical shaker (10 min at 225 rpm). The extracts are combined and methanol is added to come to a defined volume of 200 mL. After centrifugation an aliquot is evaporated to dryness and reconstituted with 2 mL methanol/water (80/20, v/v). Further dilutions are done with methanol/water (80/20, v/v) if needed. Final determination is conducted by LC MS/MS.

Extraction procedure 3 (ACN, MeOH + MeOH/Water)
A 10 g soil sample (wet soil) is extracted three times with acetonitrile, three times with methanol and three times with methanol/water (50/50, v/v) on a mechanical shaker (10 min at 225 rpm). The extracts are combined and methanol is added to come to a defined volume of 500 mL. An aliquot is evaporated to dryness and reconstituted with 2 mL methanol/water (80/20, v/v). Further dilutions were done with methanol/water (80/20, v/v) if needed. Final determination is conducted by LC MS/MS.

Quantification Procedure

The instrumental analytics of the extracts on dimoxystrobin (BAS 505 F) and its metabolites 505M98, 505M01, 505M08 and 505M09 were done according to analytical method L0189/01 by LC-MS/MS. The limit of quantification (LOQ) of the method is 0.002 mg kg^{-1}, the limit of detection (LOD) is 0.0005 mg kg^{-1}.
Calculations of results were based on peak area measurements using a calibration curve. Good linearity was observed in the range of 0.04 ng mL^{-1} to 2.0 ng mL^{-1}. The calibration curve was obtained by direct injection of BAS 505 F, 505M98, 505M01, 505M08 and 505M09 mix standards for LC-MS/MS prepared in methanol/water (80/20, v/v).

II. RESULTS AND DISCUSSION

Comparison of the three extraction methods

From each of the four field soil samples, 3 aliquots were taken and prepared according to the described extraction procedures, followed by the instrumental measurement based on residue analytical method L0189/01. As the exact residue values of the field samples were not known in advance, several dilutions of the final extracts were measured to ensure measurement solutions to be within the calibration range. Therefore, for some samples, multiple results are presented and used for mean value calculations.

The analytical investigations showed a good comparability of the extraction efficiency of the three extraction procedures for dimoxystrobin: The analytical results are in the same magnitude and within the expected variety of a residue analytical method.
For the metabolites 505M98 and 505M01 no relevant residues could be determined, therefore no comparison between the extraction procedures can be done.
The residues for the metabolites 505M08 and 505M09 were determined in the range, or slightly below, the LOQ. Taking into account the higher variety of the analytical method within this concentration range, the analytical results are also within the same magnitude and the extraction efficiency is comparable between the individual extraction procedures.

A summary of the residue results of the four field soil samples is given in Table 4.1.2-3.

Table 4.1.2-3: Extraction methods in four soil samples; summary of residue results

Analyte	Extraction Procedure (EP) No.	Soil L1501790001		Soil L1501790002		Soil L1501790003		Soil L1501790004	
		Mean C_{soil}* [mg kg^{-1}]	% [EP1]	Mean C_{soil}* [mg kg^{-1}]	% [EP1]	Mean C_{soil}* [mg kg^{-1}]	% [EP1]	Mean C_{soil}* [mg kg^{-1}]	% [EP1]
Dimoxy-strobin (Reg.No. 285028)	1	0.043		0.034		0.014		0.032	
	2	0.053	123	0.039	115	0.016	114	0.038	120
	3	0.050	117	0.037	107	0.017	121	0.036	114
505M98 M505F098 (Reg.No. 360056)	1	(0.0007)		<LOD		(0.0005)		(0.0009)	
	2	(0.0003)	n.a.	<LOD	n.a.	<LOD	n.a.	(0.0010)	n.a.
	3	(0.0010)	n.a.	<LOD	n.a.	<LOD	n.a.	<LOD	n.a.
505M01 M505F001 (Reg.No. 358104)	1	<LOD		<LOD		<LOD		<LOD	
	2	<LOD	n.a.	<LOD	n.a.	<LOD	n.a.	<LOD	n.a.
	3	<LOD	n.a.	<LOD	n.a.	<LOD	n.a.	<LOD	n.a.
505M08 M505F008 (Reg.No. 354562)	1	(0.0013)		(0.0010)		(0.0017)		(0.0018)	
	2	(0.0017)	n.a.	(0.0012)	n.a.	0.0024	n.a.	0.0025	n.a.
	3	(0.0016)	n.a.	(0.0012)	n.a.	0.0026	n.a.	0.0024	n.a.
505M09 M505F009 (Reg.No. 364563)	1	(0.0018)		(0.0012)		(0.0008)		(0.0012)	
	2	0.0024	n.a.	(0.0016)	n.a.	(0.0014)	n.a.	0.0021	n.a.
	3	0.0023	n.a.	(0.0016)	n.a.	(0.0014)	n.a.	(0.0019)	n.a.

Values in brackets are below LOQ
C_{soil} = soil concentration (mean of 3 or 6 values)
n.a. = not applicable (calculation would be based on values below LOQ, and therefore not reliable)

Matrix Effects / Quality Control Samples

To check the used field sample matrix on potential matrix effects, the field sample with the lowest residue level (L1501790003) was fortified with defined amounts of dimoxystrobin and its metabolites. Out of the difference between measured field sample residue and measured residue (fortified + field sample), the recovery of the fortified amount was calculated. The recovery values were in the expected concentration range and no relevant matrix effect from the field soil matrix could be determined (see Table 4.1.2-4).

Table 4.1.2-4: Recoveries of Quality Control Samples

Extraction Procedure (EP) No.	Final Volume [mL]	Recovery [%]				
		Dimoxy-strobin	505M98	505M01	505M08	505M09
1	50	123	100	103	104	96
	500	104	103	101	107	99
2	200	112	133	102	98	89
	2000	90	98	95	108	100
3	2	98	124	104	98	91
	20	95	97	95	109	103

Concurrent Method Validation

To check the validity of residue analytical method L0189/01, the standard soil LUFA 2.2 was fortified with solutions containing dimoxystrobin, 505M98, 505M01, 505M08 and 505M09 to obtain fortification levels at LOQ (0.002 mg kg^{-1}) and 10 times higher (0.02 mg kg^{-1}).

The results show good recovery and repeatability in the range of 97 - 105 % for dimoxystrobin, 97 - 105 % for 505M98, 102 - 110 % for 505M01, 103 - 113 % for 505M08 and 93 % - 106 % for 505M09 (see Table 4.1.2-5).

Table 4.1.2-5: Recovery experiments, LUFA 2.2 soil fortified with dimoxystrobin and its 4 metabolites; summary of validation results

Extraction Procedure (EP) No.	Forti-fication level [mg kg^{-1}]	Number of samples	Recovery [%]				
			Dimoxy-strobin	505M98	505M01	505M08	505M09
1	0.002	1	101	100	105	106	97
	0.02	1	101	101	104	103	93
2	0.002	1	97	99	107	107	99
	0.02	1	100	100	110	107	99
3	0.002	2	105, 101	105, 97	110, 102	105, 103	94, 98
	0.02	2	103, 101	105, 99	106, 106	113, 110	106, 97

III. CONCLUSION

It could be demonstrated that the three tested extraction procedures are comparable for the analytes dimoxystrobin and its metabolites 505M08 and 505M09. For the metabolites 505M98 and 505M01 no relevant residues were determined, and therefore no comparison can be done.

Water

Report: CA 4.1.2/4
Obermann M. et al., 2015a
Validation of analytical method L0191/01 for the determination of BAS 505 F (Reg.No. 285028), 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No. 354562) and 505M09 (Reg.No. 354563) in surface water and groundwater by LC-MS/MS
2014/1161841

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100

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

Principle of the methods

Method Number L0191/01:

The BASF Method allows the determination of BAS 505 F - Dimoxystrobin (Reg.No. 285028) and its metabolites 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No. 354562) and 505M09 (Reg.No. 354563) in ground- and surface water by HPLC-MS/MS. An aliquot of 10 g of the water sample was extracted by SPE. The analytes were eluted with methanol. After evaporation to dryness the residues were dissolved in methanol/water (80/20, v/v). An aliquot of the final volume was measured using HPLC-MS/MS. Due to the high specificity of HPLC-MS/MS and the different transitions proposed, no confirmatory technique is necessary. In this study, the transitions 327 → 205 and 327 → 116 were used for all analytes and matrices tested.

Recovery findings

Method L0191/01 was proved to be suitable to determine residues of BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in ground- and surface water to a limit of quantification (LOQ) of 0.025 mg/kg. The mean recovery values of the validation experiments were between 70% and 110%, which fulfils the legal requirements for mean recovery values. Detailed results of recoveries for each mass transition and matrix are given in Table 4.1.2-6. Results of stability investigations of the extracts showed that BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) are stable in the extracts of ground- and surface water over the tested time period of 7 days.

Table 4.1.2-6: Recoveries for BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in both ground- and surface water

Matrix	Test Item	Mass transition	No. of replicates	Fortification level [µg/kg]	Mean Recovery [%]	RSD [%]
Ground Water	BAS 505 F	327 → 205	5 5	0.025 0.25	91 87	2.9 1.4
		327 → 116	5 5	0.025 0.25	88 85	3.4 1.7
	505M98	327 → 205	5 5	0.025 0.25	89 85	2.4 1.8
		327 → 116	5 5	0.025 0.25	87 85	3.5 2.3
	505M01	223 → 176	5 5	0.025 0.25	93 91	2.4 2.8
		223 → 116	5 5	0.025 0.25	92 95	3.3 4.0
	505M08	357 → 205	5 5	0.025 0.25	98 99	2.7 2.9
		357 → 116	5 5	0.025 0.25	99 99	5.6 5.0
	505M09	357 → 205	5 5	0.025 0.25	98 100	5.5 4.0
		357 →116	5 5	0.025 0.25	97 96	8.1 1.1
Surface Water	BAS 505 F	327 → 205	5 5	0.025 0.25	92 90	0.5 1.3
		327 → 116	5 5	0.025 0.25	92 90	0.8 1.5
	505M98	327 → 205	5 5	0.025 0.25	92 89	1.0 1.0
		327 → 116	5 5	0.025 0.25	90 88	1.8 3.2
	505M01	223 → 176	5 5	0.025 0.25	99 97	0.7 1.5
		223 → 116	5 5	0.025 0.25	101 99	2.6 4.2
	505M08	357 → 205	5 5	0.025 0.25	103 99	1.7 4.6
		357 → 116	5 5	0.025 0.25	106 105	2.4 3.7
	505M09	357 → 205	5 5	0.025 0.25	102 101	0.8 2.0
		357 →116	5 5	0.025 0.25	103 101	3.9 5.4

Linearity
Good linearity (regression coefficients ≥ 0.998) was observed in the range of 0.025 ng/mL to 2.0 ng/mL for the two mass transitions of BAS 505 F and for the standard solutions of the metabolites 505M98, 505M01, 505M08 and 505M09.

Specificity
The method L0191/01 determines residues of BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in ground- and surface water. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of each analyte.

Limit of Quantification
The limit of quantification defined by the lowest fortification level successfully tested was 0.025 µg/kg for all analytes, an estimated 20% of the limit of detection (equivalent to 0.005 µg/kg).

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in the table above.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
It could be demonstrated that the analytical method L0191/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 505 F (Reg.No. 285028) and its metabolites 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No. 354562) and 505M09 (Reg.No. 354563) in both ground- and surface water.

Air

Report: CA 4.1.2/5
Miller C., 2015a
Validation of BASF method L269/01 for the determination of BAS 505 F in air
2015/1020131
Guidelines: 2004/10/EC of 11 February 2004, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)
GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the methods

Method Number L269/01:

BASF method L269/01 was developed for the determination of BAS 505 F (Dimoxystrobin) in air samples. The analyte is spiked to Tenax adsorption tubes. Two spiking levels (1.2 and 12 µg m^{-3}) were tested. After sucking air through the glass tubes at 35°C and a relative humidity of 80% for 6 hours at approximately 1 L min^{-1}, the tube content is extracted by ultrasonication using acetonitrile.

Final determination of dimoxystrobin is achieved by LC-MS/MS. The limit of quantification is defined as the lowest fortification level used at which acceptable recovery data was obtained corresponding to a concentration of 1.2 µg dimoxystrobin m^{-3} air.

Recovery findings

Method L269/01 was proved to be suitable to accurately determine residues of BAS 505 F in air. The mean recovery values of the validation experiments were within the acceptable range of 70% to 110% (see Table 4.1.2-7).

Results of stability investigations show that BAS 505 F is stable on Tenax sorbent material when stored at ≤ -20°C (in the dark) for a period of up to 8 days and when stored at approximately -20°C in final extract solution for a period of 7 days.

Table 4.1.2-7: Recoveries for BAS 505 F in air

Matrix	Test Item	Mass transition	No. of replicates	Fortification level [µg/cartridge]	Mean Recovery [%]	RSD [%]
Air	**BAS 505 F**	**327 → 205**	5	3.33	75	6.0
			5	33.3	93	4.5
		327 → 116	5	3.33	84	11.4
			5	33.3	95	5.0

Linearity

Good linearity (regression coefficients = 0.9997) was observed in the range of 0.005 ng/mL to 0.5 ng/mL for the two mass transitions of BAS 505 F.

Specificity

Under the described conditions method L269/01 is specific for the determination of dimoxystrobin in air. Significant interferences (>30% of LOQ) were not observed at the retention time and mass transitions of dimoxystrobin.
Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. Two mass transitions of dimoxystrobin were quantified.

Limit of Quantification

The limit of quantification defined by the lowest fortification level successfully tested was 1.2 µg m^{-3} air. The limit of detection (LOD) of the method was defined as the concentration of the lowest calibration standard chromatographed with that gave rise to a measurable chromatographic response. For this study the LOD of the method was shown to be 0.005 ng mL^{1} (equivalent to 0.5 µg per cartridge or 0.18 µg m^{-3} in air).

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in the table above.

Reproducibility

Reproducibility of the method was not determined within this validation study.

Conclusion

The method for analysis of dimoxystrobin in air uses LC-MS/MS for quantitative determination. The limit of quantification was 1.2 µg m^{-3} air.

It could be demonstrated that method L269/01 fulfils the requirements with regard to accuracy (recovery), precision (repeatability), linearity (calibration), specificity (interference), limit of quantitation (LOQ) and limit of detection (LOD), retention capacity (breakthrough) and extractability and is therefore applicable to correctly determine residues of the fungicide BAS 505 F in air.

(b)Methods in soil, water and any additional matrices used in support of efficacy studies

Not Relevant

(c)Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

Since dimoxystrobin is not classified as toxic or very toxic, methods of analysis for parent or metabolites in human body tissues or fluids are not required. Methods for concentration control in feed or other matrices are reported, where necessary, along with the respective toxicological studies.

(d) Methods in body fluids, air and any additional matrices used in support of operator, worker, resident and bystander exposure studies

Since no new exposure studies were conducted with dimoxystrobin, such methods of analysis are not required.

(e)Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residues studies

Plant matrices

A new method was developed and validated for the determination of the residues of the Z-isomer of dimoxystrobin. This method was also used for the new residue field trials.

Report:	CA 4.1.2/6 Austin R., 2014a BASF method number L0076/08 - Method validation study for the determination of residues of BAS 505 F (Dimoxystrobin) and its Z-isomer in plant matrices 2014/1233896
Guidelines:	EU Regulation 1107/2009 with Regulation 283/2013, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD-ENV/JM/MONO/(2007)17, EPA 860.1340
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the methods

Method Number L0076/08:

BAS 505 F (Dimoxystrobin) and its Z-isomer 505M98 (505M98) were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane. Final determination of BAS 505 F and 505M98 was performed by LC-MS/MS, using MRM transitions for quantification and confirmation.

Recovery findings

The method proved to be suitable for the determination of residues of BAS 505 F (Dimoxystrobin) and its Z-isomer 505M98 in various foodstuffs of plant origin (tomato, oilseed rape (seeds), beans, barley grain and orange) to achieve a limit of quantification (LOQ) of 0.005 mg/kg. The LOD (limit of detection) is 0.00125 mg/kg.

The average recoveries for the two parent-daughter ion transitions monitored were within the acceptable range of 70% to 110% with relative standard deviations (RSD) $\leq$ 20%. Results are summarized in Table 4.1.2-9.

Results of stability investigations of calibration and fortification solutions as well as stability investigations of the final volumes are summarized in Table 4.1.2-9 and in Table 4.1.2-10. The results show that stock and fortification solutions in methanol and calibration standards prepared in acetonitrile/water (50:50) are stable over the investigated time of 51 days. Stability of extracts (recoveries were within the acceptable range of 70-110%) was demonstrated for all plant matrices when stored refrigerated.

Table 4.1.2-8: Recovery results of BAS 505 F (Dimoxystrobin) and its Z-isomer 505M98

			BAS 505 F		505M98	
Matrix	**Fortification level (mg/kg)**	**Recoveries**	**m/z 327→205***	**m/z 327→116**	**m/z 327→205***	**m/z 327→116**
Tomato	0.005, 0.05	Average (%)	80.3	80.9	74.8	76.0
		RSD (%)	3.8	4.1	4.3	4.3
		n	10	10	10	10
Oilseed Rape (Seeds)	0.005, 0.05	Average (%)	87.4	84.4	79.3	79.8
		RSD (%)	4.8	5.0	3.6	4.6
		n	10	10	10	10
Beans	0.005, 0.05	Average (%)	91.1	91.2	87.9	87.3
		RSD (%)	4.3	4.7	5.1	5.5
		n	10	10	10	10
Barley Grain	0.005, 0.05	Average (%)	92.6	93.3	85.8	89.0
		RSD (%)	3.8	3.9	6.7	3.5
		n	10	10	10	10
Orange	0.005, 0.05	Average (%)	82.4	88.1	81.5	81.2
		RSD (%)	9. 1	12.0	10.0	10.0
		n	10	10	10	10

Table 4.1.2-9: Storage stability of stock, fortification and calibration standards (stored refrigerated)

Standard		**BAS 505 F Transition *m/z* 327 → 205**	**505M98 Transition *m/z* 327 → 205**
	Time interval between standard preparation (days)	**Recovery based on fresh solution (%)**	**Recovery based on fresh solution (%)**
Stock solutions	51	101	99
Fortification solutions	51	107	102
Calibration solutions	51	110	114

Table 4.1.2-10: Storage stability of BAS 505 F (Dimoxystrobin) and 505M98 in final extracts (stored refrigerated)

Matrix		**BAS 505 F Transition *m/z* 327 → 205**	**505M98 Transition *m/z* 327 → 205**
	Time interval between standard preparation (days)	**Recovery based on fortification level of 0.005 mg/kg (%)**	**Recovery based on fortification level of 0.005 mg/kg (%)**
Tomato	8	95.9	89.3
Oilseed Rape (Seeds)	11	108.0	93.9
Beans	16	97.9	92.9
Barley Grain	8	109.0	95.7
Oranges	15	94.7	95.4

Linearity

Correlation coefficients (r) ≥ 0.99 were obtained in the range of 0.05 to 10.0 ng/mL for both dimoxystrobin and its Z-isomer 505M98 (external reference standard).

Specificity

The method determines dimoxstrobin and its Z-isomer 505M98 in various foodstuffs of plant origin (tomato, oilseed rape (seeds), beans, barley grain and orange). Matrix effects were not significant (i.e. > 20%) for the analytes in plant matrices.

Limit of Quantification

The limit of quantification was defined by the lowest fortification level successfully tested, which was 0.005 mg/kg in all sample materials.

Repeatability

The relative standard deviations (RSD, %) for all matrices and all fortification levels were well below 20%. Values are shown in the table above.

Reproducibility

This study showed that the method developed in one laboratory could be successfully used in another facility.

Extractability

The extractability of dimoxystrobin and 505M98 was investigated in the BASF Study DocID 2015/1020186. Selected samples (seeds, rest of plant) from a rape metabolism study were extracted using the extraction solvent used in the analytical L0076 (MeOH/Water/HCl). Recoveries between 102% and 118% in comparison to the solvents used in the metabolism study were obtained for the analytes in rest of plant and rape seed samples.

Conclusion

The method uses LC-MS/MS for final determination of residues of BAS 505 F (Dimoxystrobin) and its Z-isomer 505M98 in foodstuffs of plant origin to achieve a limit of quantification (LOQ) of 0.005 mg/kg.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries. In addition, it could be shown by the study that the method has a high reproducibility.

Report: CA 4.1.2/7
Lehmann A.,Mackenroth C., 2001a
Validation of BASF method No. 445/0: Determination of BAS 505 F in the following plant matrices: Wheat plant without root, grain and straw, sugar beet, oilrape seed and orange
2000/1012403

Guidelines: EPA 860.1340, EEC 96/46, EEC 8064/VI/97 Guidance document on residue analytical methods

GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Principle of the methods

Method Number 445/0:

BAS 505 F (Dimoxystrobin) is extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract is centrifuged and partitioned against cyclohexane. The final determination of BAS 505 F is performed by HPLC-MS/MS. For BAS 505 F, the transition ions m/z = 327 → 205 and m/z = 327 → 116 can be used for quantification. Due to the high specificity of HPLC-MS/MS and the different transitions proposed, no confirmatory technique is necessary. In this study, the transition 327 → 205 was used for all matrices tested.

Recovery findings

The method proved to be suitable for analysis of dimoxystrobin in in plant without root, grain and straw of wheat as well as in sugar beet, oilrape seed and orange to a limit of quantification of 0.05 mg/kg. In all matrices tested, the mean recovery values were within the acceptable range of 70% to 110%. A summary of results is presented in Table 4.1.2-11.

Table 4.1.2-11: Recovery results of BAS 505 F

Matrix	Fortification level (mg/kg)	No. of tests	Average Recovery (%)	Rel. Standard Deviation (%)
Wheat, plant w/o root	0.05, 5.0	10	92.9	12.8
Wheat, grain	0.05, 5.0	10	96.0	13.6
Wheat, straw	0.05, 5.0	10	97.9	9.8
Sugar beet, root	0.05, 5.0	10	86.9	2.8
Oilrape seed	0.05, 5.0	10	89.5	6.6
Orange, fruit	0.05, 5.0	10	86.0	4.3

Linearity

Good linearity was observed in the range of 0.25 to 10.0 ng/mL for dimoxystrobin (external reference standard).

Specificity

The method determines parent dimoxystrobin in plant without root, grain and straw of wheat as well as in sugar beet, oilseed rape and orange. There were no known interferences from plant components or from reagents, solvents and glassware used.

Limit of Quantification

The limit of quantification was defined by the lowest fortification level successfully tested, which was 0.05 mg/kg in all sample materials.

Repeatability

The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%. The detailed values are shown in the table above.

Reproducibility

Not applicable (method is used as data generation method).

Extractability

The extractability of dimoxystrobin and 505M98 was investigated in the BASF Study DocID 2015/1020186. Selected samples (seeds, rest of plant) from a rape metabolism study were extracted using the extraction solvent used in the analytical 445/0 (MeOH/Water/HCl, equivalent to solvent used in L0076). Recoveries between 102% and 118% in comparison to the solvents used in the metabolism study were obtained for the analytes in rest of plant and rape seed samples.

Conclusion

The method for analysis of dimoxystrobin uses HPLC-MS/MS for final determination. The limit of quantification is 0.05 mg/kg.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries.

Animal matrices

A new method was developed and validated to include the metabolite 505M09 of dimoxystrobin. This new method fulfils the requirement mentioned in the recently EFSA Reason Opinion on MRLs (EFSA Journal 2013; 11(11):3464.

Report:	CA 4.1.2/8 Richter S., 2015a Dimoxystrobin BAS 505 F and its metabolite 505M09: Validation of the multi-residue method DFG S19 for the determination of residues in milk, egg, meat, fat, liver and kidney using LC/MS/MS - BASF method number L0232/01 2014/1315330
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, OECD-ENV/JM/MONO/(2007)17, EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)
GLP:	yes (certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the methods

Multi-Residue Method DFG S19:

Appropriate DFG S19 extraction modules E1 (milk, egg, meat, liver and kidney) and E6 (fat), clean-up modules (gel permeation chromatography: module GPC) and module LC/MS/MS (with positive ionisation: ESI) were used for quantification and confirmation. No column chromatographic fractionation (module C1) on silica gel was necessary due to the highly selective LC/MS/MS method employed.

For the metabolite 505M09 in egg low recovery results for all applicable extraction modules of the DFG S19 method were obtained. Thus extraction module E1 was slightly modified by addition of acidified water instead of water prior the extraction. The method uses the following two mass transitions for each analyte:

Dimoxystrobin: 327 m/z → 205 m/z for quantification and 327 m/z → 116 m/z for quantitative confirmation.

Dimoxystrobin metabolite 505M09: 357 m/z → 205 m/z for quantification and 357 m/z → 116 m/z for quantitative confirmation.

Recovery findings

The method proved to be suitable for measuring residues of dimoxystrobin (BAS 505 F) and its metabolite 505M09 in foodstuffs of animal origin (exemplified by milk, egg, meat, fat, liver and kidney), with a target limit of quantification (LOQ) of 0.010 mg/kg per analyte, using LC/MS/MS for quantification and confirmation.

Residues in all blank control specimens were below 20% of the LOQ (< 0.002 mg/kg). The average recoveries for the two parent-daughter ion transitions monitored were within the acceptable range of 70% to 110% with relative standard deviations (RSD) ≤ 20% except for fat with an average recovery of 69.5% for the conformation transition. A summary of results is given in Table 4.1.2-12.
Stability of the analytes in stock, fortification and calibration solutions was demonstrated for up to 6 weeks when stored refrigerated. The stability of the final extracts was demonstrated for at least 5 days by acceptable recoveries within 70 to 120% when stored refrigerated. Stability of the metabolite 505M09 in fat extracts was demonstrated by low deviation of recovery results between first injection and re-injection after refrigerated storage.

Table 4.1.2-12: Recovery results of BAS 505 F (Dimoxystrobin) and its metabolite 505M09

Fortification Level (mg/kg)	Analyte	BAS 505 F (Dimoxystrobin)											
	Daughter Ion	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z
	Matrix	Milk		Egg		Meat		Liver		Kidney		Fat	
0.010, 0.10	Average	102%	102%	109%	109%	78.9%	78.5%	99.8%	99.8%	97.4%	97.5%	105%	104%
	SD	3.4%	3.3%	3.0%	2.3%	6.3%	6.7%	5.1%	6.0%	5.2%	5.7%	2.2%	4.0%
	RSD	3.3%	3.2%	2.8%	2.1%	8.0%	8.6%	5.1%	6.1%	5.3%	5.8%	2.1%	3.8%
	n	10	10	10	10	10	10	10	10	10	10	10	10
Fortification Level (mg/kg)	Analyte	Metabolite 505M09											
	Daughter Ion	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z
	Matrix	Milk		Egg		Meat		Liver		Kidney		Fat	
0.010, 0.10	Average	91.5%	93.2%	101%	103%	79.9%	81.3%	92.1%	93.4%	89.3%	89.3%	84.6%	83.7%
	SD	3.4%	2.9%	5.9%	5.8%	6.9%	7.5%	4.9%	4.8%	5.0%	4.6%	15%	17%
	RSD	3.7%	3.1%	5.8%	5.6%	8.6%	9.2%	5.4%	5.1%	5.6%	5.2%	18%	20%
	n	10	10	10	10	10	10	10	10	10	10	10	10

Linearity

Good linearity was observed in the range of 0.015 to 1.2 ng/mL (for fat) or 0.15 ng/mL to 12 ng/mL (for milk, egg, bovine meat, liver and kidney).

Specificity

The method determines residues of dimoxystrobin and its metabolite 505M09 in animal matrices. Matrix effects were not significant (i.e. <20%) for milk, meat, egg and fat. Quantitative determination of final meat extracts was carried out by external standardization using calibration solutions in solvent. For milk, egg and fat matrix effects were insignificant too, but evaluation was done using matrix-matched standards. For kidney and liver matrix effects were significant and thus quantitative determination was carried out by external standardization using matrix matched standards.

Limit of Quantification
The limit of quantification (LOQ) of the method is 0.010 mg/kg per analyte.

Repeatability
The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%, except for fat. Values are shown in the table above.

Reproducibility
This study showed that the method developed in one laboratory could be successfully used in another facility.

Extractability
The extractability of dimoxystrobin and its metabolite M505F009 was investigated in the BASF Study DocID 2015/1125782. Selected samples (goat liver and goat kidney) from a goat metabolism study were extracted using the extraction solvent used for method L0232/01 (DFG, S19, Acetone). Recoveries between 30% and 84% were obtained for dimoxystrobin. Recoveries of 100% were obtained for M505F09.

Conclusion
The LC/MS/MS based DFG method S19 was successfully validated for the determination of dimoxystrobin (BAS 505 F) and its metabolite 505M09 in foodstuffs of animal origin (exemplified with milk, egg, meat, fat, liver and kidney) with an LOQ of 0.010 mg/kg per analyte and thus demonstrated to be applicable for enforcement and monitoring purposes.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries.

Report: CA 4.1.2/9
Abdel-Baky S.,Jones J.E., 2001a
Validation of BASF method D0006: Determination of residues of BAS 505 F and its metabolites (BF 505-8 and BF 505-11) in animal tissues (liver, kidney, fat and muscle) and milk using LC/MS/MS
2001/5000047

Guidelines: EPA 860.1340, Guidance Document of Residue Analytical Methods 8064/VI/97 rev. 4 15.12.1998

GLP: yes
(certified by United States Environmental Protection Agency)

Principle of the method

Method Number D0006:

The parent compound (BAS 505 F) and its metabolites 505M09 (BF 505-8) and 505M76 (BF 505-11) are extracted from liver, muscle, kidney and milk using 100% methanol and from fat with hexane and acetonitrile-HCI. An aliquot is taken from the extract and is purified by solid phase extraction followed by HLB SPE column chromatography. The eluent from the HLB column is diluted with buffer solution and injected directly into LC/MS/MS for analyte determination.

Recovery findings

The method proved to be suitable for measuring residues of BAS 505 F and its metabolites 505M09 and 505M76 in animal tissues (liver, kidney, fat and muscle), and milk down to a quantification limit of 0.025 ppm (0.01 ppm for milk).

The overall average recoveries of BAS 505 F, 505M09 and 505M76 in animal matrices and milk were in the range of 70 to 110%. A summary of results is given in Table 4.1.2-13.

Table 4.1.2-13: Recovery results of BAS 505 F, 505M09 and 505M76

Matrix	Fortification level (mg/kg)	n	Average Recovery (%) ± standard deviation			Rel. Standard Deviation (%)		
			BAS 505 F	505M09	505M76	BAS 505 F	505M09	505M76
Milk	0.01, 0.10	20	90 ± 7	99 ± 14	88 ± 11	8	14	12
Fat	0.025, 0.25	10	80 ± 4	94 ± 10	86 ± 9	5	11	10
Liver	0.025, 0.25	10	82 ± 11	89 ± 11	92 ± 14	13	13	15
Muscle	0.025, 0.25	10	89 ± 6	90 ± 13	83 ± 13	7	14	15
Kidney	0.025, 0.25	15	85 ± 8	101 ± 11	87 ± 7	9	11	8

Linearity

The correlation coefficients of the calibration curves were above 0.98 for the range of 0.05 to 1.0 ng/mL for BAS 505 F, 505M09 and 505M76 (external reference standard).

Specificity
The method determines BAS 505 F, 505M09 and 505M76 in animal tissues and milk samples.

Limit of Quantification
The limit of quantification of the method for all analytes in tissues (liver, kidney, fat and muscle), is 0.025 ppm and 0.01 ppm for the milk.

Repeatability
The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%. Values are shown in the table above.

Reproducibility
Not applicable (method is used as data generation method).

Extractability
The extractability of dimoxystrobin and its metabolite 505M09 was investigated in the BASF Study DocID 2015/1125782. Selected samples (goat liver and goat kidney) from a goat metabolism study were extracted using the extraction solvent used for method D0006. Recoveries between 66% and 133% were obtained for dimoxystrobin in comparison with the solvent used in the metabolism study.

Conclusion
The method for analysis of BAS 505 F (Dimoxystrobin), 505M09 (BF 505-8) and 505M76 (BF 505-11) uses LC/MS/MS for final determination. The limit of quantification has been established as 0.01 ppm for each analyte in milk and 0.025 ppm per analyte in animal tissues.
It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries.

(f)Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies

Methods for concentration control or for additional matrices are reported, where necessary, along with the respective ecotoxicological studies.

(g)Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests

Where necessary, these methods are reported along with the respective studies.

CA 4.2 Methods for post-approval control and monitoring purposes

Note: The order of the study summaries is differing compared to the information given in the application submitted for renewal of approval. In case references are summarized that were not contained in the application or in case references listed in the application are not contained in this chapter, comments are made where appropriate.

(a) Methods for the analysis in food and feed of plant and animal origin

Two validated multi-residue methods (DFG-S19, Quechers) are submitted for the determination of dimoxystrobin in plant matrices. They are supported by appropriate extractability investigations performed with sample material generated in course of the metabolism study in crops. According to the results of these extractability investigations, the DFG S19 method is recommended as monitoring method for dimoxystrobin in food of plant origin.

For food of animal origin a validated DFG S19 method is submitted and also recommended for monitoring purposes for dimoxystrobin and its metabolite. It is supported by appropriate extractability investigations performed with sample material generated in course of the metabolism study in livestock. Its corresponding ILV is also available and submitted.

Plants

Report: CA 4.2/1
Guedez-Orozco A. et al., 2015a
Validation of the BASF analytical method L0248/01: Multi-residue method (QuEChERS) for the determination of Dimoxystrobin (BAS 505 F) in plant matrices
2015/1020187
Guidelines: SANCO/3029/99 rev. 4 (11 July 2000), SANCO/825/00 rev. 8.1 (16 November 2010), OECD-ENV/JM/MONO/(2007)17 (OECD No. 72), OECD-ENV/JM/MONO/(2007)17 (OECD No. 39)
GLP: yes
(certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

The analytical method L0248/01 was developed for the determination of dimoxystrobin (BAS 505 F, Reg.No. 285028) in plant matrices.
Residues of dimoxystrobin are extracted from plant matrices with acetonitrile. For samples with a low water content (< 80 %), sample material is mixed with water prior to extraction. After the addition of magnesium sulfate, sodium chloride and buffering citrate salts (QuEChERS Extract Pack), the mixture is shaken vigorously and centrifuged in order to achieve a phase separation. An aliquot of the organic phase is cleaned-up by dispersive solid phase extraction (dSPE) employing bulk sorbents as well as magnesium sulfate for the removal of residual chlorophyll and / or carotenoids. An aliquot of the cleaned-up extract is diluted with acetonitrile/water (50/50, v/v). The final determination is performed by HPLC-MS/MS monitoring two mass transitions of dimoxystrobin.
The method was validated at two fortification levels for the plant matrices wheat grain, rape seed, tomato fruit, orange fruit and dried bean seed.
The limit of quantification of the method (LOQ) is 0.01 mg kg^{-1} and the limit of detection (LOD) is 0.002 mg kg^{-1}.

Recovery findings

The method proved to be to be suitable to determine residues of dimoxystrobin in plant matrices (wheat grain, rape seed, tomato fruit, orange fruit and dried bean seed).
Plant samples were fortified with dimoxystrobin at the limit of quantification of 0.01 mg kg^{-1} and 10 times higher (0.1 mg kg^{-1}).
Mean recovery values for all plant matrices tested (mean of five replicates per fortification level) were between 98 and 110% (see table below), which fulfils the legal requirements.

Table 4.2-1: Validation data for analytical method QuEChERS (L0248/01) for the determination of BAS 505 F residues in plant matrices

Test substance	Matrix	Forti-fication level (mg/kg)	No. of tests	Mean recovery (%)	RSD (%)	Overall Recovery (%)	RSD (%)	Mean recovery (%)	RSD (%)	Overall Recovery (%)	RSD (%)
Transition				327 → 205				327 → 116			
Dimoxystrobin BAS 505 F	Wheat grain	0.01	5	102	2	102	3	102	2	102	3
		0.1	5	101	4			101	3		
	Rape seed	0.01	5	104	5	102	4	103	4	101	3
		0.1	5	100	2			99	2		
	Tomato fruit	0.01	5	103	2	100	3	104	2	101	4
		0.1	5	98	3			99	3		
	Orange fruit	0.01	5	110	3	106	5	110	3	106	5
		0.1	5	102	2			101	2		
	Dried bean seed	0.01	5	102	4	102	3	103	4	103	3
		0.1	5	103	2			103	2		

Linearity
Good linearity was observed for the standard range and the two mass transitions tested for dimoxystrobin: The method-detector response was linear over the 0.05 – 2.5 ng mL^{-1} range ($r \geq 0.9665$) using solvent-based standards (diluted in acetonitrile/water (50/50, v/v)).

Specificity
Significant interferences (> 30% of LOQ) were not observed at the retention times and two mass transitions considered. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.

Matrix Effects
Matrix matched and solvent based standards were analyzed within this study to assess potential matrix effects. By comparing the slopes of the calibration curves resulting from solvent based and matrix matched standards, the matrix effects in the method were shown to be < 20 %. The use of matrix matched standards is therefore not needed.

Limit of Quantitation
The method has a limit of quantification (LOQ) of 0.01 mg kg^{-1}, resulting from the lowest fortification level successfully tested.

Limit of Detection
The method has a limit of detection (LOD) of 0.002 mg kg^{-1}, estimated as 20% of the limit of quantification.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were 5% or below.

Standard Stability
The storage stability of standard and fortification solutions was investigated after 0, 7, 20 and 28 days (standard solutions) and 0, 13 and 21 days (fortification solutions) of storage at approximately 4 °C in the dark. The mean recoveries (measured concentration at day 0 set to 100 %) were in an acceptable range between 102 and 121 % (standard solutions) and 112 and 123 % (fortification solutions). This demonstrates that BAS 505 F is stable in standard and fortification solutions for at least 28 or 21 days, when stored refrigerated at 4 °C.

Extract Stability
The stability of the analyte in plant extracts and final volumes was investigated after 1 and 7 days of storage at 4 °C. The mean corrected recoveries (day 0 set to 100 %) found during the experiments ranged between 96.0 % and 108 % (extracts) and 97.4 % and 108 % (final volumes). This demonstrates that dimoxystrobin is stable in extracts and final volumes for a time period of up to 7 days.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Extractability
The extractability of dimoxystrobin was investigated in the BASF Study DocID 2015/1020186. Selected samples (seeds, rest of plant) from a rape metabolism study were extracted using the extraction solvent used for QuEChERS (Acetonitrile). Recoveries of 70% and 31% were obtained for rest of plant and rape seed samples respectively.

Conclusion
The analytical BASF method L0248/01 is suitable for determining residues of dimoxystrobin (BAS 505 F, Reg. No. 285028) in wheat grain, rape seed, tomato fruit, orange fruit and dried bean seed down to a limit of quantification of 0.01 mg kg^{-1}.

It could be demonstrated that the method fulfils the requirements with regard to recoveries, linearity, specificity, limit of quantification and repeatability and is therefore applicable to correctly determine residues of dimoxystrobin in plant matrices.

An independent laboratory validation (ILV) of the QuEChERS method for BAS 505 F is not necessary. Enough validation data is available at the EURL DataPool (EU Reference Laboratories for Residues of Pesticides). Validation data for QuEChERS is provided in more than one laboratory with the required LOQ and acceptable recovery and RSD data. Therefore no additional validation by an independent laboratory is required in the different OECD crop groups (high water, high oil, high acid content). This was confirmed in the recently EFSA reason opinion on MRLs for dimoxystrobin (Review of established MRLs according to Reg. 396/2005 (Art. 12); (EFSA Journal 2013; 11 (11): 3464).

In addition to the studies listed in the application for renewal of approval, two further methods are submitted for completeness: a multi-pesticide method DFG S19 for plant matrices (CA 4.2/2) and its corresponding independent laboratory validation (CA 4.2/3). This multi residue method showed better recoveries in the extractability study, which was carried out with radiolabelled samples DocID 2015/1020186.

Report:	CA 4.2/2 Weeren R.D.,Pelz S., 2000a Validation of DFG method S 19 (extended revision) for the determination of BAS 505 F in plant materials (tomato, orange, rapeseed) 2000/1000249
Guidelines:	SANCO/825/00 rev. 6 (20 June 2000)
GLP:	yes (certified by Behoerde fuer Arbeit, Gesundheit und Soziales, Freie und Hansestadt Hamburg, Hamburg)

Principle of the method

Tomato and orange sample material was extracted with acetone/water and further purified by liquid/liquid partition, gel permeation chromatography and silica gel mini column chromatography. Rape seed was extracted with acetone and acetonitrile. The further purification was carried out as described for tomatoes and oranges. The quantitation is based on GC using a fused silica capillary column (DP-5 MS) and an electron capture detector (ECD).

Recovery findings

The average percent recoveries (n= 10) were 87%, 80% and 82% respectively in tomato, orange and rapeseed using capillary GC with ECD and 79%, 73% and 78% respectively, using capillary GC followed by MSD.

Table 4.2-2: Validation data DFG method S 19 (extended revision) for the determination of BAS 505 F in plant materials (tomato, orange, rapeseed) using GC-ECD

Crop, Commodity	Test Substance	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Rel. Standard Deviation (%)
Tomato	BAS 505 F	0.01 0.1	5 5	87 86	4.0 1.9
Orange		0.01 0.1	5 5	75 85	6.4 5.2
Rape: seed		0.02 0.2	5 5	78 86	7.1 3.3

Linearity

The linearity of the ECD detector was good in the range of 0.00200 µg/mL to 0.400 µg/mL BAS 505 F.

Specificity

No significant interferences from the sample matrix were detected at the retention time corresponding to BAS 505 F in control samples.
As confirmatory technique and as alternative technique, capillary gas chromatography with mass selective detection (MSD) was successfully used.

Limit of Quantitation

The limit of quantitation was 0.01 mg/kg in tomato and orange, 0.02 mg/kg in rapeseed. The limit of detection (LOD) was 0.02 mg/kg for tomato and orange and 0.04 mg/kg for rapeseed. It is worth to mention that for rapeseed an LOQ of 0.01 mg/kg and LOD of 0.002 mg/kg is obtainable.

Repeatability

The coefficients of variation with respect to recoveries following fortification at quantitation were in the range 4.0% to 7.1% (GC-ECD detector) in the tested matrices. The values obtained are indicative of a method having satisfactory repeatability.

Reproducibility

For each matrix, one control and one sample fortified at the LOQ and ten times that level were analysed by capillary GC with mass selective detection (MSD) for confirmation analysis. The results prove unequivocally the peak identity.

Extractability

The extractability of dimoxystrobin was investigated in the BASF Study DocID 2015/1020186. Selected samples (seeds, rest of plant) from a rape metabolism study were extracted using the extraction solvent used for DFG S19 (Acetone). Recoveries of 73% and 64% were obtained for rest of plant and rape seed samples.

Conclusion

The data presented demonstrate that using suitable extraction, cleanup and GLC conditions, the DFG Method S 19 (extended revision) permits the determination of residues of BAS 505 F in tomato, orange and rapeseed.

Report: CA 4.2/3
Reichert N., 2001a
Independent laboratory validation of a method of analysis for the determination of BAS 505 F in wheat (grain), rape (seed) and lettuce
2000/1014887
Guidelines: EEC 91/414, EEC 96/46, SANCO/825/00 rev. 6 (20 June 2000)
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Jugend, Familie und Gesundheit, Wiesbaden)

Principle of the method
Wheat and lettuce samples were extracted with a mixture of acetone/water whereas for rape seed, acetone/acetononitrile was used. The extracts were further purified as described above (see Weeren R.D., Pelz S. (1998, 2000). BAS 505 F was quantified by GC-MSD.

Recovery findings
The overall average percent recoveries (n= 10) were 83%, 92% and 76% respectively in wheat (grain), rape (seed) and lettuce (see Table 4.2-3).

Table 4.2-3: Validation data for analytical methods for the determination of BAS 505 F residues in food of plant origin

Crop, Commodity	Test Substance	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)	Rel. Standard Deviation (%)
Wheat: grain	BAS 505 F	0.01	5	83.0	3.0
		0.1	5	83.0	12.0
Rape: seed		0.02	5	97.0	8.0
		0.2	5	87.0	5.0
Lettuce		0.01	5	76.0	14.0
		0.1	5	75.0	7.0

Linearity
Linearity was good in the range of 0.2 ng/mL to 1.4 ng/mL BAS 505 F.

Specificity
Detection and quantitation were performed using GC-MSD. BAS 505 F was identified using the fragment ion m/e 116, 205 and 295. The fragment ion m/e 205 was used for quantitation. Therefore the method indicated high specificity and no additional confirmatory technique was necessary.

Limit of Quantitation
The limit of quantitation is 0.02 mg/kg for rape (seed) and 0.01 mg/kg in wheat (grain) and lettuce.

Repeatability

The coefficients of variation with respect to recoveries following fortification at quantitation were in the range 3.0% to 14% in tested matrix. The values obtained are indicative of a method having satisfactory repeatability.

Reproducibility

Due to the high specificity of the methods, reproducibility test is not necessary.

Conclusion

The method described above is adequate to determine residues of BAS 505 F in wheat (grain), rape (seed) and lettuce.

Animal

Report: CA 4.2/4
Richter S., 2015a
Dimoxystrobin BAS 505 F and its metabolite 505M09: Validation of the multi-residue method DFG S19 for the determination of residues in milk, egg, meat, fat, liver and kidney using LC/MS/MS - BASF method number L0232/01
2014/1315330

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, OECD-ENV/JM/MONO/(2007)17, EEC 91/414 Annex II (Part A Section 4), EEC 91/414 Annex III (Part A Section 5)

GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Principle of the methods

Multi-Residue Method DFG S19:

Appropriate DFG S19 extraction modules E1 (milk, egg, meat, liver and kidney) and E6 (fat), clean-up modules (gel permeation chromatography: module GPC) and module LC/MS/MS (with positive ionisation: ESI) were used for quantification and confirmation. No column chromatographic fractionation (module C1) on silica gel was necessary due to the highly selective LC/MS/MS method employed.

For the metabolite 505M09 in egg low recovery results for all applicable extraction modules of the DFG S19 method were obtained. Thus extraction module E1 was slightly modified by addition of acidified water instead of water prior the extraction. The method uses the following two mass transitions for each analyte:

Dimoxystrobin: 327 m/z → 205 m/z for quantification and327 m/z → 116 m/z for quantitative confirmation.

Dimoxystrobin metabolite 505M09: 357 m/z → 205 m/z for quantification and 357 m/z → 116 m/z for quantitative confirmation.

Recovery findings

The method proved to be suitable for measuring residues of dimoxystrobin (BAS 505 F) and its metabolite 505M09 in foodstuffs of animal origin (exemplified by milk, egg, meat, fat, liver and kidney), with a target limit of quantification (LOQ) of 0.010 mg/kg per analyte, using LC/MS/MS for quantification and confirmation.

Residues in all blank control specimens were below 20% of the LOQ (< 0.002 mg/kg). The average recoveries for the two parent-daughter ion transitions monitored were within the acceptable range of 70% to 110% with relative standard deviations (RSD) ≤ 20% except for fat with an average recovery of 69.5% for the conformation transition. A summary of results is given in Table 4.2-4.

Stability of the analytes in stock, fortification and calibration solutions was demonstrated for up to 6 weeks when stored refrigerated. The stability of the final extracts was demonstrated for at least 5 days by acceptable recoveries within 70 to 120% when stored refrigerated. Stability of the metabolite 505M09 in fat extracts was demonstrated by low deviation of recovery results between first injection and re-injection after refrigerated storage.

Table 4.2-4: Recovery results of BAS 505 F (Dimoxystrobin) and its metabolite 505M09

Fortification Level (mg/kg)	Analyte	BAS 505 F (Dimoxystrobin)											
	Daughter Ion	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z
	Matrix	Milk		Egg		Meat		Liver		Kidney		Fat	
0.010, 0.10	Average	102%	102%	109%	109%	78.9%	78.5%	99.8%	99.8%	97.4%	97.5%	105%	104%
	SD	3.4%	3.3%	3.0%	2.3%	6.3%	6.7%	5.1%	6.0%	5.2%	5.7%	2.2%	4.0%
	RSD	3.3%	3.2%	2.8%	2.1%	8.0%	8.6%	5.1%	6.1%	5.3%	5.8%	2.1%	3.8%
	n	10	10	10	10	10	10	10	10	10	10	10	10
Fortification Level (mg/kg)	Analyte	Metabolite 505M09											
	Daughter Ion	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z
	Matrix	Milk		Egg		Meat		Liver		Kidney		Fat	
0.010, 0.10	Average	91.5%	93.2%	101%	103%	79.9%	81.3%	92.1%	93.4%	89.3%	89.3%	84.6%	83.7%
	SD	3.4%	2.9%	5.9%	5.8%	6.9%	7.5%	4.9%	4.8%	5.0%	4.6%	15%	17%
	RSD	3.7%	3.1%	5.8%	5.6%	8.6%	9.2%	5.4%	5.1%	5.6%	5.2%	18%	20%
	n	10	10	10	10	10	10	10	10	10	10	10	10

Linearity

Good linearity was observed in the range of 0.015 to 1.2 ng/mL (for fat) or 0.15 ng/mL to 12 ng/mL (for milk, egg, bovine meat, liver and kidney).

Specificity

The method determines residues of dimoxystrobin and its metabolite 505M09 in animal matrices. Matrix effects were not significant (i.e. <20%) for milk, meat, egg and fat. Quantitative determination of final meat extracts was carried out by external standardization using calibration solutions in solvent. For milk, egg and fat matrix effects were insignificant too, but evaluation was done using matrix-matched standards. For kidney and liver matrix effects were significant and thus quantitative determination was carried out by external standardization using matrix-matched standards.

Limit of Quantification

The limit of quantification (LOQ) of the method is 0.010 mg/kg per analyte.

Repeatability

The relative standard deviations (RSD, %) for all commodities and all fortification levels were well below 20%, except for fat. Values are shown in the table above.

Reproducibility

This study showed that the method developed in one laboratory could be successfully used in another facility.

Extractability

The extractability of dimoxystrobin and its metabolite 505M09 was investigated in the BASF Study DocID 2015/1125782. Selected samples (goat liver and goat kidney) from a goat metabolism study were extracted using the extraction solvent used for method L0232/01 (DFG, S19, Acetone). Recoveries between 30% and 84% were obtained for dimoxystrobin. Recoveries of 100% were obtained for 505M09.

Conclusion

The LC/MS/MS based DFG method S19 was successfully validated for the determination of dimoxystrobin (BAS 505 F) and its metabolite 505M09 in foodstuffs of animal origin (exemplified with milk, egg, meat, fat, liver and kidney) with an LOQ of 0.010 mg/kg per analyte and thus demonstrated to be applicable for enforcement and monitoring purposes.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries.

Report: CA 4.2/5
Benotti M.J., 2015a
Independent laboratory validation of BASF method L0232/01 for the determination of BAS 505 F and metabolite 505M09 in animal matrices
2015/1020188

Guidelines: EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4, OECD-ENV/JM/MONO/(2007)17, EPA PR Notice 96-1

GLP: yes
(certified by United States Environmental Protection Agency)

Principle of the method

The aim of this study was to validate the analytical method L0232/01 for the determination of dimoxystrobin (BAS 505 F) and its metabolite 505M09 in animal matrices. The matrices investigated include milk, liver, kidney, eggs, fat and meat.

2.5 g or 20 g of the homogenized matrix samples are extracted with acetone, sodium chloride and ethyl acetate/cyclohexane (1/1, v/v). An aliquot of the organic upper phase is reduced to dryness. The residue is reconstituted in ethyl acetate and cyclohexane. An aliquot of the reconstituted extract is taken to dryness and reconstituted in methanol/water (1/1, v/v) or isopropanol. An aliquot of this second reconstituted extract is diluted with methanol/water (1/1, v/v) and analyzed by LC-MS/MS monitoring two mass transitions for both analytes.

The limit of quantification (LOQ) of the method is 0.01 mg kg^{-1} and the limit of detection (LOD) is 0.003 mg kg^{-1}.

Recovery findings

The method proved to be to be suitable to determine residues of dimoxystrobin and its metabolite 505M09 in animal matrices, including milk, liver, kidney, eggs, fat and meat.

All six animal matrices were fortified with the two analytes at the LOQ of 0.01 mg kg^{-1} and 10 times higher (0.1 mg kg^{-1}). Mean recovery values (mean of 5 or 6 replicates per fortification level and analyte) were between 70% and 103% for both the quantitation and confirmation transitions in each matrix. (see tables below).

Table 4.2-5: Recovery results of dimoxystrobin in animal matrices

Fortification Level (mg kg^{-1})	Analyte	BAS 505 F (dimoxystrobin)											
	Daughter Ion	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z
	Matrix	Kidney		Liver		Eggs		Beef		Milk		Fat	
0.010	Average	71%	70%	92%	91%	77%	76%	71%	70%	71%	70%	72%	70%
	RSD	3%	4%	16%	15%	12%	12%	3%	3%	4%	4%	4%	5%
	n	6	6	5	5	6	6	5	5	5	5	5	5
0.1	Average	80%	80%	103%	102%	92%	93%	73%	72%	72%	72%	79%	80%
	RSD	4%	4%	14%	14%	18%	18%	2%	2%	6%	6%	3%	2%
	n	6	6	6	6	6	6	6	6	5	5	6	6
Overall	Average	75%	75%	98%	97%	84%	84%	72%	71%	71%	71%	76%	75%
	RSD	7%	8%	15%	15%	18%	18%	3%	3%	4%	5%	6%	7%
	n	12	12	11	11	12	12	11	11	10	10	11	11

Table 4.2-6: Recovery results of 505M09 in animal matrices

Fortification Level (mg kg^{-1})	Analyte	Metabolite 505M09											
	Daughter Ion	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z	205 m/z	116 m/z
	Matrix	Kidney		Liver		Eggs		Beef		Milk		Fat	
0.010	Average	71%	70%	96%	87%	75%	73%	73%	70%	72%	71%	72%	70%
	RSD	3%	3%	6%	5%	8%	8%	2%	3%	2%	2%	9%	7%
	n	6	6	5	5	6	6	5	5	5	5	5	5
0.1	Average	74%	75%	102%	98%	84%	84%	73%	73%	80%	79%	80%	80%
	RSD	5%	4%	6%	8%	5%	5%	2%	2%	3%	3%	4%	3%
	n	6	6	6	6	6	6	6	6	5	5	6	6
Overall	Average	72%	72%	99%	93%	80%	79%	73%	72%	76%	75%	77%	76%
	RSD	5%	6%	7%	9%	8%	9%	2%	3%	6%	6%	8%	8%
	n	12	12	11	11	12	12	11	11	10	10	11	11

Linearity

Six matrix-matched calibration solutions ranging from 0.150 to 12 ng mL^{-1} (for the milk, egg, meat, liver and kidney samples) and from 0.015 to 1.2 ng mL^{-1} (for the fat samples) were used to evaluate the final extracts. Good linearity (regression coefficients ≥ 0.995) was observed for the two mass transitions monitored in all matrix samples.

Specificity

Apparent residues or interferences were not measured or were below 30% of the LOQ in unfortified control samples across all six matrices for both the quantitation and confirmation transitions of dimoxystrobin and 505M09. Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary.

Matrix Effects
Matrix effects were investigated by comparing similar concentrations of each test material in solvent to that in matrix. Results demonstrate that matrix were generally not significant (i.e. generally less than positive 20% but greater than negative 20%) for both analytes and both transitions. However, many of the lowest calibration level standards were more susceptible to matrix suppression. Additionally, some of the mid- and high-concentration calibration standards were also susceptible to suppression, particularly for the egg and beef matrices. Therefore, it is beneficial to use matrix-matched calibrations for the determination of dimoxystrobin and 505M09.

Limit of Quantification
The limit of quantification (LOQ) was defined by the lowest fortification level successfully tested. The LOQ for this method is 0.01 mg kg^{-1} corresponding to a concentration of 0.936 ng mL^{-1} in the milk, egg, meat, liver and kidney sample extracts and 0.100 ng mL^{-1} in the fat sample extracts.

Limit of Detection
The method has a limit of detection (LOD) of 0.003 mg kg^{-1} for all matrices, estimated as 30% of the LOQ. This corresponds to a concentration of 0.28 ng mL^{-1} in the milk, egg, meat, liver and kidney sample extracts and 0.03 ng mL^{-1} in the fat sample extracts.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were at or below 20%.

Standard Stability
Not analyzed in this ILV. In the validation study CA 4.2/1 (DocID 2015/1020187) was showed that BAS 505 F is stable in standard (acetonitrile/water 50/50 v/v) and fortification solutions (acetonitrile) for at least 28 or 21 days, when stored refrigerated at 4 °C.

Extract Stability
The good recovery values show that the extracts were stable during the course of the study.

Reproducibility

The independent laboratory validation was successfully completed for the analysis of dimoxystrobin and its metabolite 505M09 in all animal matrices tested.

Conclusion

The analytical method L0232/01 for analysis of dimoxystrobin (BAS 505 F) and its metabolite 505M09 in animal matrices uses LC-MS/MS for final determination, with a limit of quantification of 0.01 mg kg^{-1}.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of dimoxystrobin and 505M09 in animal matrices, including milk, liver, kidney, eggs, fat and meat.

(b) Methods for the analysis in soil and water

Soil

Report:	CA 4.2/6 Penning H. et al., 2013a Validation of analytical method L0189/01 for the determination of BAS 505 F, 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No. 354562) and 505M09 (Reg.No. 354563) in soil and sediment by LC-MS/MS 2012/1287158
Guidelines:	SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the methods

Method Number L0189/01:

The BASF Method allows the determination of BAS 505 F (Dimoxystrobin) and its metabolites 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No 354562) and 505M09 (Reg.No. 354563) in soil and sediment. Soil/sediment samples (5 g) are extracted with 50 ml methanol/water (80/20, v/v) by mechanical shaking for 30 min at 225 rpm. A 5 ml aliquot of the extract is centrifuged for 5 min at 4000 rpm (20°C). The extract is taken directly or diluted with methanol/water (80/20, v/v) to the appropriate final volume. The concentration of each analyte in the extract is measured by HPLC-MS/MS. The limit of quantification (LOQ) of the method is 0.002 mg/kg for each analyte.

Recovery findings

Method L0189/01 was proved to be suitable to determine residues of BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in soil and sediment to a limit of quantification (LOQ) of 0.002 mg/kg. The mean recovery values of the validation experiments were between 70% and 110%. Detailed results of recoveries for each mass transition and matrix are given in the Table 4.2-7.

Results of stability investigations of calibration and fortification solutions as well as stability investigations of the extracts showed that the fortification and calibration solutions of each analyte were stable (less than 10% decline) for at least 4 weeks refrigerate and that BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) are stable in the extracts of the two soils and the sediment over the tested time period of 7 days.

Table 4.2-7: Recoveries for BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in both soils and sediment

Matrix	Test Item	Mass transition	No. of replicates	Fortification level [mg/kg]	Mean Recovery [%]	RSD [%]
LUFA Soil 2.2	BAS 505 F	327 → 205	5	0.002	102.5	1.5
			5	0.02	101.0	3.1
		327 → 116	5	0.002	103.4	1.7
			5	0.02	101.5	2.3
	505M98	327 → 205	5	0.002	113.9	0.7
			5	0.02	112.4	3.0
		327 → 116	5	0.002	115.2	1.2
			5	0.02	111.5	2.6
	505M01	223 → 176	5	0.002	96.8	0.9
			5	0.02	96.1	1.3
		223 → 116	5	0.002	98.4	3.7
			5	0.02	95.6	2.8
	505M08	357 → 205	5	0.002	101.9	2.1
			5	0.02	99.3	1.6
		357 → 116	5	0.002	102.5	2.4
			5	0.02	100.4	1.2
	505M09	357 → 205	5	0.002	99.0	2.6
			5	0.02	97.8	1.8
		357 →116	5	0.002	95.0	1.9
			5	0.02	94.6	1.6
LUFA Soil 5M	BAS 505 F	327 → 205	5	0.002	98.3	6.0
			5	0.02	96.2	2.5
		327 → 116	5	0.002	98.5	6.9
			5	0.02	96.0	3.3
	505M98	327 → 205	5	0.002	108.0	5.7
			5	0.02	105.8	2.8
		327 → 116	5	0.002	111.3	6.5
			5	0.02	105.4	4.1
	505M01	223 → 176	5	0.002	92.7	4.3
			5	0.02	89.1	4.0
		223 → 116	5	0.002	93.9	5.9
			5	0.02	86.7	6.7
	505M08	357 → 205	5	0.002	97.0	3.8
			5	0.02	93.9	1.5
		357 → 116	5	0.002	97.4	3.3
			5	0.02	94.3	1.7
	505M09	357 → 205	5	0.002	95.3	5.5
			5	0.02	92.8	1.1
		357 →116	5	0.002	96.5	3.6
			5	0.02	92.6	1.5

Table 4.2-7: Recoveries for BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in both soils and sediment

Matrix	Test Item	Mass transition	No. of replicates	Fortification level [mg/kg]	Mean Recovery [%]	RSD [%]
Berghäuser Altrhein Sediment	BAS 505 F	327 → 205	5	0.002	97.5	1.0
			5	0.02	97.6	2.8
		327 → 116	5	0.002	97.8	1.5
			5	0.02	98.1	2.8
	505M98	327 → 205	5	0.002	108.1	2.3
			5	0.02	107.9	2.5
		327 → 116	5	0.002	107.5	2.9
			5	0.02	107.7	5.1
	505M01	223 → 176	5	0.002	92.6	1.2
			5	0.02	92.2	1.1
		223 → 116	5	0.002	89.0	1.4
			5	0.02	89.7	2.3
	505M08	357 → 205	5	0.002	97.6	0.8
			5	0.02	97.7	3.2
		357 → 116	5	0.002	98.1	1.6
			5	0.02	97.8	2.8
	505M09	357 → 205	5	0.002	94.4	1.1
			5	0.02	95.3	2.9
		357 →116	5	0.002	94.2	1.4
			5	0.02	95.8	4.2

Linearity

Good linearity (regression coefficients ≥ 0.99) was observed in the range of 0.04 ng/mL to 2.0 ng/mL for the two mass transitions of BAS 505 F, 505M98, 505M01, 505M08 and 505M09.

Specificity

The method L0189/01 determines residues of BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in soil and sediment. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of each analyte.

Limit of Quantification

The limit of quantification defined by the lowest fortification level successfully tested was 0.002 mg/kg for all analytes (corresponding to 0.2 ng/mL in extract). The limit of detection was an estimated 20% hereof, equivalent to 0.0004 mg/kg for all analytes.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in the table above.

Reproducibility

Reproducibility of the method was not determined within this validation study.

Extractability

The extractability of dimoxystrobin and its metabolites M505M01, 505M98, 505M08, 505M09 in soil was investigated in the BASF Study DocID 2015/1105484. Selected samples from a terrestrial field dissipation study were extracted using the extraction solvent used for method L0189/01. Recoveries between 93% and 100% were obtained for dimoxystrobin and its metabolites.

Conclusion

The method for analysis of dimoxystrobin and its metabolites 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No 354562) and 505M09 (Reg.No. 354563) uses HPLC-MS/MS for quantitative determinations. The limit of quantification was 0.002 mg/kg for each analyte.

It could be demonstrated that method L0189/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of the fungicide BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in both soil and sediment.

Report: CA 4.2/7
Obermann M. et al., 2015a
Validation of analytical method L0191/01 for the determination of BAS 505 F (Reg.No. 285028), 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No. 354562) and 505M09 (Reg.No. 354563) in surface water and groundwater by LC-MS/MS
2014/1161841

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000), EPA 850.7100

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

Principle of the methods

Method Number L0191/01:

The BASF Method allows the determination of BAS 505 F - Dimoxystrobin (Reg.No. 285028) and its metabolites 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No. 354562) and 505M09 (Reg.No. 354563) in ground- and surface water by HPLC-MS/MS. An aliquot of 10 g of the water sample was extracted by SPE. The analytes were eluted with methanol. After evaporation to dryness the residues were dissolved in methanol/water (80/20, v/v). An aliquot of the final volume was measured using HPLC-MS/MS. Due to the high specificity of HPLC-MS/MS and the different transitions proposed, no confirmatory technique is necessary. In this study, the transitions 327 → 205 and 327 → 116 were used for all analytes and matrices tested.

Recovery findings

Method L0191/01 was proved to be suitable to determine residues of BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in ground- and surface water to a limit of quantification (LOQ) of 0.025 mg/kg. The mean recovery values of the validation experiments were between 70% and 110%, which fulfils the legal requirements for mean recovery values. Detailed results of recoveries for each mass transition and matrix are given in Table 4.2-8.

Results of stability investigations of the extracts showed that BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) are stable in the extracts of ground- and surface water over the tested time period of 7 days.

Table 4.2-8: Recoveries for BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in both ground- and surface water

Matrix	Test Item	Mass transition	No. of replicates	Fortification level [µg/kg]	Mean Recovery [%]	RSD [%]
Ground Water	BAS 505 F	327 → 205	5 5	0.025 0.25	91 87	2.9 1.4
		327 → 116	5 5	0.025 0.25	88 85	3.4 1.7
	505M98	327 → 205	5 5	0.025 0.25	89 85	2.4 1.8
		327 → 116	5 5	0.025 0.25	87 85	3.5 2.3
	505M01	223 → 176	5 5	0.025 0.25	93 91	2.4 2.8
		223 → 116	5 5	0.025 0.25	92 95	3.3 4.0
	505M08	357 → 205	5 5	0.025 0.25	98 99	2.7 2.9
		357 → 116	5 5	0.025 0.25	99 99	5.6 5.0
	505M09	357 → 205	5 5	0.025 0.25	98 100	5.5 4.0
		357 →116	5 5	0.025 0.25	97 96	8.1 1.1
Surface Water	BAS 505 F	327 → 205	5 5	0.025 0.25	92 90	0.5 1.3
		327 → 116	5 5	0.025 0.25	92 90	0.8 1.5
	505M98	327 → 205	5 5	0.025 0.25	92 89	1.0 1.0
		327 → 116	5 5	0.025 0.25	90 88	1.8 3.2
	505M01	223 → 176	5 5	0.025 0.25	99 97	0.7 1.5
		223 → 116	5 5	0.025 0.25	101 99	2.6 4.2
	505M08	357 → 205	5 5	0.025 0.25	103 99	1.7 4.6
		357 → 116	5 5	0.025 0.25	106 105	2.4 3.7
	505M09	357 → 205	5 5	0.025 0.25	102 101	0.8 2.0
		357 →116	5 5	0.025 0.25	103 101	3.9 5.4

Linearity

Good linearity (regression coefficients ≥ 0.998) was observed in the range of 0.025 ng/mL to 2.0 ng/mL for the two mass transitions of BAS 505 F and for the standard solutions of the metabolites 505M98, 505M01, 505M08 and 505M09.

Specificity

The method L0191/01 determines residues of BAS 505 F and its metabolites (505M98, 505M01, 505M08 and 505M09) in ground- and surface water. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of each analyte.

Limit of Quantification

The limit of quantification defined by the lowest fortification level successfully tested was 0.025 µg/kg for all analytes, an estimated 20% of the limit of detection (equivalent to 0.005 µg/kg).

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in the table above.

Reproducibility

Reproducibility of the method was not determined within this validation study.

Conclusion

It could be demonstrated that the analytical method L0191/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 505 F (Reg.No. 285028) and its metabolites 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No. 354562) and 505M09 (Reg.No. 354563) in both ground- and surface water.

Report: CA 4.2/8
Pissot N., 2015a
Independent laboratory validation of BASF Analytical Method L0191/01: BAS 505 F (Reg.No. 285028), 505M98 (Reg.No. 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No. 354562), 505M09 (Reg.No. 354563) in surface- and groundwater by LC/MS/MS
2015/1076561

Guidelines: SANCO/825/00 rev. 8.1 (16 November 2010), EPA 850.6100, EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method, EPA 850.7100

GLP: yes
(certified by Groupe Interministeriel des Produits Chimiques, France)

In the application a different DocID was assigned to this study (2015/1020130).

Principle of the methods

Method Number L0191/01:

The aim of this study was to validate BASF analytical method L0191/01 for the determination of BAS 505 F (dimoxystrobin) and its metabolites 505M98 (Reg.No 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No. 354562) and 505M09 (Reg.No. 354563) in surface water and groundwater matrices.

An aliquot of 10 g of the water sample is extracted by SPE. The analytes are eluted with methanol. After evaporation to dryness the residues are dissolved in methanol/water (80/20, v/v) and measured using HPLC-MS/MS monitoring two mass transitions for each analyte.

The limit of quantification of the method (LOQ) is 0.025 µg kg^{-1} and the limit of detection (LOD) is 0.005 µg kg^{-1}.

Recovery findings

It was proven that the analytical method L0191/01 is suitable to determine residues of dimoxystrobin and its metabolites 505M98, 505M01, 505M08 and 505M09 in ground- and surface water. Water samples were fortified with the analytes at the limit of quantification of 0.025 µg kg^{-1} and 10 times higher (0.25 µg kg^{-1}).

As shown in the table below, the mean recovery values (mean of five replicates per fortification level and analyte) were between 75 and 106% of the nominal values, which fulfils the legal requirements.

Table 4.2-9: Results of the method validation for the determination of dimoxystrobin and its metabolites in groundwater and surface water

	Matrix	Mass Transition	Fortification Level [µg kg^{-1}]	Number of Replicates	Mean Recovery [%]	RSD [%]	Overall Recovery [%]	RSD [%]
505 F	Ground Water	327->205	LOQ	5	98	0.9	99	2.1
			10xLOQ	5	100	2.7		
		327->116	LOQ	5	97	2.2	98	2.8
			10xLOQ	5	100	3.0		
	Surface Water	327->205	LOQ	5	94	2.7	93	3.1
			10xLOQ	5	92	3.1		
		327->116	LOQ	5	95	3.6	94	2.8
			10xLOQ	5	93	1.1		
505M98	Ground Water	327->205	LOQ	5	95	1.5	89	7.5
			10xLOQ	5	83	4.6		
		327->116	LOQ	5	95	2.6	89	8.7
			10xLOQ	5	82	4.7		
	Surface Water	327->205	LOQ	5	86	3.2	81	7.3
			10xLOQ	5	76	3.1		
		327->116	LOQ	5	87	2.2	81	8.3
			10xLOQ	5	75	4.4		
505M01	Ground Water	223->176	LOQ	5	102	2.5	100	6.4
			10xLOQ	5	98	8.7		
		223->116	LOQ	5	104	5.5	102	5.9
			10xLOQ	5	101	6.6		
	Surface Water	223->176	LOQ	5	94	4.8	90	6.5
			10xLOQ	5	86	4.0		
		223->116	LOQ	5	100	8.3	93	9.9
			10xLOQ	5	87	5.6		
505M08	Ground Water	357->205	LOQ	5	106	0.7	105	2.3
			10xLOQ	5	104	2.9		
		357->116	LOQ	5	105	2.0	104	2.8
			10xLOQ	5	103	3.3		
	Surface Water	357->205	LOQ	5	93	3.6	90	4.4
			10xLOQ	5	88	2.4		
		357->116	LOQ	5	91	7.1	89	5.9
			10xLOQ	5	88	4.4		
505M09	Ground Water	357->205	LOQ	5	103	6.1	104	4.1
			10xLOQ	5	104	0.9		
		357->116	LOQ	5	100	1.6	102	2.4
			10xLOQ	5	103	2.5		
	Surface Water	357->205	LOQ	5	100	5.5	101	4.0
			10xLOQ	5	102	1.7		
		357->116	LOQ	5	100	3.1	100	2.5
			10xLOQ	5	100	2.0		

Linearity

Good linearity (r > 0.997) was observed in the range of 0.025 ng mL^{-1} to 2 ng mL^{-1} for the two mass transitions of dimoxystrobin and its metabolites 505M98, 505M01, 505M08 and 505M09.

Specificity

Under the described conditions the method is specific for the determination of dimoxystrobin and its metabolites 505M98, 505M01, 505M08 and 505M09 in ground- and surface water. Significant interferences (> 30% of LOQ) were not observed at the retention times and mass transitions of the analytes. Due to the high selectivity and specificity of HPLC-MS/MS an additional confirmatory technique is not necessary.

Matrix Effects

Solvent as well as matrix-matched standards were analysed and significant matrix effects (difference above 20%) were found. Therefore, calibration curves based on matrix-matched standards in ground- and surface water were used for the validation data.

Limit of Quantification

The method has a limit of quantification (LOQ) of 0.025 µg kg^{-1}, resulting from the lowest fortification level successfully tested.

Limit of Detection

The method has a limit of determination (LOD) of 0.005 µg kg^{-1}, estimated as 20% of the limit of quantification.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 10%.

Reproducibility

The independent laboratory validation was successfully completed for the analysis of dimoxystrobin and its metabolites in water matrices.

Conclusion

The method L0191/01 for the analysis of dimoxystrobin (BAS 505 F) and its metabolites 505M98 (Reg.No 360056), 505M01 (Reg.No. 358104), 505M08 (Reg.No. 354562) and 505M09 (Reg.No. 354563) in ground- and surface water uses HPLC-MS/MS for final determination, with a limit of quantification of 0.025 µg kg^{-1}.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of dimoxystrobin and its metabolites 505M98, 505M01, 505M08 and 505M09 in ground- and surface water.

(c) Methods for the analysis in air

Report: CA 4.2/9
Miller C., 2015b
Validation of BASF method L269/01 for the determination of BAS 505 F in air
2015/1020131

Guidelines: 2004/10/EC of 11 February 2004, SANCO/825/00 rev. 8.1 (16 November 2010), SANCO/3029/99 rev. 4 (11 July 2000)

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Principle of the methods

Method Number L269/01:

BASF method L269/01 was developed for the determination of BAS 505 F (Dimoxystrobin) in air samples. The analyte is spiked to Tenax adsorption tubes. Two spiking levels (1.2 and 12 µg m^{-3}) were tested. After sucking air through the glass tubes at 35°C and a relative humidity of 80% for 6 hours at approximately 1 L min^{-1}, the tube content is extracted by ultrasonication using acetonitrile. Final determination of dimoxystrobin is achieved by LC-MS/MS. The limit of quantification is defined as the lowest fortification level used at which acceptable recovery data was obtained corresponding to a concentration of 1.2 µg dimoxystrobin m^{-3} air.

Recovery findings

Method L269/01 was proved to be suitable to accurately determine residues of BAS 505 F in air. The mean recovery values of the validation experiments were within the acceptable range of 70% to 110% (Table 4.2-10)

Results of stability investigations show that BAS 505 F is stable on Tenax sorbent material when stored at ≤ -20°C (in the dark) for a period of up to 8 days and when stored at approximately -20°C in final extract solution for a period of 7 days.

Table 4.2-10: Recoveries for BAS 505 F in air

Matrix	Test Item	Mass transition	No. of replicates	Fortification level [µg/cartridge]	Mean Recovery [%]	RSD [%]
Air	BAS 505 F	327 → 205	5	3.33	75	6.0
			5	33.3	93	4.5
		327 → 116	5	3.33	84	11.4
			5	33.3	95	5.0

Linearity

Good linearity (regression coefficients = 0.9997) was observed in the range of 0.005 ng/mL to 0.5 ng/mL for the two mass transitions of BAS 505 F.

Specificity

Under the described conditions method L269/01 is specific for the determination of dimoxystrobin in air. Significant interferences (>30% of LOQ) were not observed at the retention time and mass transitions of dimoxystrobin.

Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique was not necessary. Two mass transitions of dimoxystrobin were quantified.

Limit of Quantification

The limit of quantification defined by the lowest fortification level successfully tested was 1.2 µg m^{-3} air. The limit of detection (LOD) of the method was defined as the concentration of the lowest calibration standard chromatographed with that gave rise to a measurable chromatographic response. For this study the LOD of the method was shown to be 0.005 ng mL^{-1} (equivalent to 0.5 µg per cartridge or 0.18 µg m^{-3} in air).

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in the table above.

Reproducibility

Reproducibility of the method was not determined within this validation study.

Conclusion

The method for analysis of dimoxystrobin in air uses LC-MS/MS for quantitative determination. The limit of quantification was 1.2 µg m^{-3} air.

It could be demonstrated that method L269/01 fulfils the requirements with regard to accuracy (recovery), precision (repeatability), linearity (calibration), specificity (interference), limit of quantitation (LOQ) and limit of detection (LOD), retention capacity (breakthrough) and extractability and is therefore applicable to correctly determine residues of the fungicide BAS 505 F in air.

(d) Methods for the analysis in body fluids and tissues

Since dimoxystrobin is not classified as toxic or very toxic, methods of analysis for parent or metabolites in human body tissues or fluids are not required. Methods for concentration control in feed or other matrices are reported, where necessary, along with the respective toxicological studies.

Dimoxystrobin

Document M-CA, Section 5

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

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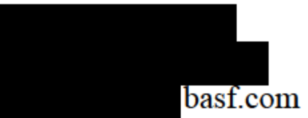

Version history[1]

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

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

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CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

CA 5.1 Studies on Absorption, Distribution, Metabolism and Excretion in Mammals

CA 5.1.1 Absorption, distribution, metabolism and excretion by oral exposure

Studies already peer-reviewed and presented in the original Annex I dossier:

For the determination of the toxicokinetic properties of dimoxystrobin, two studies are available (see table below) with the test substance radiolabelled in the benzyl or phenyl ring system. One study (BASF DocID 1999/12013) investigated absorption, distribution, excretion and metabolism in rats after single oral low dose (15 mg/kg bw) and single oral high dose (150 mg/kg bw) while the other study (BASF DocID 1999/11642) covers dose groups for blood pharmacokinetics, tissue distribution and biliary excretion after single oral low (10 mg/kg bw) or high doses (100 mg/kg bw). Both studies have been part of the previous evaluation and are therefore not submitted again in this dossier. For reasons of convenience, a short summary of the main conclusions and an overview about the integration of rat metabolism data into the TTC concept (see M-CA 6.7 and 6.9) is given below.

Category of test	Dose ranges	Results	Reference (BASF DocID)
Metabolism of ^{14}C-BAS 505 F (^{14}C-285028) in rats	– 10/100/150 mg/kg bw/d (single ^{14}C-exposure) – 10 mg/kg bw ^{14}C-spike after 14 d pre-treatment (unlabelled) – 15 and 150 mg/kg bw/d (single ^{14}C-exposure sacrifice after 8h, plasma, liver, kidney analysis) – 10 and 100 mg/kg bw/d (single ^{14}C-exposure, bile fluid analysis)	BAS 505 F was rapidly and intensively metabolised to a large number of biotransformation products. Combinations of Phase I biotransformation and conjugation reactions led to the large number of observed metabolites. No major differences were observed with regard to sex and dose level.	1999/12013
^{14}C-BAS 505 F - Study of the Biokinetics in rats	10 and 100 mg/kg bw	Radioactivity excretion half-life 11.4 - 18.6 h. Faecal excretion 57 - 82% of applied dose Biliary excretion 34 - 55% of applied dose. Bioavailability was 58 - 71% (high dose level) and 85 - 90% (low dose level).	1999/11642

An overview of all metabolites identified in rat metabolism studies is presented in the table below.

Substance Code	Study	Occurrence (Compartment)	Chemical structure
505M01	rat	urine, feces	
505M02	rat	feces	
505M08	rat	urine, feces	
505M09	rat	urine, feces, bile, plasma, liver, kidney	
505M10	rat	urine	
505M11	rat	urine	
505M12	rat	urine	
505M13	rat	urine	
505M14	rat	urine	
505M15	rat	urine	

Substance Code	Study	Occurrence (Compartment)	Chemical structure
505M17	rat	urine, feces	
505M18	rat	urine, feces	
505M19	rat	urine	
505M20	rat	urine	
505M21	rat	urine	
505M22	rat	urine	
505M23	rat	urine	
505M24	rat	urine	
505M26	rat	urine, feces	
505M28	rat	urine	

Substance Code	Study	Occurrence (Compartment)	Chemical structure
505M29	rat	urine, feces	
505M30	rat	urine, feces	
505M31	rat	urine	
505M32	rat	urine	
505M33	rat	urine, bile	
505M34	rat	urine	
505M37	rat	urine, feces	
505M38	rat	urine	
505M39	rat	urine, feces, bile	
505M40	rat	urine	

Substance Code	Study	Occurrence (Compartment)	Chemical structure
505M41	rat	urine, feces	
505M42	rat	urine, feces, bile	
505M44	rat	urine, feces	
505M46	rat	urine	
505M50	rat	bile	
505M53	rat	urine	
505M55	rat	urine	
505M57	rat	urine	
505M59	rat	urine	
505M60	rat	urine	

Substance Code	Study	Occurrence (Compartment)	Chemical structure
505M63	rat	feces	
505M64	rat	urine	
505M66	rat	urine	
505M67	rat	urine, feces, liver, kidney	
505M81	rat	bile	

Dosing and dose groups

The biokinetics of ^{14}C-BAS 505 F (BASF DocID ID 1999/11642) in male and female Wistar rats (Strain Chbb-THOM) were investigated at dose levels of 10 mg/kg bw and 100 mg/kg bw ("low" and "high" dose, respectively). For the isolation and identification of metabolites (BASF DocID 1999/12013), male and female Wistar rats were orally dosed in a separate study with [^{14}C]-BAS 505 F at a nominal dose level of 10 mg/kg, 100 mg/kg and 150 mg/kg body weight.

Excretion balance
In all dose groups and for both labelled forms of the test substance, the total amount of radioactivity was almost completely excreted, predominantly via the faecal route: 120 hours after administration 57 - 82% of the dose were recovered from faeces and 14 - 39% from urine. The renal excretion was more pronounced in female than in male animals (22 - 39% vs. 14 - 22% of dose after 120 hours). No radioactivity was detectable in the exhaled air. Already within the first 24 hours after dosing, 41 - 62% of the administered radioactivity was found in faeces and 8 - 35% in urine. Radioactivity remaining in tissues and organs 120 hours post dosing was below 0.6% of the dose (< 2µg eq./g). The overall recovery of radioactivity was in the range of 93-100 %. Within 48 hours after administration of 10 and 100 mg/kg bw of both ^{14}C-BAS 505 F, 34 - 55% of the administered radioactivity were excreted with the bile.

The amount of radioactivity excreted via bile and urine essentially reflects the absorbed proportion of the dose. Based on the amount of radioactivity excreted from 0 to 48 hours, the extrapolated total excretion and hence the bioavailability is assumed to be in the range of 85 - 90% at the low dose level and 58 - 71% at the high dose level with indications of saturation with increasing dose.

Pharmacokinetics

After oral administration at both dose levels a first peak plasma level was reached 0.5 hours post dose and second peak occurred after ca. 8 hours at the low dose level and between 8 and 24 hours at the high dose level. The second peak plasma level can be explained with a slow dissolution of initially undissolved (suspended) test material in the gut. The plasma level maxima were 0.77 – 1.00 µg eq/ after dosing of 10 mg/kg bw and 1.67 - 1.72 µg eq/g after dosing of 100 mg/kg. Up to 24 hours after dosing, the concentrations in whole blood were lower than plasma concentrations and thus indicate that the radioactivity preferably stayed in the plasma and was not bound to erythrocytes or other blood cells. After reaching the second peak plasma level, radioactivity concentrations declined monophasically with a half-life of 11.4 to 18.6 hours. The plasma AUC-values increased less than proportionally with the dose indicating a lower absorption at the high dose level.

Tissue distribution

At the low dose level, animals that were sacrificed 0.5 hours after dosing (at the first and, at this dose level, more pronounced plasma peak) showed highest radioactivity concentrations in the gastro-intestinal tract. Tissues and organs which showed radioactivity concentrations higher than or close to the plasma concentration were kidney, liver, lung, fat tissue, thyroid, pancreas, adrenals, ovaries, uterus. At later time points the concentrations declined continuously in all tissues and dropped to values below 1 µg equiv./g at 32 hours post dose, except for the gastro-intestinal tract. At the high dose level, animals that were sacrificed 8 hours after dosing (close to the second and at this dose level more pronounced plasma peak) showed highest radioactivity concentrations in the gastro-intestinal tract. Tissues and organs which showed radioactivity concentrations higher than or close to the plasma concentration were kidney, liver, fat tissue, thyroid, pancreas, adrenals, and ovaries. At 24 hours after dosing, according to the long lasting peak plasma level, the concentration dropped in the gastro-intestinal tract but remained more or less unchanged in the tissues except for fat, where the concentration had increased. From 48 hours onwards, the concentrations declined continuously in all tissues and dropped to values below 2.5 µg equiv./g at 68 hours post dose, except for the gastro-intestinal tract.

Proposed metabolic pathway

The overall picture is that BAS 505 F was mainly metabolised by hydroxylation of the phenyl ring and by oxidation of the aromatic methyl groups to the corresponding benzyl alcohols and subsequently to the carboxylic acids. The cleavage of the benzyl ether bond was observed as well as the N-demethylation and O-demethylation. Combinations of these reactions and the conjugation of the resulting OH-groups with glucuronic acid led to the large number of observed metabolites. The fact that faeces contained several radioactive components that cannot be derived from biliary metabolites indicates a considerable contribution of microbial intestinal metabolism. As no unchanged parent compound was found in plasma, a pronounced first pass metabolism has to be concluded. Comparison of metabolite patterns in samples from animals especially generated for the isolation of metabolites with those obtained from the biokinetic study showed that metabolite patterns were essentially the same. The metabolic pathway is presented below (Figure 5-1).

Figure 5-1: Proposed metabolic pathway of dimoxystrobin in rats

Study submitted in this supplementary dossier (not yet peer-reviewed):

The following study has been performed in addition to the rat metabolism studies but has not been peer-reviewed during the last Annex I inclusion process. The objective of the new study was to quantify the metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) in rat excreta. As both metabolites became of interest for a detailed assessment of the metabolic behavior of BAS 505 F in rats, urine and faeces samples of study BASF DocID 1999/12013 were reanalyzed.

Report:	CA 5.1.1/1 [redacted] 2006a Quantification of BF 505-7 and BF 505-8 in rat excreta 2004/1006526
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Comment:
A rat metabolism study with ^{14}C-BAS 505 F was performed from August 1996 to March 1999 (BASF DocID 1999/12013). In this study the applied HPLC systems did not allow separate quantification of metabolites 505M08 and 505M09. As both metabolites became of interest for a detailed assessment of the metabolic behavior of BAS 505 F in rats, urine and faeces samples of study 36603 were reanalyzed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: a) Reg.No. 354562 (radiolabeled)
b) Reg.No. 354562 (unlabeled)
c) Reg.No. 354563 (radiolabeled)
d) Reg.No. 354563 (unlabeled)

Lot/Batch #: a) 695-1018
b) 01196-241
c) 664-1013
d) 01196-245

Purity: a) >96% (chemical/radiochemical; benzyl-U-^{14}C)
b) 97.8%
c) >99% (radiochemical; phenyl-U-^{14}C), >85% (chemical)
d) 99.6%

Metabolite code: a) BF 505-7 (505M08)
b) BF 505-7 (505M08)
c) BF 505-8 (505M09)
d) BF 505-8 (505M09)

Stability of test compound: NA

B. STUDY DESIGN AND METHODS

1. Dates of work: August 06, 2003 – February 6, 2004

The urine and faeces samples reanalyzed in this study were sampled from 10 male and 10 female Wistar rats treated with single oral dose of 150 mg/kg benzyl- or phenyl-labeled BAS 505 F (dose group DX in study 36603). The test substance was suspended in 1% carboxymethylcellulose in water containing 10% Cremophor EL. Urine and faeces from dose group DX were collected after 24, 48, 72, and 96 h. In this re-analysis the metabolites 505M08 and 505M09 were quantified in pooled faeces (0-48 h) and pooled urine (0-72 h) samples.

Urine samples were investigated by the application of several HPLC methods without any work up / purification steps. For the re-analysis of faeces samples, methanol extracts of pool samples were also measured by means of HPLC. For radioactivity measurement aliquots of liquid samples were mixed with scintillator and measured in a liquid scintillation counter. For the detection of radioactivity in extraction residues of faeces, five aliquots of the extraction residue were dried and combusted, followed by determination of the radioactivity.

II. RESULTS AND DISCUSSION

Storage stability

Before quantification, all pool samples were analyzed applying the chromatographic conditions of study 36603 and the received metabolic profiles were compared to the original profiles. Since the metabolic profiles of study 36603 and this study were qualitatively and quantitatively comparable, the storage stability of samples was proven.

Extractability

The extracts of faeces homogenates from dose group DX (label B) amounted to 100.12% (71.56% dose) and 80.58% (54.44% dose) of the faeces activity for male and female rats, respectively. The extracts of faeces homogenates from dose group DX (label P) amounted to 93.51% (76.73% dose) and 89.77% (52.75% dose) of the faeces activity for male and female rats, respectively. Therefore, the measured extractabilities of the generated pool samples were in good accordance with the values described in the main study report.

Table 5.1.1-1: Extractability of faeces samples with methanol after dosing of rats with [benzyl-U-^{14}C]- or [phenyl-U-^{14}C]-BAS 505 F

Matrix	Faeces	Methanol extract		Residue	
	% dose	% dose	% faeces activity	% dose	% faeces activity
Benzyl-U-^{14}C label					
Male rats (0-48 h)	71.47	71.56	100.12	9.87	13.81
Female rats (0-48 h)	67.56	54.44	80.58	9.98	14.77
Phenyl-^{14}C label					
Male rats (0-48 h)	82.06	76.73	93.51	13.38	16.30
Female rats (0-48 h)	58.76	52.75	89.77	7.61	12.95

Metabolism

Since the goal of this study was the quantification of 505M08 and 505M09, the evaluation of the chromatograms concentrated on these metabolites and the peaks of additional metabolites were not assigned.

In the urine samples from DX (label B) dose group 505M08 amounts to 11.64% and 11.08% of the total peak area corresponding to 1.36% and 2.61% dose for male and female rats, respectively. 505M09 amounts to 31.57% and 53.68% of the total peak area corresponding to 3.69% and 12.66% dose for male and female rats, respectively.

505M08 and 505M09 are also the main metabolites in the pool samples of faeces extracts of dose group DX (label B). 505M08 amounts to 7.82% and 6.12% of the total peak area corresponding to 5.60% and 3.33% dose for male and female rats, respectively. 505M09 amounts to 25.61% and 21.48% of the total peak area corresponding to 18.33% and 11.69% dose for male and female rats, respectively.

The sum of the amounts of 505M08 in pooled urine and faeces samples accounts for 6.96% and 5.94% dose in male and female rats of dose group DX (label B), respectively. The sum of the amounts of 505M09 in the same dose groups is 22.02% and 24.35% dose in male and female rats, respectively.

Similarly, 505M08 and 505M09 are the main metabolites in urine samples from dose group DX (label P). 505M08 amounts to 13.78% and 11.36% of the total peak area corresponding to 1.49% and 2.33% dose for male and female rats, respectively.
505M09 amounts to 32.95% and 59.23% of the total peak area corresponding to 3.57% and 12.15% dose for male and female rats, respectively. 505M08 and 505M09 are also the main metabolites in the pool samples of faeces extracts of dose group DX (label P). 505M08 amounts to 9.35% and 6.09% of the total peak area corresponding to 7.17% and 3.21% dose for male and female rats, respectively. 505M09 amounts to 22.33% and 25.34% of the total peak area corresponding to 17.13% and 13.36% dose for male and female rats, respectively.
The sum of the amounts of 505M08 in pooled urine and faeces samples accounts for 8.66% and 5.54% dose in male and female rats of dose group DX (label P), respectively. The sum of the amounts of 505M09 in the same dose groups is 20.70% and 25.51% dose in male and female rats, respectively.

It could be demonstrated that 505M08 and 505M09 can be found in significant amounts in urine and faeces samples of rats.

Table 5.1.1-2: Summary of metabolites identified in urine and faeces from dose group DX (label B) and DX (label P)

Designation	Females			Males		
	Urine (0-72 h) [% dose]	Faeces (0-48 h) [% dose]	Sum [% dose]	Urine (0-72 h) [% dose]	Faeces (0-48 h) [% dose]	Sum [% dose]
Benzyl-U-^{14}C label						
505M08	2.61	3.33	5.94	1.36	5.60	6.96
505M09	12.66	11.69	24.35	3.69	18.33	22.02
Phenyl-^{14}C label						
505M08	2.33	3.21	5.54	1.49	7.17	8.66
505M09	12.15	13.36	25.51	3.57	17.13	20.70

III. CONCLUSION

It could be demonstrated by re-analysing urine and faeces samples from study 36603 (BASF DocID 1999/12013) that 505M08 and 505M09 are found in significant amounts in urine and faeces samples of rats treated with BAS 505 F by the oral route.

CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

According to the new data requirements for active ingredients of plant protection products as set out in Commission Regulation (EU) No. 283/2013 (1 March 2013, OJ L93, 1ff, 3.4.2013), "comparative *in vitro* metabolism studies shall be performed on animal species ... and on human material ...in order to determine the relevance of the toxicological animal data and to guide in the interpretation of findings and in further definition of the testing strategy..." (Section 5, Toxicological and metabolism studies, point 5.1.1., page 22). In the absence of validated test methods or guidance documents, and in agreement with the RMS Hungary, this data requirement is waived in accordance to SANCO Guidance Document SANCO/10181/2013-rev 2.1 (13 May 2013).

Overall conclusion

Dimoxystrobin has been extensively studied for absorption, distribution, metabolism and excretion. Taking all studies into consideration, the following general conclusions can be drawn:

- Dimoxystrobin is rapidly excreted via urine and feces.
- The majority of the radioactivity was excreted via feces (57 - 82% of the dose) and smaller amounts via urine (14 - 39% of the dose). The renal excretion was more pronounced in female animals. The bioavailability is in the range of 85 - 90% at the low dose level and 58 - 71% at the high dose level.
- There is very little evidence of any cumulative potential of dimoxystrobin. Throughout the time course of the experiments, highest radioactivity concentrations were found in the GI tract. Tissues and organs which showed radioactivity concentrations higher than or close to the plasma concentration were kidney, liver, lung, fat tissue, thyroid, pancreas, adrenals, ovaries, uterus.
- The metabolite patterns in feces, urine, bile, liver, kidney and plasma were largely comparable for both sexes and for all dose groups investigated.
- In total 3 major transformation steps were observed in rats:
    - Hydroxylation of the aromatic ring system and oxidation of the methyl groups on the phenyl moiety
    - Cleavage of the ether bond between the ring systems
    - Side chain modifications (Demethylation reactions, oxidation reactions)
- The combination of these reactions followed by conjugation steps results in a huge number of metabolites.

When the information from previous and new studies is reviewed in total, the following endpoints are proposed (Table 5.1.2-1). In this context, the metabolites being considered for dietary exposure assessment according to the TTC concept are designated as “significant”. After the conduct of the assessments (see M-CA 6.7 and 6.9), none of them was identified as “relevant”.

Table 5.1.2-1: Proposed endpoints

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of absorption	85-90% within 5d at 10 mg/kg
Distribution	Extensive: highest levels in gut, liver, kidney, lung, fat, thyroid, pancreas, adrenals, ovaries, uterus
Potential for accumulation	Very little potential for accumulation at 10 mg/kg bw
Rate and extent of excretion	65-83% (mainly in faeces) within 24h at 10 mg/kg bw
Metabolism in animals	Extensive. 45 identified metabolites in rat
Toxicologically significant compounds (animals, plants)*	Dimoxystrobin and metabolites

*In this context, the metabolites being considered for dietary exposure assessment are designated as “significant”. After the conduct of the assessments (see M-CA 6.7 and 6.9), none of them was identified as “relevant”.

CA 5.2 Acute Toxicity

Studies evaluated in the draft assessment report (DAR, July 2003):
Dimoxystrobin (BAS 505 F) has been tested in various species and via different routes of administration. All studies are scientifically valid. The studies listed in Table 5.2-1 have been evaluated and peer-reviewed during the previous Annex I inclusion process.

Table 5.2-1: Summary of already peer-reviewed acute toxicity studies with dimoxystrobin

Route/species/sex	Dose range	Vehicle	Result	Reference (BASF DocID)
Oral Rat, Wistar (CHBB:THOM), m/f	2000, 5000 mg/kg bw	0.5% aqueous Tylose (CMC)	LD_{50} > 5000 mg/kg bw	1998/11002
Dermal Rat, Wistar (CHBB:THOM), m/f	2000 mg/kg bw	0.5% aqueous Tylose (CMC)	LD_{50} > 2000 mg/kg bw	1998/11001
Inhalation (dust) Rat, Wistar (CHBB:THOM), m/f	0.51, 1.28, 5.9 mg/L	None	LC_{50} = 1.7 mg/L (males: 1.9 mg/L; females: 1.3 mg/L)	1997/10971
Skin irritation Rabbit, (NZW), m/f	0.5 g/animal	None	Non irritant	1998/10999
Eye irritation Rabbit, (NZW), m	0.1 mL bulk volume/animal	None	Non irritant	1998/11000
Skin sensitization, Maximisation Test Guinea pig, Dunkin Hartley (Crl:(HA)BR), f	Intradermal: 5% in mixture Freund's adjuvant /Tylose Epidermal: 50% in Tylose	1% aqueous Tylose	Not sensitizing	1998/10998

Studies submitted in this supplementary dossier (not yet peer-reviewed):
In accordance with the requirements of Commission Regulation SANCO/11802/2010 an in vitro NRU Phototoxicity Test in Balb/c 3T3 cells has been performed and is described in detail in chapter M-CA 5.2.7. The respective studies are listed in Table 5.2-2.

Table 5.2-2: Summary of not yet peer-reviewed acute toxicity studies with dimoxystrobin

Type of study	Test substance	Result Classification	Reference (BASF DocID)
In vitro NRU Phototoxicity Test in Balb/c 3T3 cells	dimoxystrobin	Not phototoxic	2014/1083465

Dimoxystrobin has very low acute toxicity by the oral and dermal route of administration.

Dimoxystrobin is harmful after inhalation (H332) yielding an LC_{50} of 1.3/1.9 mg/L (m/f).

Dimoxystrobin is not irritant to the skin or eye.

In a Maximisation Test dimoxystrobin was not sensitizing.

No indications for a phototoxic potential was observed in an in vitro NRU Phototoxicity Test in Balb/c 3T3 cells:

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the EFSA conclusion (EFSA Scientific Report, 2005, 46, 1-82, Conclusion on the peer review of dimoxystrobin):

Rat LD_{50} oral:	> 5000 mg/kg bw
Rat LD_{50} dermal:	> 2000 mg/kg bw
Rat LC_{50} inhalation:	1.3 mg/L
Skin irritation:	Not irritating
Eye irritation:	Not irritating
Skin sensitization (test method used and result):	Not sensitizing (M & K maximization test)

The proposed endpoints based on all available studies are shown below in Table 5.2-3.

Table 5.2-3: Proposed acute toxicity endpoints of dimoxystrobin*

Study type/species	**Results**	**Classification**	
		EU Dir. 67/548/EEC 2001/59 EC	**Reg. EC 1272/2008 (CLP)**
Acute oral toxicity, rat	LD_{50} > 5000 mg/kg bw	-	-
Acute dermal toxicity, rat	LD_{50} > 2000 mg/kg bw	-	-
Acute inhalation toxicity, rat	LC_{50} = 1.3 mg/L	R20	H332
Dermal irritation, rabbit	Not irritating	-	-
Eye irritation, rabbit	Not irritating	-	-
Maximization test, guinea pig	Not sensitizing (M & K maximization test)	-	-
In vitro NRU Phototoxicity Test, Balb/c 3T3 cells	**Not phototoxic**	-	-

* new endpoints from the current agreed EU endpoints are marked in bold

CA 5.2.1 Oral

The acute oral toxicity study of dimoxystrobin was evaluated and peer-reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Dimoxystrobin has low acute oral toxicity.

CA 5.2.2 Dermal

The acute dermal toxicity study of dimoxystrobin was evaluated and peer-reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Dimoxystrobin has low acute dermal toxicity.

CA 5.2.3 Inhalation

The acute inhalation toxicity study of dimoxystrobin was evaluated and peer-reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Dimoxystrobin showed adverse effects after inhalation yielding an LC_{50} of 1.3 mg/L, and thus has to be classified as "Harmful by inhalation" (Category 4) according to the EU CLP (1272/2008) with the risk phrase H332.

CA 5.2.4 Skin irritation

The acute skin irritation study of dimoxystrobin was evaluated and peer-reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Dimoxystrobin is not irritant to the skin.

CA 5.2.5 Eye irritation

The acute eye irritation study of dimoxystrobin was evaluated and peer-reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Dimoxystrobin is not irritant to the eye.

CA 5.2.6 Skin sensitisation

The skin sensitization study (Maximization Test) of dimoxystrobin was evaluated and peer-reviewed during the previous Annex I inclusion process (for further details please see Table 5.2-1). Dimoxystrobin is not sensitizing to the skin.

CA 5.2.7 Phototoxicity

Report: CA 5.2.7/1
Cetto V.,Landsiedel R., 2014a
BAS 505 F (Dimoxystrobin) - In vitro 3T3 NRU phototoxiciy test
2014/1083465

Guidelines: OECD 432 (2004) In vitro 3T3 NRU Phototoxicity test, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.41 No. L 142

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

Executive Summary

Dimoxystrobin (Batch: OP-No. 13; purity 98.5%) was tested for its ability to induce phototoxic effects in Balb/c 3T3 cells in vitro. The photo-cytotoxicity was estimated by the means of the Neutral Red Uptake (NRU) method. A pretest and one main experiment were carried out with and without irradiation with an UVA source. Vehicle and positive controls were included into the experiment.
Based on an initial range-finding phototoxicity test for the determination of the experimental concentrations, the following concentrations were tested in this study with and without UVA irradiation: 0, 1.0, 2.2, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4 µg/mL.
Precipitation was seen at 100 µg/mL onward, with and without irradiation. In the absence and the presence of UVA irradiation cytotoxicity was noted and EC_{50} values were calculated. Based on the results of the present study, the test substance was predicted to have no phototoxic potential as indicated by Photo-Irritancy-Factor (PIF) value of 0.9. The threshold for a negative response is ≤ 2. The positive control chlorpromazine led to the expected increased cytotoxicity with UVA irradiation as indicated by a PIF value of 24.6.
Thus, under the experimental conditions of this study, dimoxystrobin is not considered to be a phototoxic substance in the in vitro 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material BAS 505 F (dimoxystrobin)

Description:	Solid, beige
Lot/Batch #:	OP-No. 13
Purity:	98.5% (tolerance ± 1.0%)
Stability of test compound:	Expiry date: 01.11.2015
Solvent used:	Dimethylsulfoxide (DMSO)

2. Control Materials:

Vehicle control:	DMSO 1% (v/v) in PBS
Positive control compounds:	Chlorpromazine (CPU) was dissolved in DMSO; 8 concentrations tested - 1.9 to 180 µg/mL without radiation, 0.03 to 3.2 µg/mL with radiation

3. Test organisms: BaIb/c 3T3, clone A31: fibroblast cell line isolated from the muscle tissue of a mouse embryo. The Balb/c 3T3 cell line which was used in this experiment was obtained from the "European Collection of Cell Cultures" Salisbury, Wiltshire SP4 OJG, UK on 09 Aug 2006 and is stored at -196°C (liquid nitrogen).

4. Culture media and reagents:

Culture medium: Dulbecco's Modified Eagle's Medium (DMEM) supplemented with
- 10% (v/v) newborn calf serum (NCBS)
- 4 mM L-glutamine
- 100 IU penicillin
- 100 µg/mL streptomycin

Neutral Red solution:
- 0.4 g Neutral Red powder (NR; Sigma N4638)
- 100 mL deionized water

Neutral Red medium:
- 1 mL Neutral Red solution
- 79 mL culture medium (DMEM incl. supplements) (incubated overnight at 37° C with 5% CO_2 and filtered with a 0.22 µm filter prior to use)

Other solutions and reagents:
- phosphate buffered saline (PBS) without Ca/Mg
- trypsin/EDTA solution (0.05%; 0.02%)
- Neutral Red desorb solution
  (1 mL acetic acid, 50 mL ethanol, 49 mL deionized water)

5. Irradiation source: The Sol 500 solar simulator (Dr. Hönle AG, 82166 Gräfelfing, Germany) used with filter H1 produced wavelength > 320 nm. The exposure rates were determined with UV-meter RM-21 (Dr. Gröbel GmbH, 76275 Ettlingen, Germany).

6. Test concentrations:

Pretest: Concentrations of 4.6 to 1000 µg/mL with and without irradiation were used. The EC_{50} values determined were 84.1 µg/mL without and 70.7 µg/mL with UVA irradiation.

Main NRU test: Based on the results of the pretest the following concentrations were used in the main study:

Without UVA: 1.0, 2.2, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4 µg/mL

With UVA: 1.0, 2.2, 4.6, 10.0, 21.5, 46.4, 100.0, 215.4 µg/mL

B. TEST PERFORMANCE:

1. Dates of experimental work: 28-Jan-2014 to 24-Feb-2014

2. Treatment and NRU Phototoxicity Test:

Two 96 well-plates per substance (test substance or positive control) were used for cultivation of cells (1.5 x 10^5 cells/well). After an attachment period of about 24 hours the cells were washed once with 100 µL PBS and subsequently treated with the respective substance (8 concentrations each with 6 replicates of the test substance or the positive control) and the vehicle control. After pre-incubation for 1 hour in the dark (5% (v/v) CO_2, ≥90% humidity; 37°C) one 96 well-plate per substance was irradiated for 50 minutes with UVA (UV intensity underneath the lid 1.5 - 2.1 mW/cm^2 = 5 J/cm^2) whereas the respective reference plate was kept in the dark for the same period. Thereafter the test- respectively control-substance was removed and the cells washed at least once with 100 µL PBS. After replenishing the wells with culture medium the cells were incubated overnight under the conditions indicated above. The medium was removed 24 hours after the start of treatment and after washing with 100 µL PBS the wells were filled with 100 µL medium containing 50 µg/mL Neutral Red. Subsequently the plates were incubated for another 3 hours. Each step was performed under light protected conditions in the lab to prevent uncontrolled photo activation. Finally, the cells were washed again with 100 µL and the dye was extracted by 100 µL Neutral Red desorb solution. Cytotoxicity was determined by measuring the Neutral Red Uptake by means of a microplate reader (Perkin Elmer, Waltham, Massachusetts, US; Wallac 1420 multilabel counter) equipped with a 550 nm filter to read the absorption of the extracted dye. The absorption shows a linear relationship with the number of surviving cells.

3. Evaluation/Assessment

3.1 Cytotoxicity

The mean absorbance values obtained for each test group of every plate were used to calculate the percentage of cell viability relative to the respective vehicle control, which is arbitrarily set at 100 %.

$$\textit{Viability}^{\S}\ [\%] = \frac{\text{Absorbance}_{\text{mean}}\ \text{of the test group}}{\text{Absorbance}_{\text{mean}}\ \text{of the vehicle control}} \times 100$$

§ The authors of the study denominate the above quotient as ‘cytotoxicity’, which is strictly speaking not correct. Thus, in this summary the appropriate term ‘viability’ is used. This applies also to Table 5.2.7-1 to Table 5.2.7-2.

In case of cytotoxicity, an EC_{50} value (concentration at which the viability is reduced by 50% relative to the respective vehicle control) was calculated by a linear interpolation method (linear dose-response curve). Therefore two viability values were needed: one between 100% and 50% and one between 50% and 0%. From these two points the concentration that inhibits the Neutral Red uptake by 50% of the respective control was calculated.

For the assessment of the phototoxic potential of a compound two prediction models are currently available:

- The Photo-Irritancy-Factor (PIF) prediction model for substances which allow the comparison of two equi-effective concentrations (EC_{50}) in the concurrently performed experiments in the presence and absence of light. This model includes two special cases: Case 1 accounts for situations in which an EC_{50} can only be calculated in the presence of UVA irradiation. Case 2 accounts for situations where an EC_{50} cannot be calculated in absence and presence of irradiation. These special cases do not apply to this study. Even though described in the report these cases are not described in this summary.
- The Mean Photo Effect prediction model which is used if no EC_{50} was obtained in the absence and presence of UV light. This is not the case in this study. Even though described in the report this prediction model is not described in this summary.

3.2 Photo-Irritancy-Factor (PIF)

For substances which induce a 50 % cytotoxicity (EC_{50}) in the presence and absence of light the Photo-Irritancy-Factor (PIF) is calculated based on comparison of the EC_{50} values in the absence (-UVA) and presence (+UVA) of UVA irradiation.

$$PIF = \frac{EC50\ (-UVA)}{EC50\ (+UVA)}$$ resulting in the following classification rules:

PIF ≥ 5	phototoxic potential predicted
2 < PIF < 5:	probable phototoxic potential predicted
PIF ≤ 2:	no phototoxic potential predicted

3.3 Other parameters

pH:
The pH was measured at least for the two top doses and for the vehicle controls with and without irradiation.

Osmolarity:
Osmolarity was measured at least for the two top doses and for the vehicle controls with and without irradiation.

Solubility:
Test substance precipitation was checked immediately after treatment and at the end of treatment.

Cell morphology:
Test cultures of all test groups were examined microscopically before staining with NRU, which allows conclusions to be drawn about attachment of the cells.

4. Statistics:

No special statistical tests were performed.
Mean absorbance values and standard deviations were calculated from the single values using calculation software (e.g. MS Excel). The calculations were made using the unedited values. For the report the values were rounded, therefore there may be deviations in the given relative values. If technical errors occurred in single wells (outlier) at least 4 single values per test group were sufficient for calculating reliable mean values. Outliers are defined as values that have half or double the value of the respective mean.

5. Acceptance criteria:

The assay has to be considered valid if the following criteria are met:

- The vehicle control needs to fulfill the following criteria:
  - The mean OD_{550} value (with and without UVA irradiation) should be > 0.3.
  - Cell viability after irradiation should be at least 80% of the concurrent non-irradiated vehicle control.
  - The standard deviation of the mean values of both vehicle control rows should not exceed ± 15%.
- The positive control chlorpromazine needs to fulfill the following criteria:
  - the EC_{50} value should be in the ranges:
    With irradiation (+UVA): 0.1 - 2.0 µg/mL
    Without irradiation (-UVA): 7.0 - 90.0 µg/mL
  - and the PIF ≥ 6.

II. RESULTS AND DISCUSSION

A. TREATMENT CONDITIONS

Osmolarity and pH values were not influenced by test substance treatment. In this study, in the absence and the presence of UVA irradiation precipitation in culture medium was observed at test substance concentrations of ≥ 100 µg/mL.

B. CYTOTOXICITY OF THE TEST SUBSTANCE

In a pretest concentrations of 4.6, 10, 21.5, 46.4, 100.0, 214.4, 464.2, and 1000 µg/mL with and without irradiation were tested. Cytotoxicity was observed at 100 µg/mL onward, and precipitation was observed at concentrations > 100 µg/mL. The EC_{50} values determined were 84.1 µg/mL without and 70.7 µg/mL with UVA irradiation. A PIF of 1.2 was calculated, indicating no phototoxic potential of dimoxystrobin.

After treatment with the test substance in the main experiment, clear cytotoxic effects indicated by Neutral Red absorbance values of below 50% of control were observed in the main experiment in the absence and the presence of UVA irradiation (see Table 5.2.7-1).
Without UVA irradiation, there was a decrease in the cell number at concentrations ≥ 100 µg/mL (EC_{50}: 105.5 µg/mL).
With UVA irradiation, there was a decrease in the cell number at concentrations ≥ 100 µg/mL (EC_{50}: 121.8 µg/mL). Cell morphology changes were restricted to concentrations of 100 µg/mL or higher with and without UVA irradiation.

Based on the EC_{50} values a PIF of 0.9 were calculated, indicating no phototoxic potential for dimoxystrobin.

Table 5.2.7-1: Mean relative viability of dimoxystrobin with (+) and without (-) UVA irradiation in Balb 3T3 cells

Test group	UVA irradiation*	Precipitation**	Mean $OD_{corr.}$ ***	Relative viability [% of control]	
				Mean	SD
Vehicle control 1	-	-	0.448	-	5.7
Vehicle control 2	-	-	0.467	-	8.7
Vehicle mean	-	-	0.457	100.0	7.4
Dimoxystrobin					
1.0 µg/mL	-	-	0.486	106.3	3.2
2.2 µg/mL	-	-	0.487	106.6	4.5
4.6 µg/mL	-	-	0.487	106.6	4.4
10.0 µg/mL	-	-	0.481	105.1	3.6
21.5 µg/mL	-	-	0.439	95.9	7.8
46.4 µg/mL	-	-	0.392	85.6	6.5
100.0 µg/mL	-	+	0.239	52.2	10.3
215.4 µg/mL	-	+	0.031	6.7	4.9
Vehicle control 1	+	-	0.441	-	5.7
Vehicle control 2	+	-	0.490	-	3.6
Vehicle mean	+	-	0.466	100.0	7.1
Dimoxystrobin					
0.5 µg/mL	+	-	0.471	101.1	2.1
1.0 µg/mL	+	-	0.481	103.3	2.2
2.2 µg/mL	+	-	0.477	102.4	2.7
4.6 µg/mL	+	-	0.467	100.3	3.3
10.0 µg/mL	+	-	0.441	94.7	5.1
21.5 µg/mL	+	-	0.389	83.6	4.6
46.4 µg/mL	+	-	0.276	59.2	16.9
100.0 µg/mL	+	+	0.049	10.6	6.6

*: Irradiation with Sol 500 solar simulator for 50 minutes (approx. 5 J/cm^2)
**: Precipitation in PBS at the end of exposure period
***: Mean OD corrected: mean absorbance (test group) minus mean absorbance (blank)

C. CYTOTOXICITY OF THE POSITIVE CONTROL

After treatment with the positive control chlorpromazine clear cytotoxic effects indicated by Neutral Red absorbance values of below 50% of control were observed in the absence and the presence of UVA irradiation (see Table 5.2.7-2).
In the experiment without UVA irradiation, there was a decrease in viability at ≥ 30.0 µg/mL (EC_{50}: 24.2 µg/mL). With UVA irradiation, there was a decrease in viability at ≥ 0.8 µg/mL (EC_{50}: 1.0 µg/mL). Cell morphology was distinctively changed in both experiments at ≥ 30 µg/mL and ≥ 0.8 µg/mL without and with UVA irradiation.

Based on the EC_{50} values PIF's of 24.6 was obtained, indicating a strong phototoxic potential, thus confirming the sensitivity of the test system.

Table 5.2.7-2: Mean relative viability of Chlorpromazine with (+) and without (-) UVA irradiation in Balb/c 3T3 cells

Test group	UVA irradiation	Mean OD *	Mean $OD_{corr.}$ **	Relative viability [% of control]	
				Mean	SD
Blank	-	0.036	-	-	-
Vehicle control 1	-	0.453	0.417	-	4.2
Vehicle control 2	-	0.494	0.458	-	7.4
Vehicle mean	-	0.473	0.437	100.0	7.7
Chlorpromazine					
1.9 µg/mL	-	0.518	0.482	110.2	3.8
3.8 µg/mL	-	0.530	0.494	113.0	3.7
7.5 µg/mL	-	0.486	0.451	103.0	4.9
15.0 µg/mL	-	0.455	0.419	95.7	7.1
30.0 µg/mL	-	0.127	0.091	20.8	4.1
60.0 µg/mL	-	0.036	0.000	0.0	0.3
90.0 µg/mL	-	0.036	0.000	0.1	0.2
180.0 µg/mL	-	0.036	0.000	0.1	0.1
Blank	+	0.036	-	-	-
Vehicle control 1	+	0.487	0.451	-	4.7
Vehicle control 2	+	0.530	0.494	-	2.7
Vehicle mean	+	0.508	0.472	100.0	6.0
Chlorpromazine					
0.03 µg/mL	+	0.501	0.465	98.4	1.6
0.05 µg/mL	+	0.512	0.476	100.8	3.8
0.10 µg/mL	+	0.530	0.494	104.6	4.2
0.20 µg/mL	+	0.513	0.477	101.1	4.3
0.40 µg/mL	+	0.480	0.444	94.0	6.3
0.80 µg/mL	+	0.340	0.304	64.4	14.7
1.60 µg/mL	+	0.040	0.004	0.7	1.6
3.20 µg/mL	+	0.036	0.000	0.0	0.2

*: Mean absorbance at 550 nm of 6 wells, in general
**: Mean absorbance (test group) minus mean absorbance (blank)

III. CONCLUSIONS

According to the results of the present study, dimoxystrobin is not considered to be a phototoxic substance in the in vitro 3T3 NRU Phototoxicity Test.

CA 5.3 Short-Term Toxicity

Studies evaluated in the draft assessment report (DAR, July 2003):
Studies evaluated in the dimoxystrobin draft assessment report of Rapporteur Member State United Kingdom (July, 2003) consisted of: short-term toxicity studies (90 days) with oral administration in three different species (rats, mice, dogs). In addition a 1-year dog study and a 28-day dermal toxicity study in rats were evaluated. The available studies are presented in tabular form below (see Table 5.3-1) and have been briefly summarized. These studies have been evaluated by European authorities and United Kingdom as RMS and were considered to be acceptable.

Table 5.3-1: Summary of reviewed dimoxystrobin short-term toxicity studies

Study	Dosages (mg/kg bw/ day)	NOAEL (mg/kg bw/day)	Main adverse effect	Reference (BASF DocID)
3-month feeding Wistar rats 0, 50, 300, 1500, 4500 ppm	M: 3, 21, 103, 311; F: 4, 24, 121, 357	50 ppm 3.0 (M); 4.0 (F)	4500 ppm, 1500 ppm: reduced body weight (gain); reduced food consumption; altered haematological and clinical chemical values; thickening of duodenal mucosa; 300 ppm: thickening of duodenal mucosa	1999/11155, *2011/1150025*#
3-month feeding B6C3F1 mouse 0, 1000, 4000, 8000 ppm	M: 206, 1187, 2867; F: 318, 1535, 3715	1000 ppm 206 (M); 318 (F)	8000 ppm, 4000 ppm: reduced body weight (gain); altered clinical chemical values; thickening of duodenal mucosa, erosion/ulceration of glandular stomach	1999/10388, *2011/1150024*#
3-month feeding Beagle dog 0, 200, 600, 1200 ppm	M: 6.1, 18.5, 36.8; F: 6.4, 18.9, 37.7	600 ppm 18.5 (M); 18.9 (F)	1200 ppm: diarrhoea, reduced body weight gain and food consumption; altered haematological and clinical chemical values	1999/11676, *2011/1150023*#
12-month feeding Beagle dog 0, 200, 400, 800 ppm	M: 5.3, 11.0, 22.3 F: 5.7, 11.2, 22.7	400 ppm 11.0 (M) 11.2 (F)	800 ppm: diarrhoea, clinical-chemical alterations	2000/1012307
4-week dermal Wistar rats	0, 50, 200, 1000	1000	No systemic toxicity No local signs	1999/11640

References (BASF DocIDs) in italic describe additional histopathological evaluations of the bone marrow within the 3-months studies in rats, mice and dogs and are submitted the first time in this supplemental dossier. As these report amendments do not affect the outcome and interpretation of the studies they are listed in this table.

Studies submitted in this supplementary dossier (not yet peer-reviewed):
No new standard subacute or subchronic studies were conducted with dimoxystrobin. Since the latest European evaluation and this AIR 3 submission, additional iron stainings in the fixed bone marrow - archived from each of the subchronic rat, mice and dog studies (see above) - were conducted. Duodenum thickening is an effect after dimoxystrobin administration in repeated dose toxicity studies in rats and mice with proven evidence of accompanied effects on iron supply and haematology. However mechanistic studies on serum iron levels and specific haematological investigations had only be conducted in rats. The idea of the additional iron stainings in the available bone marrow tissue of the 90-day studies of rats, mice and dogs, was to correlate the observed effects in the duodenum in to an assumed lower iron supply in the body compared to controls and vice versa. As serum iron levels can only be conducted in a running studies, the (assumed lower) iron storages in bone marrow of the treated animals compared to controls should serve as a surrogate measure for serum iron levels.
Detailed summaries of the investigations as described in the amendments can be found in this chapter.

Further, historical control data have been collected for the biological range of organ weights of control rats and mice from short-term studies running in the same lab (BASF DocID 2015/1171112 and 2015/1171113).

For the convenience of the reviewer brief summaries of the respective studies are provided under the respective chapters.

Summary of the short term toxicity studies:

"The short-term toxicity of dimoxystrobin was investigated in dietary 3-month studies in rats and mice. In addition, 3-month and 12-month dietary studies were conducted in dogs. However the need for the 12-month dog study is questionable on animal welfare reasons because the 3-month studies with dimoxystrobin indicated that the rat was the most sensitive species. The short-term toxicity following dermal exposure was determined in a 28-day study in rats.

Oral studies
The signs of toxicity observed in the three species tested were overall similar and consisted of reduced body weight gain at the high dose levels (diarrhoea was also seen in dogs). The observed clinico-chemical findings typically included reduced total protein, globulin and albumin, which can be linked to the reduced body weight gain.
Decreased alanine aminotransferase was a notable finding in rats. In addition, rats and dogs showed haematological changes indicative of a mild microcytic hypochromic anaemia (with minimal evidence of such an effect in mice at an extremely high dose level).
The duodenum was a target organ in rats and mice. Thickening of the duodenal wall was seen on gross examination. Histopathologically, a dose-dependent thickening of the duodenal mucosa was observed (slight to moderate in rats, slight to severe in mice). In affected animals, the intestinal villi were elongated, partly slightly broadened and branched. It is notable that macroscopic findings were not always confirmed microscopically.

From the mechanistic studies in rats and mice (see Chapter MCA 5.8) it is known, that duodenum thickening and duodenum weight increases occur very rapidly after the start of dimoxystrobin administration (already 2 days after start of exposure) as an adaptive response. With regard to NOAEL setting, for the 90-day and also later for the chronic studies, the histopathological observation of duodenal mucosa thickening is considered to be the more relevant parameter.

No substance-related histopathological lesions were seen in dogs.

Dermal study
In a 4-week dermal toxicity study in rats no substance-related systemic or local toxicity was detected up to the highest dose level tested (1000 mg/kg bw/day).

Inhalation study
No short-term inhalation study was conducted with dimoxystrobin and none is needed for this application.

For rats, a short-term NOAEL of 50 ppm (3 mg/kg bw/d (m), 4 mg/kg bw/d (f)) has been established. For dogs, the short-term NOAEL is 400 ppm, equivalent to about 11 mg/kg bw/d, based on the 3-month and 1-year feeding study. For mice, the NOAEL in the 3-month study was 1000 ppm (206 mg/kg bw/d (m); 318 (f) mg/kg bw/d).

Overall, the lowest relevant NOAEL for short-term oral toxicity is 3 mg/kg bw/d, respectively 4 mg/kg bw/d in males and females, based on the thickening of duodenal mucosa after 91 days from the 3-month study in rats.

Based on the studies previously peer-reviewed, the following EU agreed endpoints are given in the DAR and the EFSA conclusion (EFSA Scientific Report, 2005, 46, 1-82, Conclusion on the peer review of dimoxystrobin):

Short term toxicity	
Target / critical effect:	Duodenal (mucosal) thickening, 3-month rat Decreased serum iron, 7-day rat
Lowest relevant oral NOAEL / NOEL:	3-month rat: 50 ppm (3 mg/kg bw/d) 7-day rat: 50 ppm (*c.* 4 mg/kg bw/d)
Lowest relevant dermal NOAEL / NOEL:	>1000 mg/kg bw/day
Lowest relevant inhalation NOAEL/NOEL	No data- not required for this application.

The proposed endpoints based on all available studies are shown below.

Target organ / critical effect	Duodenal (mucosal) thickening, 3-month rat Decreased serum iron, 7-day rat
Relevant oral NOAEL	3-month, rat: 50 ppm (3-4 mg/kg bw/d) 7-day, rat: 50 ppm (*c.* 4 mg/kg bw/d)
Relevant dermal NOAEL	28-day, rat: >1000 mg/kg bw/day
Relevant inhalation NOAEL	No data - not required

CA 5.3.1 Oral 28-day study

See 90-day oral administration studies, chapter M-CA 5.3.2

CA 5.3.2 Oral 90-day study

Studies in rats

Note: The main rat 90-day study report (BASF DocID 1999/11155) was already submitted and reviewed in the course of the previous registration of dimoxystrobin. This study serves as the basis for the assessment of the report amendment submitted under M-CA 5.3.2/1. Amendment 1 provides the result of an additional iron staining conducted in the bone marrow.

Three month toxicity study in rats by dietary administration (BASF DocID 1999/11155)

Guidelines: In compliance with OECD guideline 408 (adopted 1981)
Deviations: NA
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and Methods:
Dimoxystrobin (batch/purity: N 6 lot 3004: 98.8%) was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 50, 300, 1500, and 4500 ppm for 3 months.
Food consumption and body weight were determined each week. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week.
Ophthalmological examinations were carried out prior to the start and towards the end of dosing.
Urinalysis, clinico-chemical and hematological examinations were carried out at the end of the administration period.
All animals were subjected to gross-pathological assessment, followed by histopathological examinations. As enlargement of the duodenum was observed, the thickness of the duodenal mucosa (tip of villi to smooth muscle) was measured at 4 locations in all animals.

Results:
The stability and homogeneous distribution of the test substance in the diet were confirmed by analysis. The correctness of the concentrations was demonstrated analytically.

Based on food consumption and body weight data average daily compound intakes of 3, 21, 103, and 311 mg/kg bw/d in male and of 4, 24, 121, and 357 mg/kg bw/d in female rats were calculated at dietary dose levels of 50, 300, 1500 and 4500 ppm, respectively.

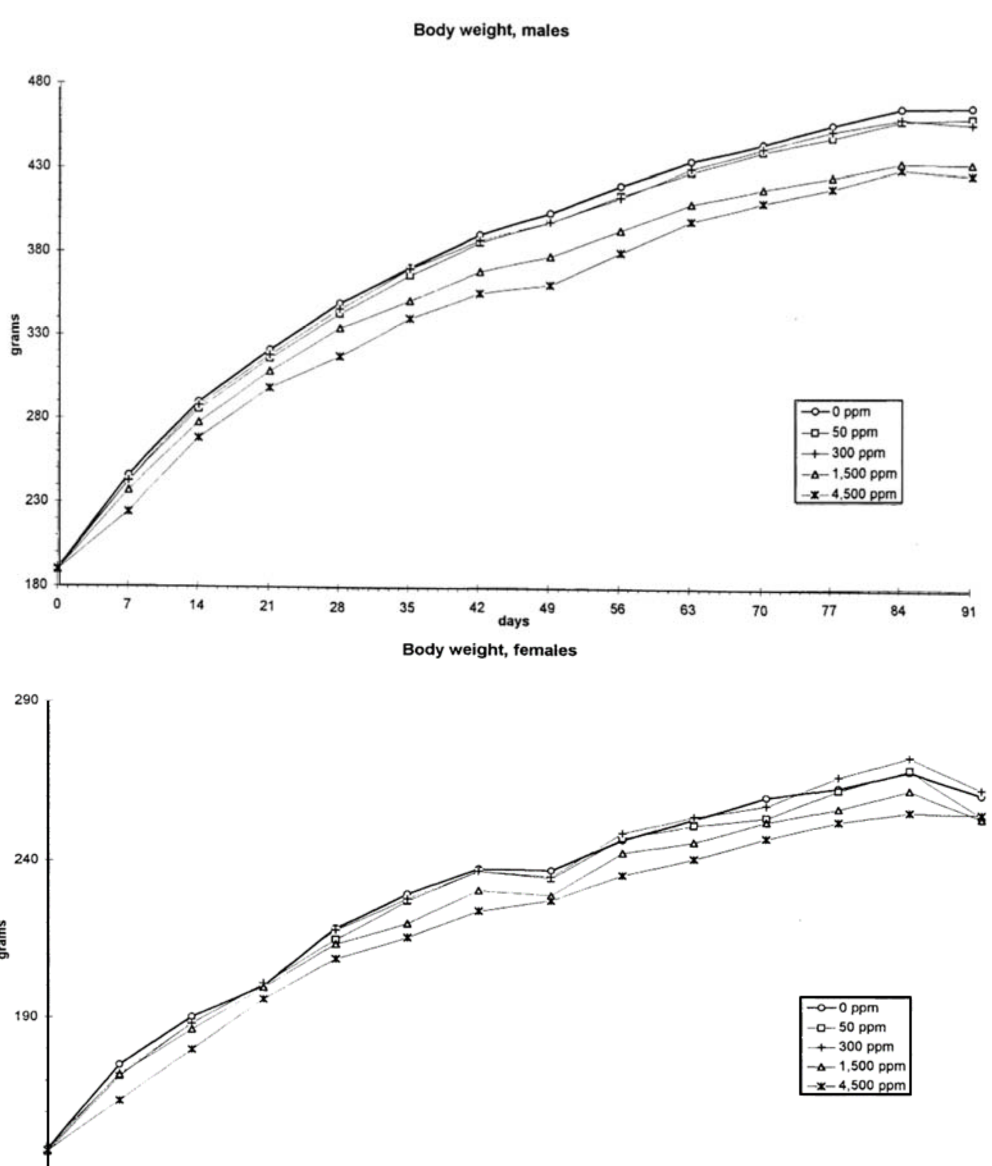
There were no mortalities or clinical signs of toxicity in any of the dose groups.

Dose-dependent and statistically significant reduced body weight and body weight gain occurred in males at ≥ 1500 ppm.
Food consumption was statistically significantly impaired in males at 4500 ppm on days 7, 14 and 49, and in females on days 7 and 35, and in males and females at 1500 ppm on day 7.

Table 5.3.2-1: Body weights and body weight gain of rats administered dimoxystrobin for at least 91 days

Dose	Males					Females				
(ppm)	0	50	300	1,500	4,500	0	50	300	1,500	4,500
Body weight [g];	467.9	461.4	458.1	434.2*	427.8**	260.6	254.0	262.4	253.2	254.6
% of control	100	98.6	97.9	92.8	91.4	100	97.5	100.7	97.1	97.7
Body weight gain [g];	277.6	271.8	267.1	244.4*	237.7**	112.2	106.7	114.4	104.2	106.9
% of control	100	97.9	96.2	88.0	85.6	100	95.1	102.0	92.8	95.3
* P ≤ 0.05; ** P ≤ 0.01; F-test (ANOVA, 2-sided) + Dunnett's test (2-sided)										

Figure 5.3.2-1: Body weights of male and female rats administered dimoxystrobin for at least 91 days



The following statistically significant changes in haematology and clinical chemistry were considered to be test substance-related effects:

Table 5.3.2-2: Clinical chemical and hematological findings in rats administered dimoxystrobin for at least 91 days

Dose (ppm)	0	50	300	1,500	4,500
Hematology:					
Mean corpuscular volume [fl], m/f (1)	50.5 / 52.4	51.3 / 52.6	49.3 / 52.0	49.0 / 51.0*	47.8*** / 50.7**
Mean corpuscular hemoglobin [fmol], m/f (1); f (2)	1.11 / 1.16 1.16	1.12 / 1.17 1.16	1.09 / 1.17 1.17	1.09 / 1.15 1.15	1.04** / 1.13** 1.13***
Hemoglobin [mmol/l], f (1)	9.4	9.6	9.4	9.4	9.1**
Mean corpuscular hemoglobin concentration [mmol/l], f (2)	22.87	23.01	23.06	22.89	22.48**
Prothrombin time [sec], m (1)	27.3	28.9	27.8	28.5	29.8**
Enzymes:					
Alanine amino transferase [µkat/l] m/f (1) m/f (2)	0.91 / 0.84 0.98 / 0.99	0.94 / 0.80 1.14 / 0.90	0.83 / 0.72* 0.98 / 0.97	0.73** / 0.62*** 0.99 / 0.82**	0.60*** / 0.65** 0.77** / 0.82***
Serum cholinesterase [µkat/l], f (1)	48.69	45.05	51.13	52.41	31.12***
Clinical chemistry:					
Total protein [g/l], m/f (1)	65.83 / 67.90	64.81 / 68.35	62.88* / 67.27	61.95* / 67.55	61.05**/ 62.93**
Globulins [g/l], m/f (1)	31.07 / 28.93	30.08 / 29.40	29.37 / 28.74	28.13** / 28.37	26.86***/ 26.88*
Albumin [g/l], f (1)	38.97	38.95	38.53	39.18	36.05**
Cholesterol [mmol/l], f (1)	1.64	1.58	1.58	1.81	2.03**

* P ≤ 0.05; ** P ≤ 0.02; *** P ≤ 0.002; (1): Changes on day 49; (2) Changes on day 88;
m = males; f = females

Mean corpuscular volume (MCV) was slightly decreased in 4500 ppm males and in females from 1500 ppm onwards (3-5%). Mean corpuscular haemoglobin was slightly decreased in 4500 ppm males and females (3-6%).
Haemoglobin (3%) and mean corpuscular haemoglobin concentration were slightly decreased in 4500 ppm females (2%). Prothrombin time was slightly prolonged in 4500 ppm males.
Alanine amino transferase activity was decreased in males ≥ 1500 and in females ≥ 300 ppm. However in females the effect did not follow a dose response. Therefore the biological relevance of this effect is questionable.
Serum cholinesterase activity (but not erythrocyte cholinesterase activity) was decreased in 4500 ppm females

In clinical chemistry total protein was decreased in males at ≥ 300 ppm and in females at 4500 ppm. Globulins were decreased in males at ≥ 1500 ppm and in females 4500 ppm males. Furthermore, 4500 ppm females showed decreased albumin and increased cholesterol

The above slight changes in red blood parameters were indicative of a mild hypochromic microcytic anaemia. Alanine aminotransferase and cholinesterase activities in rats are correlated with dietary protein intake.
There were no test substance-related effects seen in urinalysis and ophthalmoscopy.

Organ weight determinations revealed no adverse test substance-related findings (see Table 5.3.2-3).

Table 5.3.2-3: Selected mean absolute and relative organ weights of rats administered dimoxystrobin for at least 91 days

Sex		Males				Females			
Organ weight [g]+	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #
Terminal weight [g]	0	443.10				244.91			
	50	436.26	(-1.5)			238.71	(-2.5)		
	300	432.28	(-2.4)			242.96	(-0.8)		
	1,500	407.56**	(-8.0)			228.14	(-6.8)		
	4,500	402.10**	(-9.3)			232.90	(-4.9)		
Spleen [g]	0	0.860		0.194		0.491		0.200	
	50	0.782	(-9.1)	0.179	(-7.7)	0.484	(-1.4)	0.203	(-1.5)
	300	0.876	(1.9)	0.203	(4.6)	0.533	(8.6)	0.219	(9.5)
	1,500	0.830	(-3.5)	0.205	(5.7)	0.513	(4.5)	0.255	(27.5)
	4,500	0.732**	(-14.9)	0.183	(-5.7)	0.505	(2.9)	0.217	(8.5)
Testes (m) [g] / Ovaries (f) [mg]	0	3.517		0.796		107.2		0.044	
	50	3.467	(-1.4)	0.798	(3.8)	81.3**	(-24.2)	0.034**	(-22.7)
	300	3.531	(0.4)	0.821	(3.1)	92.7**	(-13.5)	0.038**	(-13.6)
	1,500	3.712	(5.5)	0.915**	(15.0)	81.5**	(-24.0)	0.036**	(-18.2)
	4,500	3.490	(-0.8)	0.870	(9.3)	113.6	(6.0)	0.049**	(11.4)

+Duodenum weight is not a standard parameter in this study type
* P ≤ 0.05; ** P ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may not calculate exactly due to rounding of figures

The decreased absolute spleen weights in males treated with 1,500 ppm do not represent a treatment-related effect as it is the consequence of the reduced terminal body weight of these animals. Relative testes weights were statistically significantly increased in 1500 ppm males. No dose response was determined and furthermore no histopathological correlate was found. Testes weights of treated animals were moreover covered by historical control data, with only the relative weights of the 1500 ppm dose group exceeding to a small extent (absolute 2.918-3.871 g; relative 0.681-0.884%; BASF DocID 2015/1171112).Therefore, the effect was regarded as incidental. The decreased weights of the ovaries in females of dose groups 50 – 1,500 ppm are considered to be incidental, because there was no such effect in the high dose females treated with 4,500 ppm and no dose-response relationship could be observed. Furthermore, there was no histopathological correlate. From historical control data it is evident that the mean absolute ovary weights in the control animals were high leading to the statistically significant decrease in treated low and mid dose females (absolute 84.2-107.2 mg, relative 0.034-0.047 g; BASF DocID 2015/1171112).

Duodenum: A dose-dependent incidence of thickening of the duodenal mucosa was seen at ≥ 300 ppm macroscopically in males and females (only one male and one female animal each at 300 ppm) (see Table 5.3.2-4). The macroscopically thickening of duodenal wall corresponded in all males and nine females of the 4500 ppm dose group histopathologically with a thickening of the duodenal mucosa. The degree of severity was slight in 3 males and all females and moderate in 7 males. In the 1500 ppm dose groups thickening of the duodenal mucosa could be observed in 3 males (slight) and 2 females (minimal or slight). In the 300 ppm dose groups the macroscopic finding could not clearly be correlated to thickening of the mucosa. Microscopic measurements confirmed the dose-dependent thickening (as shown by the mean thickness at each dose) at 300 ppm and above in females and at 1500 ppm and above in males. Thickening was characterized by the villi being elongated, partly slightly broadened and branched.

Table 5.3.2-4: Selected macroscopic and microscopic findings (graded) in rats administered dimoxystrobin for at least 91 days

Dose	Males					Females				
(ppm)	0	50	300	1,500	4,500	0	50	300	1,500	4,500
Duodenum	10/	10/	10/	10/	10/	10/	10/	10/	10/	10/
- Thickening of wall	-	-	1	4	10	-	-	1	3	10
- Thickening of mucosa	-	-	-	3	10	-	-	1	2	9
Grade										
1									1	
2				3	3			1	1	9
3					7					
Animals examined/animals affected										

Additional mucosa thickness measurement had been conducted in this study, the values are given below:

Table 5.3.2-5: Mean thickness of duodenal mucosa [mm]

	Mean thickness [mm]	
Groups	male	female
Controls	0.66	0.55
50 ppm	0.65	0.55
300 ppm	0.66	0.62
1500 ppm	0.77	0.64
4500 ppm	0.98	0.78

Conclusion:
The NOAEL was 50 ppm (3 mg/kg bw/day in males and 4 mg/kg bw/day in females) based on an increase in thickness of the duodenal mucosa at 300 ppm (21 mg/kg bw/day in males, 24 mg/kg bw/day in females).
Reduced alanine aminotransferase activity in serum, and reduced serum protein, were also observed at 300 ppm. They were of limited magnitude and are not considered adverse findings in the absence of a toxicologically significant effect on body weight or food consumption at this dose level.
Further investigation indicates that at least some of the effects of dimoxystrobin (i.e. on haematology and the duodenum) are due to an effect on iron kinetics and that there is a compensatory increase in surface area for absorption of iron by the duodenum.

Additional data not peer-reviewed

Report: CA 5.3.2/1
[redacted] 2011a
BAS 505 F (Reg.No. 285 028 F) - Subchronic oral toxicity study in Wistar rats - Administration in the diet for 3 months
2011/1150025

Guidelines: EEC 87/302, OECD 408, EPA 82-1, JMAFF

GLP: yes
(certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Material and Methods:

At request of the sponsor the following additional examinations were performed and reported in this amendment: Bone marrow of all animals of control and high dose group was investigated for ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron by light microscopy after Turnbull stain. The severity of iron storage in the bone marrow was semi-quantitatively estimated (grade 1: minimal, up to grade 3: moderate).

Results:

After 3 months of treatment with the test substance, the number of male Wistar rats with iron storage in the bone marrow was decreased in test group 4 (4500 ppm), but the severity of iron storage was only minimal in all affected males. In female Wistar rats, the number of animals with iron storage as well as the severity of iron storage in the bone marrow was slightly decreased in test group 4 (4500 ppm).

Table 5.3.2-6: Iron storage in bone marrow of rats administered dimoxystrobin for at least 91 days

Dose	Males		Females	
(ppm)	0	4,500	0	4,500
Bone marrow - Iron storage	10/ 7	10/ 2	10/ 10	10/ 4
Grade 1 2 3	 7 	 2 	 4 5 1	 4

For the reduced number of male Wistar rats with minimal iron storage (2/10 in the 4500 ppm group compared to 7/10 in the control group) a treatment-related effect could not be ruled out.
In female Wistar rats, the number of affected animals was reduced in test group 4 (4500 ppm). In addition, the severity of iron storage was slightly decreased in these animals when compared with control females. The reduced iron storage in female Wistar rats of test group 4 (4500 ppm) was related to treatment.

The mean values of the severity of iron storage in the bone marrow of males are 0.7 in the controls and 0.2 in the 4500 ppm group. For female animals, mean values of 1.7 and 0.4 were observed in the control and 4500 ppm groups, respectively. Thus it can be concluded, that there is some evidence for lower iron storage in the bone marrow of rats treated with 4500 ppm ppm dimoxystrobin compared to controls, with females being more affected than males.

Studies in mice

Note: The main mouse 90-day study report (BASF DocID 1999/10388) was already submitted and reviewed in the course of the initial registration of dimoxystrobin. This study serves as the basis for the assessment of the report amendment submitted under M-CA 5.3.2/2. Amendment 1 provides the result of an additional iron staining conducted in the bone marrow

Three month toxicity study in mice by dietary administration (BASF DocID 1999/10388)

Guidelines: In compliance with OECD guideline 408 (adopted 1981)
Deviations: NA
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and Methods:
Dimoxystrobin (batch/purity: N 6 lot 3004: 98.8%) was administered to groups of 10 male and 10 female B6C3F1 Crl BR mice at dietary concentrations of 0, 1000, 4000, and 8000 ppm over a period of 3 months.
Food consumption and body weight were determined once a week. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week.
Clinico-chemical and haematological examinations were carried out at the end of the administration period in all mice (fasted). All animals were subjected to gross-pathological assessment and weights determined for 7 organs. All control and high dose animals were subject to an extensive histopathological examination. In addition stomach, duodenum, liver, gall bladder, lungs, and kidneys were examined from all animals in other dose groups; and all gross lesions were examined in all affected animals from all groups. Adrenals were also examined from all females in other dose groups. As enlargement of the duodenum was observed, the thickness of the duodenal mucosa (tip of villi to smooth muscle) was measured at 4 locations in all animals.

Results:
There were no mortalities or substance-related clinical signs of toxicity in any of the dose groups.

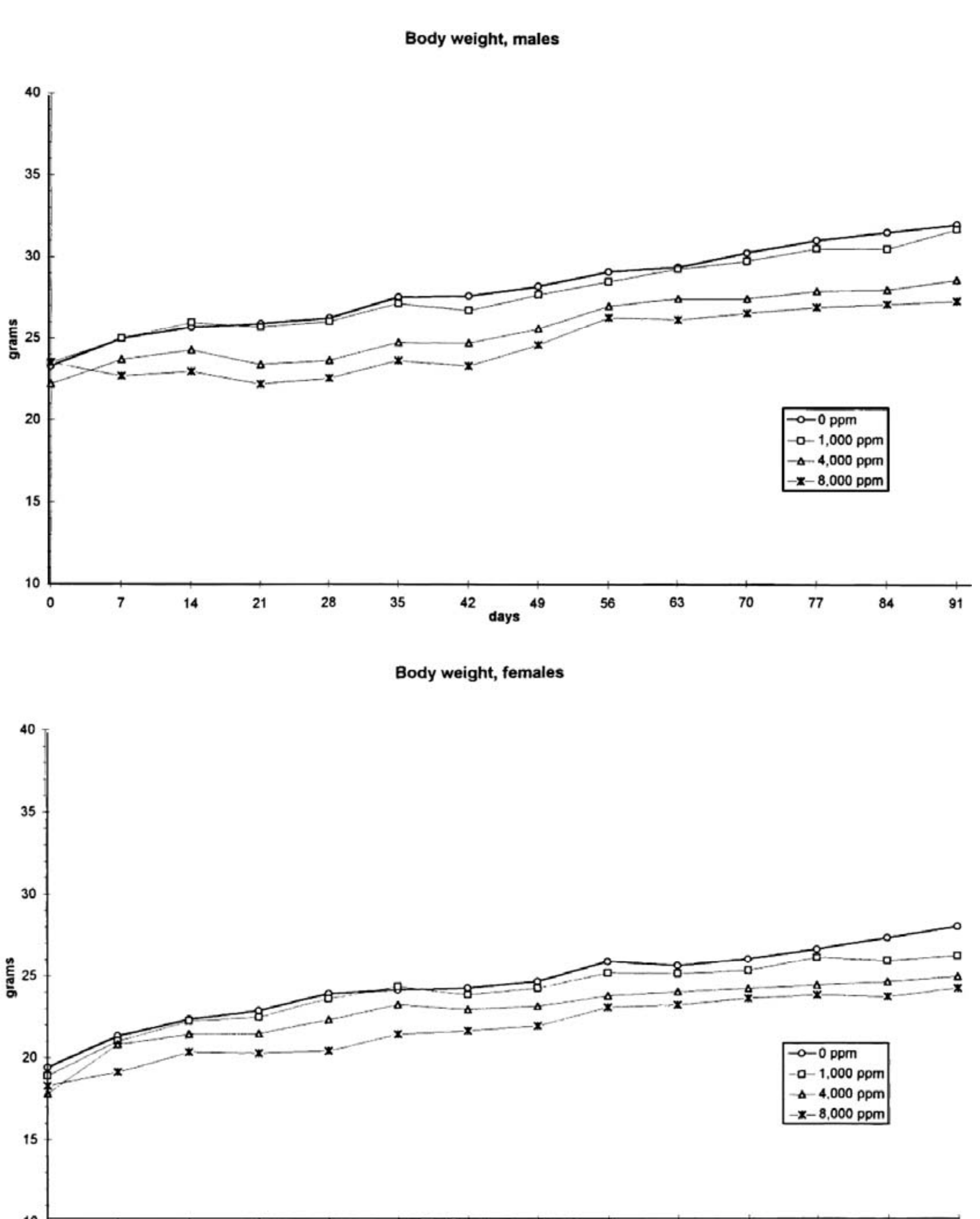
Based on food consumption and body weight data average daily compound intakes of 206, 1,187, and 2,867 mg/kg bw/d in male and of 318, 1,535, and 3,715 mg/kg bw/d in female mice were calculated at dietary dose levels of 1000, 4000 and 8000 ppm, respectively. As food spilling was observed in all groups irrespective of dose, these values may not represent the real compound intakes.

Body weight gain was clearly adversely reduced at ≥ 4000 ppm (males) and at 8000 ppm (females). There was a dose-related reduction in overall body weight in males and females from 4000 ppm onwards. Food consumption values were increased in both sexes at the two top dose levels on several occasions, most probably related to spillage of food rather than to a substance-related effect. There was no clear effect on food efficiency.

Table 5.3.2-7: Body weights and body weight gain of mice administered dimoxystrobin for at least 91 days

Dose	Male				Female			
(ppm)	0	1,000	4,000	8,000	0	1,000	4,000	8,000
Body weight;	32.0	31.7	28.6**	27.3**	27.8	26.0	24.7**	24.0**
% of control	100.0	99.0	89.3	85.2	100.0	93.8	88.9	86.5
Body weight gain;	8.8	8.3	6.4**	3.8**	8.4	7.2	6.9	5.7**
% of control	100.0	94.1	73.2	43.6	100.0	85.1	81.9	67.3
** P ≤ 0.01								

Figure 5.3.2-2: Body weights of male and female mice administered dimoxystrobin for at least 91 days



There was a slight (3%) statistically significant reduction in mean corpuscular haemoglobin in high dose males.
Clinico-chemical findings, which are considered to be test substance-related adverse effects, including decreased total protein, albumin, globulins, and triglycerides were seen at 4000 ppm and above. An increase in urea was seen in females at ≥ 4000 ppm.

Table 5.3.2-8: Clinical chemical findings in mice administered dimoxystrobin for at least 91 days

Dose (ppm)	0	1,000	4,000	8,000
Total protein [g/l], m / f	64.59 / 63.12	65.41 / 60.90	59.01*** / 59.03**	57.96*** / 58.52**
Albumin [g/l], m	37.56	37.55	34.81***	34.39***
Globulins [g/l], m/f	27.04 / 24.12	27.52 / 23.17	24.20*** / 22.35*	23.59** / 21.57**
Triglycerides [mmol/l], m / f	1.71 / 1.74	1.98 / 1.16	0.97*** / 0.99*	0.84*** / 0.87**
Urea [mmol/l], f	7.23	7.09	8.71*	9.06**
* P ≤ 0.05; ** P ≤ 0.02; *** P ≤ 0.002; m=males; f=females				

Statistically significant changes in some absolute and relative organ weights were seen primarily at 4000 ppm and above. Changes were considered secondary to the reduced terminal body weight.

Table 5.3.2-9: Selected mean absolute and relative organ weights of mice administered dimoxystrobin for at least 91 days

Sex		Males				Females			
Organ weight [g]	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #
Terminal weight [g]	0	27.99				23.99			
	1,000	27.54	(-1.6)			22.11	(-7.8)		
	4,000	24.48**	(-12.5)			20.50**	(-14.5)		
	8,000	23.22**	(-17.0)			19.84**	(-17.3)		
Liver [mg]	0	1063.9		3.815		1034.3		4.331	
	1,000	1121.6	(5.4)	4.081	(7.0)	1033.5	(-0.1)	4.671	(7.9)
	4,000	1078.1	(1.3)	4.407**	(15.5)	1015.4	(-1.8)	4.954**	(14.4)
	8,000	1054.1	(-0.9)	4.544**	(13.6)	979.8	(-5.3)	4.934**	(14.0)
Kidney [mg]	0	474.3		1.701		360.7		1.515	
	1,000	470.7	(-0.8)	1.713	(-2.3)	353.3	(-2.1)	1.601	(7.6)
	4,000	439.9	(-7.3)	1.795	(5.9)	325.5**	(-9.8)	1.588	(9.9)
	8,000	402.7**	(-15.1)	1.737	(19.1)	314.2**	(-12.9)	1.585	(13.9)
Testes (m) / Ovaries (f) [mg]	0	236.1		0.847		29.6		0.124	
	1,000	228.5	(-3.2)	0.831	(-1.9)	26.1*	(-11.8)	0.118	(-4.8)
	4,000	228.5	(-3.2)	0.937**	(10.6)	25.9*	(-12.5)	0.127	(2.4)
	8,000	224.9	(-4.7)	0.970**	(14.5)	21.9**	(-26.0)	0.111	(-10.5)
Brain [mg]	0	480.2		1.727		495.2		2.086	
	1,000	472.9	(-1.1)	1.720	(-0.4)	494.9	(-0.1)	2.244	(7.6)
	4,000	475.2	(-0.9)	1.952**	(13.0)	495.1	(0.0)	2.422**	(16.1)
	8,000	472.0	(-1.7)	2.037**	(18.0)	487.0	(-2.3)	2.461**	(18.0)
Adrenal glands [mg]	0	5.2		0.019		13.4		0.057	
	1,000	5.6	(7.7)	0.020	(5.3)	15.6	(16.4)	0.071	(24.6)
	4,000	5.5	(5.8)	0.023*	(21.1)	10.5*	(-21.6)	0.051	(-10.5)
	8,000	5.4	(3.8)	0.023*	(21.1)	9.4**	(-29.9)	0.047*	(-17.5)

* P ≤ 0.05; ** P ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may not calculate exactly due to rounding of figures

In particular, a slight decrease in relative ovary weight at 1,000 ppm and above could be related to the decrease in terminal body weight. In the absence of histopathological findings in the ovary at 8000 ppm, it is not considered a substance-related adverse finding. Furthermore, the absolute and relative ovary weight of treated females are well covered by historical control data (absolute ovary weights 20.0-29.6 mg, relative ovary weights 0.086-0.133%; BASF DocID 2015/1171113). Therefore, the effect is regarded as incidental.
Adrenals of females showed decreases in both absolute and relative weight at 4000 and 8000 ppm. The slight decrease in relative weight at 4000 ppm was not statistically significant. Both absolute and relative adrenal weights of mid and high dose females are covered by historical control data (absolute adrenal weights 9.2-18.9 mg, relative adrenal weights 0.04-0.087%; BASF DocID 2015/1171113). The reduced weight was associated with decreased vacuolization (lipid) in the X-zone of the adrenal cortex of females at 4000 and 8000 ppm, which is specific to mice and of unknown function. This effect is considered as consequence of the reduction of terminal body weight associated with reduced triglycerides in affected animals. This assessment is further confirmed by the fact, that neither adrenal weight changes nor pathological effects were detected in the chronic mouse study (see Chapter M-CA 5.5).
The increased relative weight of adrenals in males is considered not treatment related as only a single animal of the mid-dose group (#27) and two animals of the high dose group (#32,33) exceed the range of relative adrenal weight of the concurrent control group [0.014 – 0.024 mg]. Furthermore adrenal weights are in the range of historical control data (absolute adrenal weights 4.0-6.5 mg, relative adrenal weights 0.015-0.022%; BASF DocID 2015/1171113). No correlate to the adrenal findings was seen in the chronic mouse study, making a treatment related effect less likely. Erosion/ulceration of the glandular stomach showed a dose-related increased incidence at ≥ 4000 ppm.
Statistically significant increases in liver and brain weights relative to body weight in top dose males and females and relative testes weights in males were associated with a decrease in body weight. There were no substance-related gross or histopathological findings.

Thickening of duodenal mucosa was seen (grossly and microscopically) in 4000 and 8000 ppm males and females, with an increased incidence and severity at 8000 ppm (where thickening was slight to severe). Microscopic measurements confirmed the dose-dependent thickening (as shown by the mean thickness at each dose) at these 2 dose levels. Thickening was characterised by the villi being elongated, partly slightly broadened and branched.

Table 5.3.2-10: Selected macroscopic and microscopic findings (graded) in mice administered dimoxystrobin for at least 91 days

Dose	Males				Females			
(ppm)	0	1,000	4,000	8,000	0	1,000	4,000	8,000
Duodenum	-	10/	10/	10/	-	10/	10/	10/
- Thickening of wall		-	9	10		-	9	10
- Thickening of mucosa		-	5	9		-	2	6
Grade								
1	-	-	4		-	-	2	
2			1	3				3
3				4				3
4				2				
Adrenal cortex -X-zone: lipid decrease	-	-	-	-	-	-	2	10
Stomach -erosion/ ulcer	-	-	2	4	1	1	3	6

Animals examined/animals affected

Conclusion:

It is considered that no clear substance-related adverse effects were seen at 1000 ppm. The proposed NOAEL is therefore 1000 ppm (206 mg/kg bw in males and 318 mg/kg bw in females) based on reduced weight gain, clinico-chemical findings, and histopathological changes in the adrenals, duodenum and stomach at 4000 ppm (about 1187 mg/kg bw/day in males and 1535 mg/kg bw/day in females, although these calculated dose levels may be slightly high due to food spillage).

Additional data not peer-reviewed

Report: CA 5.3.2/2
[redacted] 2011b
BAS 505 F (Reg.No. 285 028 F) - Subchronic oral toxicity study in B6C3F1/CrlBR mice - Administration in the diet for 3 months
2011/1150024

Guidelines: EEC 87/302, OECD 408, EPA 82-1, JMAFF

GLP: yes
(certified by Ministerium fuer Arbeit, Soziales und Gesundheit, Postfach 3180, 55021 Mainz)

Material and Methods:

At request of the sponsor the following additional examinations were performed and reported in this amendment: Bone marrow of all animals of control and high dose group was investigated for ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron by light microscopy after Turnbull stain. The severity of iron storage in the bone marrow was semi-quantitatively estimated (grade 1: minimal, up to grade 4: severe).

Results:

After 3 months of treatment with the test substance, all male and female mice showed minimal to severe iron storage in the bone marrow. The severity of the iron storage was decreased in male and female animals of the 8000 ppm dose group. The significantly reduced iron storage in males and females of 8000 ppm dose group is considered to be treatment-related.

Table 5.3.2-11: Iron storage in bone marrow of mice administered dimoxystrobin for at least 91 days

Dose	Males		Females	
(ppm)	0	8,000	0	8,000
Bone marrow - Iron storage	10/ 10	10/ 10	10/ 10	10/ 10
Grade				
1		3		2
2		5	2	4
3	6	2	5	4
4	4		3	

The mean values of the severity of iron storage in the bone marrow of males are 3.4 in the controls and 1.9 in the 8000 ppm group. In females, mean values of 3.1 and 2.2 were observed in the control and 8000 ppm groups, respectively. Thus it can be concluded, that there is some evidence for lower iron storage in the bone marrow of mice treated with 8000 ppm ppm dimoxystrobin compared to controls.

Studies in dogs

Note: The main dog 90-day study report (BASF DocID 1999/11676) was already submitted and reviewed in the course of the initial registration of dimoxystrobin. This study serves as the basis for the assessment of the report amendment submitted under CA 5.3.2/3. Amendment 1 provides the result of an additional histopathological evaluation of the bone marrow. To take into account that the majority of the data were already reviewed, the following study summary will be more comprehensive than for other studies already reviewed, but will be less detailed than the summary of new studies.

Three month toxicity study in dogs by dietary administration (BASF DocID 1999/11676)

Guidelines: In compliance with OECD guideline 409 (adopted 1981)
Deviations: NA
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Dimoxystrobin (batch/purity: N 6 (Lot No. 3004): 98.8%) was administered to groups of five male and five female pure bred Beagle dogs at dietary concentrations of 0, 200, 600 and 1200 ppm for 3 months. Food consumption of the animals was determined daily and their body weight once a week. The animals were examined at least once each working day for any signs of toxicity and a check for any moribund or dead animals was made twice a day. Clinical chemistry and haematological examinations as well as urinalyses were carried out once before and two times during the administration period from fasted dogs. Ophthalmological examinations were carried out 3 days before the beginning of the administration period and on study day 95. All animals were subjected to gross-pathological assessment, organ weighing (9 organs) followed by extensive histopathological examination.

Results:
Based on food consumption and body weight data average daily compound intakes of 6.1, 18.5, and 36.8 mg/kg bw/d in male and of 6.4, 18.9, and 37.7 mg/kg bw/d in female dogs were calculated at dietary dose levels of 200, 600 and 1200 ppm, respectively.

There were no mortalities in any of the dose groups.

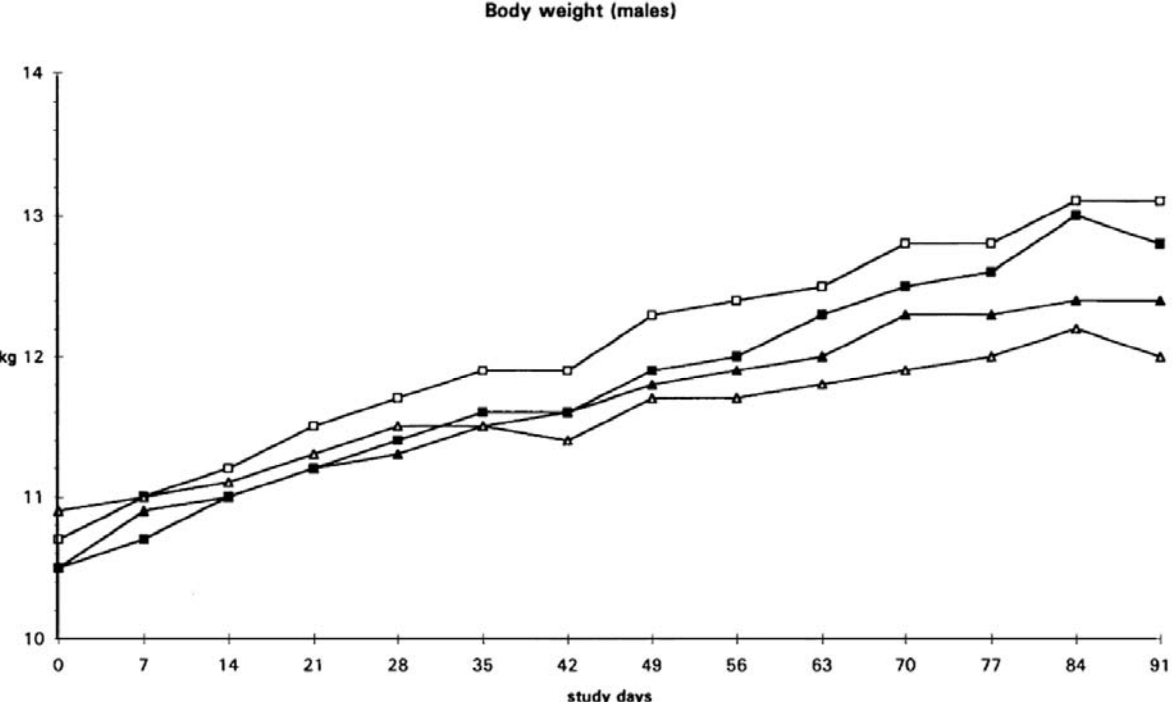
At the high dose level (1200 ppm) diarrhea was observed in male and female animals on several occasions up to week 12, as well as vomiting during the first two weeks of treatment. These clinical findings were assessed as being test substance-related.

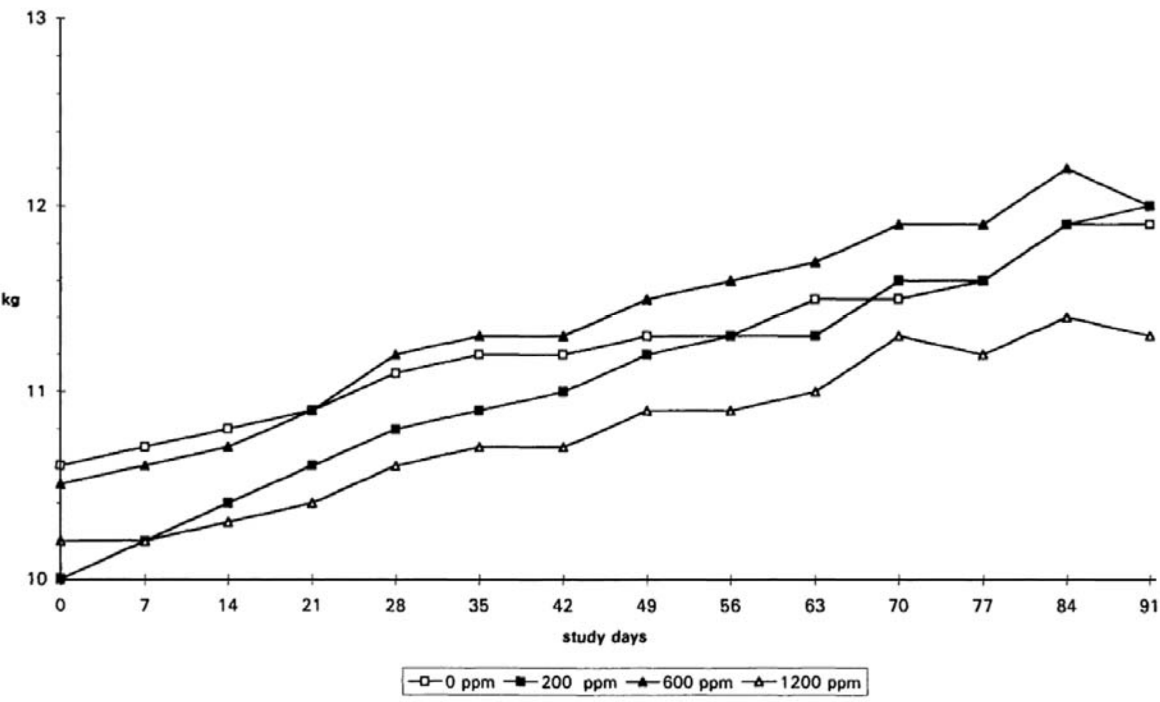
High dose males showed a statistically significant reduction in body weight gain, which was seen consistently from day 42, and is assessed to be treatment-related.
A slight reduction in food consumption in females of the high dose group (overall mean of 96% of food consumed, compared with 100% for all other groups) was considered to be treatment-related. No ophthalmological lesions were noted.

Table 5.3.2-12: Body weights and body weight gain of dogs administered dimoxystrobin for at least 91 days

Dose	Males				Females			
(ppm)	0	200	600	1,200	0	200	600	1,200
Body weight	13.1	12.8	12.4	12.0	11.9	12.0	12.0	11.3
Body weight change	2.4	2.3	1.9	1.1*	1.3	2.0	1.5	1.1
* P ≤ 0.05								

Figure 5.3.2-3: Body weights and body weight gain of dogs administered dimoxystrobin for at least 91 days





Slight, statistically significant reductions (by 2-7%) in some red blood cell parameters were seen at 1200 ppm. These changes might be due to extremely mild microcytic hypochromic anaemia. Some changes in clinical chemistry parameters (electrolyte and protein levels) were also seen at 1200 ppm.

Table 5.3.2-13: Clinical chemical and hematological findings in dogs administered dimoxystrobin for at least 91 days

Dose (ppm)	0	200	600	1,200
Hematology:				
Mean corpuscular volume [fl], m (2)	66.9	66.1	66.8	62.7*
Mean corpuscular hemoglobin [fmol], m (1) m (2)	1.45 1.45	1.43 1.38	1.44 1.44	1.35** 1.35*
Mean corpuscular hemoglobin concentration [mmol/l], m (1)	21.65	21.50	21.22* #	21.13**
Clinical chemistry:				
Potassium [mmol/l], m (1) m (2)	4.60 4.51	4.66 4.87**	4.55 4.77	4.98** 5.07**
Chloride [mmol/l], m (1)	113.2	113.7	113.8	116.3**
Sodium [mmol/l], m (1)	146.8	146.8	146.7	145.1**
Total protein [g/l], f (2)	57.64	57.79	57.93	53.94**
Albumin [g/l], m (1)	33.54	33.24	33.45	30.33**
Globulins [g/l], m / f (1) m / f (2)	28.92 / 25.11 30.26 / 25.71	27.81 / 26.99 27.51 / 27.35	27.62 / 25.76 26.72 / 27.31	26.97 / 23.83 ## 26.33 / 23.71 ##

* $P \leq 0.05$; ** $P \leq 0.02$; (1): Changes on day 43; (2): Changes on day 92;
m = males; f = females;
#: considered to be incidental in nature, because it is an isolated finding that was seen in one sex and in one interval only, and the difference between the control and test group value is only slight in the magnitude of change;
##: not statistically significant, but considered treatment-related

There were no treatment-related changes in the urine parameters measured. However, a reduction in mean urine volume in high dose females at the end of the study was seen.

Organ weight determinations revealed no test substance-related findings. A statistically significant increase in brain weight relative to body weight in top dose males was associated with a decrease in body weight. There were no substance-related gross or histopathological findings. Notably, no thickening of the duodenal mucosa was detected.

Table 5.3.2-14: Selected mean absolute and relative organ weights of dogs administered dimoxystrobin for at least 91 days

Sex		Males				Females			
Organ weight [g]	Dose [ppm]	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #	Absolute weight	Δ%	Relative weight [% of bw]	Δ% #
Terminal weight [g]	0	13380				12360			
	200	13260	(-0.9)			12140	(-1.8)		
	600	12800	(-4.3)			12180	(-1.5)		
	1,200	12120	(-9.4)			11240	(-2.6)		
Brain [g]	0	81.616		0.611		74.720		0.607	
	200	78.810	(-3.4)	0.597	(-2.3)	79.232	(6.0)	0.653	(7.6)
	600	82.346	(0.9)	0.647	(5.9)	80.940	(8.3)	0.667	(9.9)
	1,200	83.662	(1.0)	0.694*	(13.6)	76.806	(2.8)	0.692	(14.0)

* P ≤ 0.05; ** P ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may not calculate exactly due to rounding of figures

Conclusion:
The NOAEL in this study was 600 ppm (18.5 mg/kg bw in males and 18.9 mg/kg bw in females) based on diarrhoea, reduced body weight gain and food consumption, slight changes in red blood cells parameters and changes in clinical chemistry parameters at 1200 ppm (36.8 mg/kg bw/day in males and 37.7 mg/kg bw/day in females). The slightly increased incidence of diarrhoea at 600 ppm is considered to be a finding of no toxicological importance.

Additional data not peer-reviewed

Report: CA 5.3.2/3
[redacted] 2011c
Amendment No. 1 - BAS 505 F (Reg.No. 285 028) - Testing for toxicity in beagle dogs - Administration in the diet for 3 months
2011/1150023

Guidelines: EEC 87/302 B, OECD 409, EPA 82-1, MAFF Testing Guideline for Toxicology Studies (1985)

GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Material and Methods:
At request of the sponsor the following additional examinations were performed and reported in this amendment: Bone marrow of all animals of control and high dose group was investigated for ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron by light microscopy after Turnbull stain. The severity of iron storage in the bone marrow was semi-quantitatively estimated (grade 1: minimal, up to grade 2: slight).

Results:
After 3 months of treatment with the test substance, some male and female Beagle dogs showed minimal or slight iron storage in the bone marrow (sternum). In males, 3 dogs of the control group and one high dose male (1200 ppm) showed iron storage in the bone marrow (sternum). In females, each 2 dogs were affected in the control group and in test group 3 (1200 ppm). In most of these animals the severity of iron storage was only minimal. The slight differences observed are considered incidental.

Table 5.3.2-15: Iron storage in bone marrow of dogs administered dimoxystrobin for at least 91 days

Dose	Males		Females	
(ppm)	0	1200	0	1200
Bone marrow - Iron storage	5/ 3	5/ 1	5/ 2	5/ 2
Grade 1 2	 3 	 1 	 1 1	 2

The mean values of the severity of iron storage in the bone marrow of males are 0.6 and 0.2 in the control and 1200 ppm group. Furthermore, mean values of 0.6 and 0.4 were observed in the control and 1200 ppm groups of female animals, respectively. Thus there is no clear evidence for a treatment-related change in iron storage in bone marrow of dogs.

Overall conclusion on additional iron staining conducted in bone marrow

Taken together the results of the histopathological evaluations of bone marrow in the 3-month studies in rats, mice and dogs, it could be shown, that iron storage was affected by treatment with dimoxystrobin in mice and female rats. Both male and female mice showed a decrease in iron storage in the bone marrow after treatment with 8000 ppm dimoxystrobin, which was considered to be related to treatment.
Additionally, treatment of female rats with 4500 ppm dimoxystrobin resulted in a slight decrease in the number of animals with iron storage as well as the severity of iron storage in the bone marrow. Male rats were less affected, only minimal iron storage was observed in all affected animals. Therefore, a treatment-related effect in the reduced number of male Wistar rats with minimal iron storage could not be ruled out.
In dogs no treatment-related effect on iron storage could be observed. The slight differences observed were considered incidental. The results of the additional iron staining are well correlated with the observed effects on duodenum thickening, with effects seen in mice and rats and not in dogs.

The effects of dimoxystrobin on iron metabolism and the associated duodenum toxicity are discussed in more detail in chapter M-CA5.5 of this dossier.

One year toxicity study in dogs by dietary administration (BASF DocID 2000/1012307)

Guidelines: In line with OECD guideline 452 (adopted 1981)
Comment: The study design was very similar to the 90-day dog study.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Dimoxystrobin (batch/purity: N 15: 98.4%) was administered to groups of 5 male and 5 female pure bred Beagle dogs at dietary concentrations of 0, 200, 400 and 800 ppm for about 12 months.

Results:
No mortality occurred during the study.

At the high dose vomitus was observed during the first week of administration in 2 males and 4 females, and diarrhoea in all males and 4 females. The very low incidence of diarrhoea in controls and at 200 and 400 ppm provides no evidence for a substance-related effect. In addition, vomitus in one female at 400 ppm in the second week is not considered a clear substance-related adverse effect even though no vomitus was recorded in controls.

No ophthalmological lesions were noted.

There were no clear substance-related effects on food consumption or on food efficiency. There were no effects on body weight or body weight gain.

No clear adverse treatment-related changes were observed during haematological investigations. The largest reductions in MCV and MCH at the top dose were on day 98 but these were reductions of only 2-4% and are therefore not considered clear adverse findings (they were not statistically significant). Decreased concentrations in total protein, albumin and globulins were detected in the high dose animals of either sex throughout the study. Although most of these findings were not statistically significantly different from the corresponding controls, they do provide evidence of a trend towards decreased values. Decreased cholesterol levels, probably due to increased incidence of diarrhea, were also found in the sera of the high dose dogs.

Table 5.3.2-16: Clinical chemical and hematological findings in dogs administered dimoxystrobin for 12 months

Dose (ppm)	0	200	400	800
Hematology:				
Mean corpuscular volume [fl], m (1) f (1)	 65.2 65.6	 64.6 67.3	 64.9 64.4	 63.7 64.3
Mean corpuscular hemoglobin [fmol], m (1) f (1)	 1.40 1.41	 1.40 1.44	 1.37 1.39	 1.35 1.38
Mean corpuscular hemoglobin concentration [mmol/l], m (1)	21.44	21.69	21.36	21.14
Clinical chemistry:				
Sodium [mmol/l] m (2)	146.0	146.1	148.8**	145.2
Potassium [mmol/l], f (2)	4.47	4.55	4.88*	4.91**
Calcium [mmol/l], m (2)	2.65	2.78*	2.71	2.60
Total protein [g/l], m / f (1) m / f (2)	 58.03 / 59.35 57.21 / 62.09	 58.79 / 59.68 60.71* / 61.91	 57.91 / 59.23 58.98 / 61.62	 52.70 / 53.85** 51.75 / 54.81**
Albumin [g/l], m / f (1) m / f (2)	 34.64 / 36.02 30.58 / 30.35	 34.85 / 35.70 31.81 / 32.15	 34.30 / 35.99 31.38 / 31.48	 31.22* / 33.11** 28.34 / 28.21*
Globulins [g/l], m / f (1) m / f (2)	 23.39 / 23.33 26.63 / 31.74	 23.94 / 23.98 28.90 / 29.75	 23.61 / 23.24 27.60 / 30.14	 21.47 / 20.73 23.41* / 26.61
Cholesterol [mmol/l] m (1)	5.00	5.33	4.82	3.73**
* P ≤ 0.05; ** P ≤ 0.02; (1): Changes on day 98; (2): Changes on day 365 (m) / 366 (f); m = males; f = females;				

Mean serum alkaline phosphatase activity was decreased (by 27-41%) in high dose males at all sample times during exposure but the differences were not statistically significant.

No effects on urinalysis were seen.

Organ weight determinations showed no statistically significant differences and there were no substance-related gross or histopathological findings. Notably no thickening of the duodenal mucosa was observed.

Conclusion:
The NOAEL in this study was 400 ppm (males: 11.0 mg/kg bw; females: 11.2 mg/kg bw) based on an increased incidence of diarrhoea and decreased serum protein and cholesterol at 800 ppm (males 22.3 mg/kg bw/day, females 22.7 mg/kg bw/day).

CA 5.3.3 Other routes

Regarding other routes of exposure, the applicant performed a 28-day study by the dermal route.

Four week toxicity study in rats by dermal application (BASF DocID 1999/11640)

Guidelines: In compliance with OECD guideline 410 (adopted 1981)
Comments: The study exceeded the requirements of OECD guideline 410 (1981) by including open field clinical observations and a more extensive histopathological examination.
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Dimoxystrobin (batch/purity: N 15: 98.4%) was administered to groups of 10 male and 10 female Wistar rats by dermal route (6 hours/day; 5 days/week, semi-occlusive dressing) for about 4 weeks at doses of 0 (vehicle control, 0.5% carboxymethyl cellulose (CMC) and 0.5% Cremophor EL), 50, 200 and 1000 mg/kg bw. The nominal dimoxystrobin concentrations were 0, 10, 40 and 200 g/l.

Results:
No animal died and no substance-related effects were observed during clinical examinations (including skin) or ophthalmoscopy.
There were no clear substance-related adverse effects on body weight or body weight gain. There was no substance-related effect on food consumption or food efficiency.
There were no substance-related effects on haematology or clinical chemistry. Urinalysis revealed no convincing substance-related effect.
No substance-related lesions were seen on gross or microscopic examination (including treated skin).

Conclusion:
No substance-related effects were observed. The NOAEL was therefore 1000 mg/kg bw/day in both sexes.

CA 5.4 Genotoxicity Testing

Studies evaluated in the draft assessment report (DAR, July 2003):
Dimoxystrobin (BAS 505 F) has been tested both *in vitro* and *in vivo* in a comprehensive battery of mutagenicity and genotoxicity tests using bacterial and mammalian cells as well as whole animals to assess the genotoxic potential. The available studies are presented in tabular form in Table 5.4.1-1 and have been briefly summarized. These studies have already been evaluated by European authorities and the United Kingdom as Rapporteur Member State (European Commission Peer Review Program) and were considered to be acceptable.

Studies submitted in this supplementary dossier (not yet peer-reviewed):
No additional data on genotoxicity of dimoxystrobin was generated.
Additional genotoxicity studies on the representative formulation BAS 540 01 F can be found in the chapter M-CP 7.1.7 of this dossier.

Dimoxystrobin has been tested both *in vitro* and *in vivo* in a series of mutagenic and genetic toxicity tests using bacterial and mammalian cells and whole animals to assess its potential to induce mutations and to damage chromosomes and DNA. All of these tests were clearly negative.

In summary the available data on genotoxicity of dimoxystrobin does not indicate a genotoxic potential.

Thus, the conclusion for relevant endpoints for the current re-registration remains as follows:

In vitro studies	No genotoxic potential
In vivo studies	No genotoxic potential
Potential for genotoxicity	No genotoxic potential

CA 5.4.1 In vitro studies

Table 5.4.1-1: *In vitro* mutagenicity studies with dimoxystrobin

Test System	Test Object	Conc.	Purity	Results	Reference
In vitro Mutagenicity in bacterial cells (Ames test) (with and without Metabolic activation)	*S. typhimurium* TA-98-100-1535-1537 *E. coli WP2 uvrA*	20-5000 µg/plate (plate incorporation assay) 4-2500 µg/plate (pre-incubation assay)	98.8%	Negative	BASF DocID 1998/10411 1999/10454
In vitro Mutagenicity in mammalian cells (with and without metabolic activation)	CHO/HPRT	1st experiment: 1.6-50 µg/mL (-S9, 4 h), 15.6-500 µg/mL (+S9, 4 h) 2nd experiment: 3.1-50 µg/mL (-S9, 18 h), 12.5-200 µg/mL (+S9, 4 h)	98.4%	Negative	BASF DocID 1999/10539
In vitro cytogenicity Chromosome aberration assay (with and without activation)	Chinese hamster V79 cells	1st experiment: 6-25 µg/mL (-S9, 4/18 h), 12-50 µg/mL (+S9, 4/18 h) 2nd experiment: 3-12 µg/mL (-S9, 18/18 h), 12 µg/mL (-S9, 18/28 h), 12-50 µg/mL (+S9, 4/28 h)	98.4%	Negative (Not clastogenic and no clear effect on chromosome number)	BASF DocID 1999/11417
In vitro DNA damage and repair, UDS	Rat hepatocytes	1st experiment: 0.16-10 µg/mL 2nd experiment: 0.25-2 µg/mL	98.4%	Negative	BASF DocID 1999/10523

CA 5.4.2 In vivo studies in somatic cells

Table 5.4.2-1: *In vivo* mutagenicity studies with dimoxystrobin

Test System	Test Object	Conc.	Purity	Results	Reference
In vivo cytogenicity micronucleus assay	Mouse bone marrow cells	500-2000 mg/kg bw (gavage)	98.4%	Negative	BASF DocID 1998/10412 1998/10628

CA 5.4.3 In vivo studies in germ cells

No studies were available. No studies were required.

CA 5.5 Long-Term Toxicity and Carcinogenicity

Studies evaluated in the draft assessment report (DAR, July 2003):
Dimoxystrobin has been tested in rats and mice in chronic toxicity and carcinogenicity feeding studies. All studies are scientifically valid. The studies listed in Table 5.5-1 have been evaluated and peer reviewed during the previous Annex I inclusion process. For the convenience of the reviewer, these studies are summarized below.

Table 5.5-1: Summary of long-term toxicity/carcinogenicity studies conducted with dimoxystrobin

Study	NOAEL (mg/kg bw/d)	Critical effects	Reference
24 month chronic toxicity Wistar rats 0, 50, 150, 500 ppm	150 ppm 7-9 mg/kg bw/day	500 ppm: Reduced body weight gain, impairment of food consumption and duodenal thickening. Oncogenicity: No increase in tumor incidences	BASF DocID 2000/1016865; 2002/1013942
24 month carcinogenicity study Wistar rats 0, 50, 150, 500 ppm	150 ppm 7-10 mg/kg bw/day No substance-related oncogenic response. Thus NOAEL for tumors = 500 ppm.	500 ppm: Reduced body weight gain impairment of food consumption and duodenal thickening. Oncogenicity: No evidence of carcinogenicity	BASF DocID 2000/1016866; 2002/1013943
18 month carcinogenicity study B6C3F1/Rj mice 0, 25, 200, 1000 ppm (males) 0, 25, 100, 500 ppm (females)	25 ppm (m) 4 mg/kg bw/day (m) 100 ppm (f) 20 mg/kg bw/day (f)	500 ppm (females), 1000 ppm (males): Reduced body weight gain, erosions/ulcers in the glandular stomach, duodenal hyperplasia + adenoma and/or adenocarcinoma, thickening of duodenal mucosa. 200 ppm (males): Reduced body weight gain	BASF DocID 2001/1000021; *2011/1040675#*

#Reference (BASF DocID 2011/1040675) in italic describes additional statistical evaluations within the carcinogenicity study in mice and are submitted the first time in this supplemental dossier. As these report amendments do not affect the outcome and interpretation of the studies they are listed in this table.

Mechanistic studies reported in the original dossier can be found in chapter M-CA 5.8.2 of this dossier.

Studies submitted in this supplementary dossier (not yet peer-reviewed):
No new long-term or carcinogenicity studies have been performed. Only, one study amendment to the carcinogenicity study in mice with additional statistical evaluations has been added. Brief study summaries, including study amendments are provided below.

Mechanistic studies performed with dimoxystrobin not reported in the original dossier are summarized in M-CA 5.8.2. An overall assessment of the mode of action of duodenal toxicity of dimoxystrobin can be found at the end of chapter M-CA 5.5.

Evaluation in the DAR:
"Chronic toxicity and carcinogenicity studies with rats exposed for up to two years were conducted in parallel at the same laboratory. A maximum tolerated dose was achieved at the high dose level for females (overall body weight gain depression of up to 15.5%). An acceptable top dose was also used for males because there was a slight (<10%) reduction in overall body weight gain. Food consumption was also reduced at the top dose.
There were reductions in two clinico-chemical parameters, alkaline phosphatase and alanine amino transferase, in the chronic rat study. This effect is considered to be characteristic of this class of compounds in the rat and is likely to be related to reduced food absorption. Reductions in activity of these two enzymes were seen at the top and middle dose. Effects at the middle dose are not considered to be adverse in the absence of adverse effects on body weight gain or food consumption (indeed the degree of reduction in enzyme activity at all dose levels does not suggest a clear adverse response).
Macroscopic and histopathological investigations of the rats revealed slight diffuse thickening of duodenal mucosa in both sexes at the high dose. Histologically the duodenal mucosa was nearly comparable to controls, only differing by having elongated and partly slightly broadened villi (there is no mention in the text of the study reports of the branched villi seen in the 3-month rat study). It is possible that this thickening of the duodenal mucosa should be regarded as an adaptive effect, rather than as an adverse effect. A low incidence of thickening of the mucosa was also detected grossly in males at 50 ppm (2 males) and 150 ppm (3 males) but this was not confirmed microscopically. It is notable that at the top dose (500 ppm) many more animals were identified grossly with a thickened mucosa than were identified on histological examination; it is possible that this is related to the diffuse nature of the mucosal thickening.
There was no convincing evidence of a substance-related oncogenic response in rats. The slightly increased incidence of c-cell adenoma of the thyroid in female rats at the top dose is considered to be an incidental finding.

A carcinogenicity study in B6C3F1 mice used a top dose of 500 ppm for females which was a maximum tolerated dose (13% reduction in body weight gain) and a top dose of 1,000 ppm for males which exceeded a maximum tolerated dose (34% reduction in body weight gain). However, there was no test substance-related increase in mortality or clinical signs of toxicity. Reduced body weight gain was also seen in mid-dose males.

In the duodenum of top dose mice, there were increased incidences of adenoma and adenocarcinoma, as well as focal hyperplasia (characterized by a higher density of villi and increased lengths of crypts, but not graded as to severity) which was interpreted as a pre-neoplastic lesion. A diffuse, and in most cases minimal or slight, thickening of duodenal mucosa was also seen in top dose mice. The histological appearance of this diffuse thickening, which was different to the focal hyperplasia, was essentially the same as that seen in the rat chronic/carcinogenicity studies. It is possible that the diffuse thickening should be regarded as an adaptive response (rather than as an adverse response).
The incidence of erosions or ulcers in the glandular stomach was increased in top dose male and female mice. The same lesions were seen in the mouse 90-day study. However, it is noteworthy that dimoxystrobin is not classifiable as a skin or eye irritant.

Since dimoxystrobin is non-genotoxic, it was considered a category 3 carcinogen and labelled with R40 based on the clear substance-related increase in duodenal tumors in mice. Evidence for a proposed mode of action for formation of duodenal tumours following exposure to dimoxystrobin has been provided. The relevance of the duodenal tumours in mice for humans could not be dismissed at that point in time."

Based on the data provided in 2003, the list of EU agreed endpoints specified:

Target/critical effect:	Reduced body weight gain and/or increased duodenal thickening. Duodenal tumours in mice and thyroid adenomas in rats.
Lowest relevant NOAEL	18-month mouse: 25 ppm (4 mg/kg bw/day)
Carcinogenicity:	Duodenal tumours in mice. Non-genotoxic mechanism proposed (increased proliferation of duodenal mucosa to increase surface area for absorption of iron in response to reduced serum iron levels) **Carc cat 3: R40**

Submission of not yet peer-reviewed studies in this supplementary dossier:

In this dossier an amendment to the already submitted long-term study in mice is submitted. Statistics of microscopic findings was performed in all protocol organs. The additional investigations and the results are provided in this amendment (BASF DocID 2011/1040675). Further, historical control data have been collected for the biological range of organ weights of control rats and mice from long-term studies running in the same lab (BASF DocID 2015/1171114 and 2015/1171115)

Mechanistic studies, related to the mode of action for effects on the duodenum, conducted with dimoxystrobin and previously not reported in the original dossier are summarized in chapter MCA 5.8.2. A local reversible thresholded mechanism for the induction of duodenum tumors, correlated with lower iron supply in mice is proposed (see below).

As no new long-term toxicity or carcinogenicity studies are submitted in this supplementary dossier, the conclusion for relevant endpoints for the current renewal of approval remains as follows:

Long-term effects (target organ/critical effect)	Reduced body weight gain and/or increased duodenal thickening.	
Relevant long-term NOAEL	18-month mouse: 25 ppm (4 mg/kg bw/day)	
Carcinogenicity (target organ, tumour type)	Slightly increased incidence of duodenal adenoma/adenocarcinoma in mice. Non-genotoxic mechanism proposed (local reversible interaction with duodenum receptors leading to lower iron absorption and thresholded cell proliferation of duodenal mucosa to increase surface area for absorption of iron)	
Relevant NOAEL for carcinogenicity	18-month mouse: F: 100 ppm (20 mg/kg bw/day)	

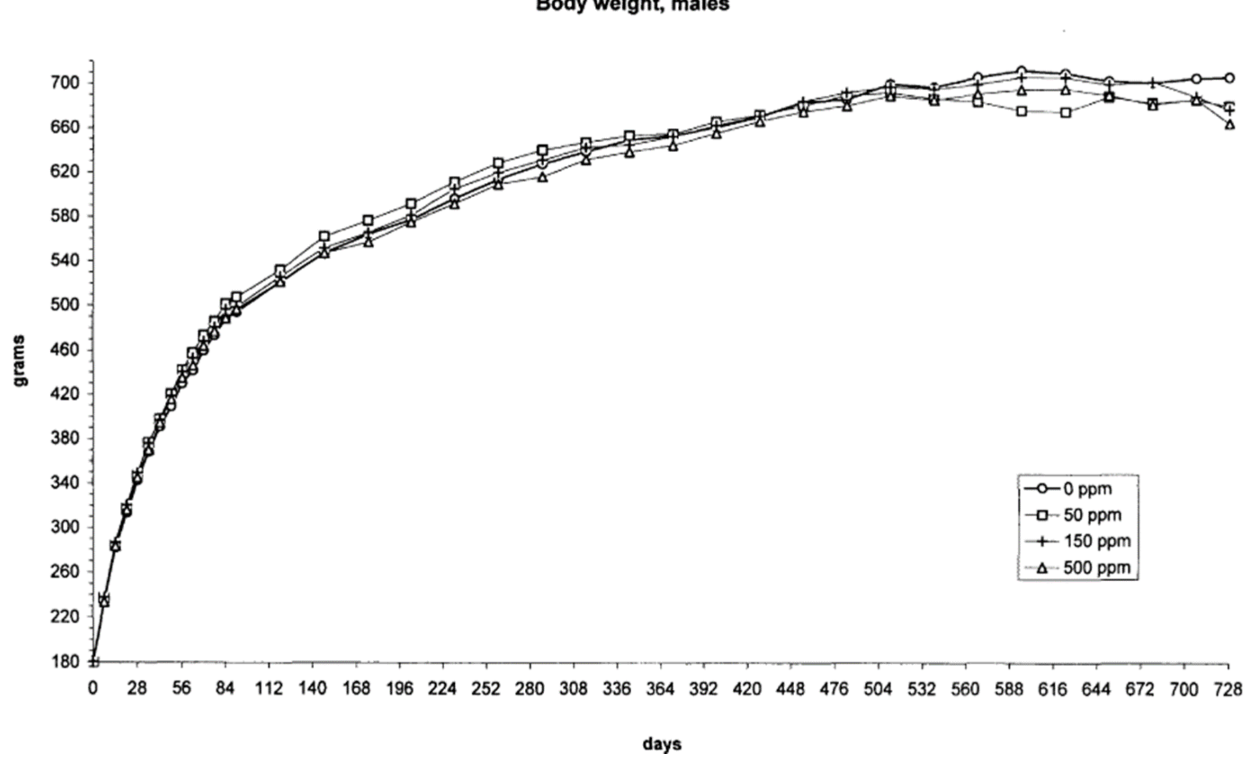
Dimoxystrobin (BAS 505 F) – Chronic toxicity study in Wistar rats. Administration in the diet for 24 months (BASF DocID 2000/1016865 and 2002/1013942)

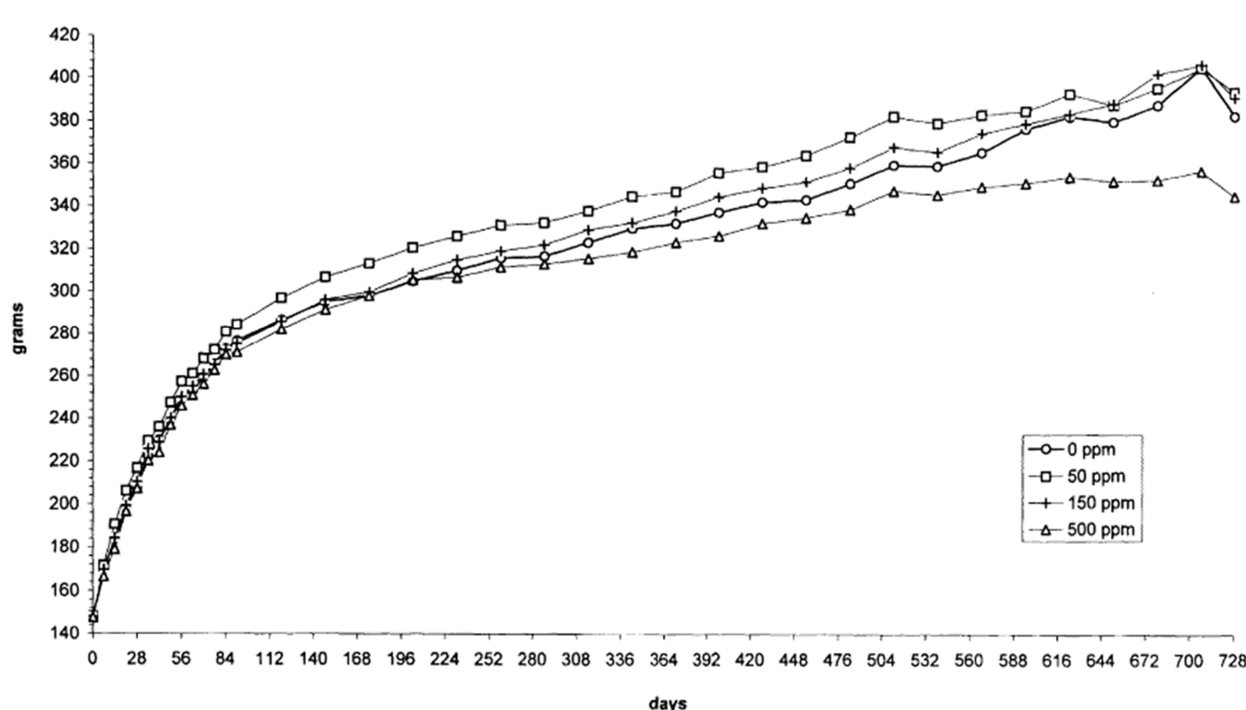
Guidelines: Equivalent to OECD guideline 452 (adopted 1981)
Deviations: No
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Materials and Methods:
Dimoxystrobin (batch/purity: N 6: 98.8%; N 15: 98.4%) was administered to groups of 20 male and 20 female Wistar rats at dietary concentrations of 0, 50, 150 and 500 ppm for 24 months. Mean test substance intakes were 2.3, 6.8, and 22.5 mg/kg bw/day for males and 3.0, 8.9, and 30.1 mg/kg bw/day for females.
Food consumption and body weights were determined once a week during the first 13 weeks, thereafter at 4-week intervals. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive clinical examinations and palpations of the animals were performed once a week. Ophthalmological examinations were carried out prior to the start and towards the end of dosing. Urinalysis, clinico-chemical and haematological examinations were carried out 3, 6, 12, 18, and 24 months after start of the administration period on all surviving animals.
The animals were subjected to gross-pathological assessment and organ weights determined for 6 organs (namely liver, kidneys, testes/ovaries, brain, adrenal glands) from animals surviving to termination. Specifically organ weights of 15, 16, 15 and 17 male animals were determined from dose groups 0, 50, 150 and 500 ppm and of 14, 17, 14, 18 females. All control and high dose animals (and intercurrent decedents) were subject to an extensive histopathological examination. In addition stomach, duodenum, liver, lungs and kidneys were examined from all animals in other dose groups; and all gross lesions were examined in all affected animals from all groups.

Results:
There was no test substance-related increase in mortality, or clinical signs of toxicity or ophthalmological effects. Food consumption and body weight gain were slightly lower in high dose females compared to controls. At study termination body weight/body weight change were impaired in high dose females (9.8%/15.5% below controls). For all treated males food consumption and body weights were comparable to controls. Figure 5.5-1 shows body weight developments in male and female rats during the entire study period.

Figure 5.5-1: Body weights of rats administered dimoxystrobin for 2 years – chronic toxicity study





There were no substance-related adverse effects on haematology. Statistically significant reductions in alkaline phosphatase and/or alanine amino transferase were observed in high dose rats of both sexes (by 10-31%). These reductions in enzyme activity changes are not considered adverse findings, where they occur in the absence of adverse effects on body weight gain or food consumption. There were no treatment-related effects on urinalysis.

No test-substance related changes in organs weights could be detected.

Table 5.5-2: Selected mean absolute and relative organ weights of rats administered dimoxystrobin for 2 years – chronic toxicity group

Sex		Males				Females			
Organ weight [mg]	Dose [ppm]	Absolute weight (SD)	Δ%	Relative weight [% of b.w.] (SD)	Δ% #	Absolute weight (SD)	Δ%	Relative weight [% of b.w.] (SD)	Δ% #
Terminal weight [g]	0	680.4 (104.0)				363.3			
	50	649.8 (54.9)	(-4.5)			370.5	(2.0)		
	150	647.1 (88.5)	(-4.9)			369.4	(1.7)		
	500	635.8 (65.1)	(-6.6)			324.7*	(-10.6)		
Testes [g] [g]	0	4.157 (0.95)		0.622 (0.163)					
	50	6.405 (5.89)	(54)	0.973 (0.821)	(56)				
	150	7.641 (11.04)	(84)	1.378 (2.441)	(121)				
	500	10.034 (12.46)	(141)	1.594 (1.914)	(156)				
Ovaries [mg] [g]	0					119.4 (63.25)		0.034 (0.02)	
	50					119.1 (74.43)	(-0.3)	0.033 (0.022)	(-3)
	150					132.0 (108.90)	(10)	0.037 (0.036)	(9)
	500					464.1 (1427.24)	(289)	0.152 (0.479)	(347)
Liver [g] [g]	0	20.811 (7.24)		3.042 (0.834)		12.146 (2.55)		3.348 (0.44)	
	50	18.680 (4.10)	(-10.8)	2.863 (0.508)	(-5.9)	11.946 (1.23)	(-1.6)	3.242 (0.335)	(-3.2)
	150	19.319 (3.81)	(-7.2)	2.98 (0.369)	(-2.0)	11.584 (1.66)	(-4.6)	3.149 (0.293)	(-5.9)
	500	19.309 (3.88)	(-7.2)	3.07 (0.73)	(0.9)	10.215* (1.19)	(-15.9)	3.153 (0.247)	(-5.8)

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may be not calculated exactly due to rounding of figures

Increases in testes weights at high dose males (not statistically significant) depend on high individual values of a few animals. Among the 17 male high dose animals three males showed extraordinary high values (#62 with 51 g, #64 with 23 g, #73 with 25 g). Excluding these three animals from the calculation of the mean a value of 5.11 g can be calculated, which is well covered by the historical control data for mean testes weights, ranging between 3.7-7.8 g (BASF DocID 2015/1171114) (see Table 5.5-2). All of these animals showed Leydig cell adenomas. However the incidences for histopathological findings in the testes for controls and high dose animals in this study were not different, as indicated in the following table.

Table 5.5-3: Comparison of incidences for histopathological findings in testes after chronic dimoxystrobin administration to male rats

Group	Control	500 ppm dose group
Calcification, focal	8	11
Degeneration, focal	11	10
Degeneration, diffuse	6	5
Arteritis	0	1
Leydig cell hyperplasia	15	15
Leydig cell adenoma	11	13

All findings noted in the testes were either single observations or they were biologically equally distributed between controls and treatment groups. All of them were considered to be incidental or spontaneous in origin and without any relation to treatment. Therefore the increased testes weights in high dose animals, which were not statistically significant, are considered incidental.
The increase in absolute and relative ovary weight (not statistically significant) at the top dose was attributed to one female (#151) which showed an incidental massive abscess-forming inflammation (absolute ovary weight 6173 mg, compared to rest of the group 38-228 mg, which is clearly covered by historical control data ranging between 68.8 and 295.1 mg for the absolute means). Most females (control and treated animals) with high individual ovary weights showed cysts, inflammation or sex cord stromal hyperplasia histopathologically. Also for the females, the type and incidences of histopathological findings is not different between controls and treated animals, as the following table shows:

Table 5.5-4: Comparison of incidences for histopathological findings in ovaries after chronic dimoxystrobin administration to female rats

Group	Control	500 ppm dose group
Cyst(s)	9	9
Inflammation	1	1
Hyperplasia, sex cord, focal	0	1
Hyperplasia, sex cord, diffuse	3	4

All findings in the ovaries were biologically equally distributed between control and high dose females. Therefore, the increased ovary weights in high dose females are considered incidental.
A statistically significant reduction in liver weight (by 16%) of top dose females was attributed to reduced body weight (by 11%). There was no significant effect on liver weight relative to body weight and no histopathological correlates (see Table 5.5-2). Furthermore, the absolute and the relative mean liver weights are well covered by collected mean historical control data (absolute liver weights 10.1-14.6 g, relative 2.871-3.767%; BASF DocID 2015/1171114).

Slight and diffuse thickening of the duodenum was determined grossly at the high dose in males and females (6 males, 3 females) and in one medium dose male (see Table 5.5-5). On histological examination thickening of the mucosa was seen in 7 high dose males and in one high dose female, not in the mid dose male animals. The thickening of the mucosa was diffuse and slight, and histologically the mucosa was nearly comparable to controls. Only intestinal villi were elongated and partly slightly broadened. No duodenal tumours were observed.

Table 5.5-5: Selected macroscopic and microscopic findings in rats administered dimoxystrobin for at least 24 months

	Males				Females			
Dose (ppm)	0	50	150	500	0	50	150	500
No. of animals	20	20	20	20	20	20	20	20
Duodenum								
- Thickening of wall	0	0	1	6	0	0	0	3
- Thickening of mucosa	0	0	0	7	0	0	0	1
Uterus								
- Mass					2	2	2	6
- Polyp, endometrial					2	0	0	0
- Sarcoma, endometrial					1	1	1	2
- hemangiosarcoma					1	0	0	0
- Cervical fibrosis					4	5	7	7

The incidence of rats with uterine masses was increased at the top dose (see Table 5.5-5). Most of these masses were found to be due to cervical fibrosis, endometrial polyps, endometrial sarcomas or haemangiosarcoma, which individually were comparable between control and treated groups and showed no convincing evidence of a substance related response.

Table 5.5-6: Thyroid gland findings in female rats administered dimoxystrobin for at least 24 months

Thyroid gland	males				females			
Dose [ppm]	0	50	150	500	0	50	150	500
No- of animals*	20	7	5	20	20	20	20	20
c-cell adenoma	4	2	0	2	1	4	1	5
c-cell carcinoma	-	3	-	-	-	-	-	-

*The thyroids of the low and mid dose females were subsequently histopathologically assessed (see amendment to the study), not the males.

After a full histopathological evaluation of the thyroid of all females of all groups (see BASF DocID 2002/1013942) there was no evidence for a treatment-related increased in c-cell adenoma in female rats (see Table 5.5-6), as there was no dose-relationship observed and the incidences are fully covered by the historical control data range with up to 6/20 c-cell adenoma (BASF DocID 2015/1171114). In males the incidence of c-cell adenoma was highest in the control group. C-Cell carcinoma were only found in low dose males. Missing a dose-response relationship, the findings were regarded as incidental. For further discussion see summary of carcinogenicity study below.

Conclusion:

A clear substance-related adverse effect (reduced body weight gain and reduced food consumption) was seen in females at the top dose level of 500 ppm. No effect on food consumption and body weights was detected in treated males. An increased incidence of slight thickening of the duodenal mucosa in both sexes at 500 ppm was observed. The single incidence of grossly identified duodenal thickening at 150 ppm in one male, which was not confirmed on histological examination, is not considered an effect of clear toxicological concern. Thus the NOAEL is 150 ppm (7 and 9 mg/kg bw/day for males and females) based on reduced body weight and food consumption in females and increased duodenal thickening at 500 ppm (23 and 30 mg/kg bw/day for males and females) in both sexes.

Dimoxystrobin (BAS 505 F) – Carcinogenicity study in Wistar rats. Administration in the diet for 24 months (BASF DocID 2000/1016866 and 2002/1013943)

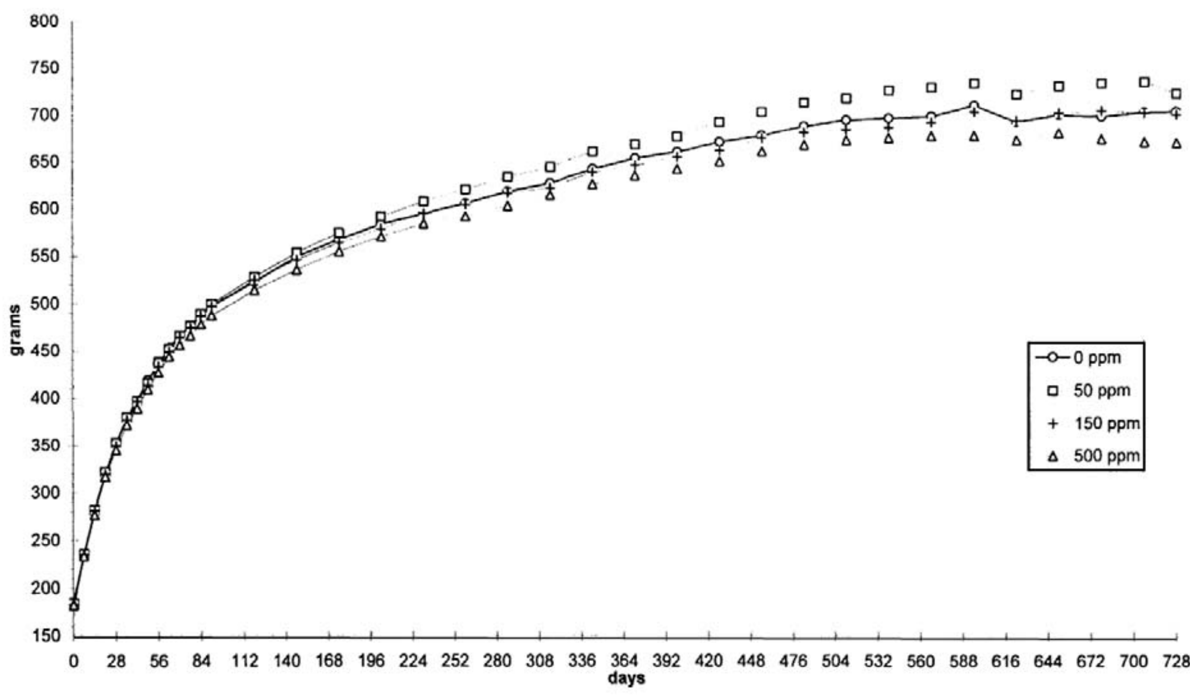
Guidelines: Equivalent to OECD guideline 452 (adopted 1981)
Deviations: No
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Materials and Methods:
Dimoxystrobin (batch/purity: N 6: 98.8%; N 15: 98.4%) was administered to groups of 50 male and 50 female Wistar rats at dietary concentrations of 0, 50, 150, and 500 ppm for 24 months. Mean test substance intakes were 2.2, 6.9, and 22.5 mg/kg bw/day for males and 3.1, 9.5, and 31.5 mg/kg bw/day for females.

Food consumption and body weight were determined once a week during the first 13 weeks, thereafter at 4-week intervals. The animals were examined for signs of toxicity or mortality at least once a day; moreover, comprehensive additional clinical examinations and palpations of the animals were performed once a week. Differential blood counts were determined for all surviving high dose and control animals at the end of the study and also from all animals killed in extremis during the study. The animals were subjected to gross-pathological assessment and organ weights determined for 6 organs (namely liver, kidneys, testes/ovaries, brain, adrenal glands) from animals surviving to termination. All control and high dose animals (and intercurrent decedents) were subject to an extensive histopathological examination. In addition, stomach, duodenum, liver, lungs and kidneys were examined from all animals in other dose groups; and all gross lesions were examined in all affected animals from all groups.

Results:
There was no test substance related increase in mortality (84% of the high dose males and females survived to termination, compared to 68 and 60% of control males or females; in each sex survival was highest at the top dose). There were no substance-related clinical signs of toxicity. Food consumption at 500 ppm was statistically significant decreased (but only by -4 to -8%) on several days in males from day 28 to day 203 and in females from day 56 to day 567.
Body weight gain in high dose females was slightly lower than controls from day 343, with a 10-12% reduction in overall gain (as measured from day 0) being seen at most measurements from day 455 onwards.
There was a slight reduction in overall body weight gain of high dose males, which was never statistically significant and only reached a 6% reduction on 3 days (at and near termination). The effect is considered to be treatment-related because of the response seen in high dose females. Figure 5.5-2 shows body weight developments in male and female rats during the entire study period.
There were no effects on food efficiency or, at termination, on differential blood counts.

Figure 5.5-2: Body weights of rats administered dimoxystrobin for 2 years – carcinogenicity study



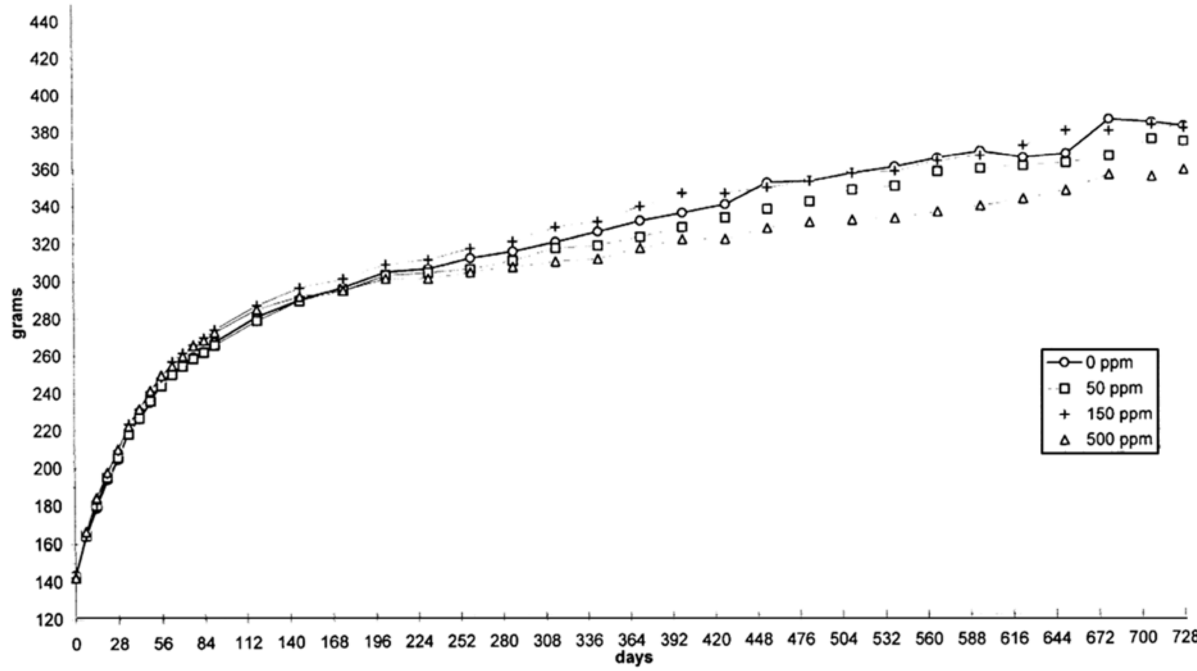


Table 5.5-7: Selected mean absolute and relative organ weights of rats administered dimoxystrobin for 2 years – carcinogenicity group

Sex		Males				Females			
Organ weight [mg]	Dose [ppm]	Absolute weight (SD)	Δ%	Relative weight [% of b.w.] (SD)	Δ% #	Absolute weight (SD)	Δ%	Relative weight [% of b.w.] (SD)	Δ% #
Terminal weight [g]	0	677.7 (73.3)				362.2 (59.29´)			
	50	699.5 (84.4)	(3.2)			353.9 (46.74)	(-2.3)		
	150	682.9 (99.9)	(0.8)			356.3 (59.32)	(-1.6)		
	500	645.2 (84.30)	(-4.8)			334.7 (37.96)	(-7.6)		
Testes [g] [g]	0	4.151 (1.52)		0.616 (0.225)					
	50	4.662 (2.52)	(12.3)	0.679 (0.392)	(10.2)				
	150	4.423 (1.52)	(6.6)	0.657 (0.222)	(6.7)				
	500	5.660** (3.01)	(36.4)	0.887** (0.456)	(44.0)				
Adrenals [mg] [mg]	0	89.7 (42.11)		0.013 (0.006)		154.8 (91.98)		0.045 (0.032)	
	50	82.0 (16.95)	(-8.6)	0.012 (0.002)	(-7.7)	314.3 (930.16)	(103)	0.109 (0.371)	(142.9)
	150	85.2 (20.77)	(-5.0)	0.013 (0.051)	(0)	236.4* (290.16)	(52.7)	0.067 (0.083)	(48.9)
	500	143.2 (375.21)	(59.6)	0.021 (0.049)	(61.5)	127.6 (40.50)	(-17.6)	0.038 (0.01)	(-15.6)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may be not calculated exactly due to rounding of figures

Absolute and relative testes weights were increased statistically significantly at the top dose (see Table 5.5-7). All mean values calculated for the treatment group are fully covered by the mean historical control ranges of 3.67-7.75 g for absolute and 0.565-1.146% for relative testes weights; BASF DocID 2015/1171114). Also the incidence of macroscopically determined masses and cystic degeneration in the testes were increased at the top dose (see Table 5.5-8). The masses correlated in the majority with unilateral or bilateral Leydig cell adenomas and cystic degeneration correlated in the majority with diffuse tubular degeneration. There was some evidence for an increased incidence of Leydig cell hyperplasia at the top dose (70% vs. 44% in control). However, this is a common lesion in ageing rats, and the incidence was similar to what has been observed in control animals of the parallel running chronic study (75%). Further the total number of Leydig cell adenomas in the testes did not differ between treated and control animals. Hence, overall it is considered that the increased testis weight at the top dose is incidental and not a substance-related adverse effect.

Table 5.5-8: Selected macroscopic and microscopic findings in rats administered dimoxystrobin for 24 months

Dose	Males				Females			
(ppm)	0	50	150	500	0	50	150	500
Duodenum	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
- Thickening of wall	0	2	2	19	0	0	0	12
- Thickening of mucosa	0	0	0	7	0	0	0	2
Testes	**50**	**35**	**36**	**50**				
- Diffuse tubular degeneration	10	11	5	11				
- Masses	10	13	10	18				
- Cystic degeneration	3	3	7	13				
- Leydig cell hyperplasia	22	18	28	35				
- Leydig cell adenomas	24	18	22	27				

Bold: No. of animals/organs examined

A non-statistically significant increase in adrenal weight in high dose males (see Table 5.5-7) is attributed to one male with very large adrenals (#371 with 2479 mg, compared to a range of 64-241 mg in the rest of the group). Excluding this animal, which showed an adrenal adenocarcinoma from the calculation, the mean absolute adrenal weight value decreases to 82.8 mg, which is covered by historical control data (absolute adrenal weights 78.4-537.1 mg, relative adrenal weights 0.011-0.084%). This is therefore considered not to be a substance-related response. In females an increase in adrenal weights was observed in the low and mid-dose animals (statistically significant in the mid dose only), while the top dose showed decreased mean adrenal weights. Increased adrenal weights were mainly attributed to single animals (low dose female #481 with 5789 mg adrenal weight compared to weight range of 75-586 mg; mid dose female #562 with 1821 mg adrenal weight compared to weight range of 88-618 mg within the respective group). Furthermore, the adrenal weights of treated animals were covered by historical control data (absolute adrenal weights 117.7-319.7 mg, relative adrenal weights 0.031-0.115%; BASF DocID 2015/1171114).

A slight increase in the incidence of focal fatty changes in adrenals of mid and high dose females was regarded to be an incidental background lesion. This assessment is based on the fact, that no increased incidence in the high dose group of the chronic study was detected and the combined incidences for females from both studies showed no dose response (controls and low dose 4/70, medium dose 14/69, high dose 10/70) (see Table 5.5-9). Furthermore, in another rat carcinogenicity study (study no. 97090) conducted in the same lab at the same timeframe in the same rat strain focal fatty change was actually highest in the control animals with 16/50 incidences (16, 12, 14, and 16/50 in control, low, mid and high dose, respectively). In males of the dimoxystrobin study the occurrence of focal fatty change in the adrenals was highest in the control group (see Table 5.5-9). Hence, this is not substance-related effect, but just demonstrating the high biological variability of this parameter.

Table 5.5-9: Additional findings in rats administered dimoxystrobin for 24 months – combined results from chronic and carcinogenicity study

Dose	Males				Females			
(ppm)	0	50	150	500	0	50	150	500
Adrenals	**50/20**	**50/11**	**50/12**	**50/20**	**50/20**	**50/20**	**50/19**	**50/20**
- focal fatty change	18/7	10/2	10/3	13/7	1/3	2/2	11/3	8/2
Liver	**50/20**	**50/20**	**50/20**	**50/20**	**50/20**	**50/20**	**50/20**	**50/20**
- adenoma	2/1	3/1	7/3	3/2	1/1	1/0	2/0	4/1
- adenocarcinoma	3/0	3/1	1/1	4/2	0/2	0/1	1/0	0/0

Bold: No. of animals/organs examined (Carcinogenicity study/chronic study)

A slight increase in the incidence of hepatocellular adenoma was seen in high dose females (4/50, controls 1/50) (see Table 5.5-9), however when the results of both studies are combined no statistically significant increase for high dose females was seen (5/70 in the top dose vs 2/70 in the controls) and there was no increase in the overall incidence of hepatocellular adenoma plus carcinoma (5/70 = 7% in the top dose vs 4/70 = 6% in controls). The incidence at the top dose was also within the range of historical control data (hepatocellular adenoma no. 0-5, 0-20%; BASF DocID 2015/1171114). Hence, this is considered not a substance-related response.
In males, there was no good evidence of a dose-related increase in the overall incidence (both studies) for hepatocellular adenoma plus carcinoma (controls 6/70 = 9%, low dose 8/70 = 11%, medium dose, 12/70 = 17%, high dose 11/70 = 16%). Again the incidences were well covered by historical control data (hepatocellular adenoma no. 0-11, 0-30%; BASF DocID 2015/1171114). Hence, this is considered not a substance-related response.

Thickening of the duodenal wall was seen in all male and in female rats of the top dose (see Table 5.5-8). On histological examination however thickening of the duodenal mucosa was seen only at the high dose. The pathology and severity were as described for the chronic rat study, where slight and diffuse thickening of the duodenum wall was determined in high dose males and females and one medium dose male. Thickening of the duodenal mucosa was seen only in the high dose animals.
No substance related duodenal tumours were seen.

An increased incidence of c-cell adenoma in the thyroid was detected in high dose female rats (see Table 5.5-10). One low dose female also had a c-cell adenocarcinoma. After combining the results from both the chronic and carcinogenicity studies, there was a slightly statistically significant increase in the incidence of c-cell adenoma in high dose females ($p < 0.05$ Fishers Exact test, one sided), without a dose-response.

Table 5.5-10: Adenoma and carcinoma findings (thyroid) in female rats administered dimoxystrobin for 24 months

Thyroid gland	**Males**				**females**			
24 months carcinogenicity study	**0**	**50**	**150**	**500**	**0**	**50**	**150**	**500**
c-cell adenoma	7/50 (14%)	1/17 (14%)	0/11 (0%)	5/50 (10%)	6/50 (12%)	7/50 (14%)	6/50 (12%)	11/50 (22%)
c-cell carcinoma	0	1	0	0	0	1	0	0
Combined 24 months chronic and carcinogenicity study								
c-cell adenoma	11/70 (16%)	3/37 (8%)	0/16 (0%)	7/70 (10%)	7/70 (10%)	11/70 (15.7%)	7/69 (10%)	16/70* (22.9%)
c-cell carcinoma	0	4	0	0	0	1	1	0

*p ≤ 0.05 (Fishers Exact test, one sided, see Monograph)

The significance of the increase in c-cell adenomas was only slight: $p \leq 0.05$. There is literature available recommending a probability value of $p \leq 0.01$ for a high dose effect with a common tumour, i.e. one with a background frequency of 1% or more (Haseman et al., 1986; BASF DocID 1986/1002811).
The incidence of c-cell adenomas was in the range of the historical control data for incidences in chronic studies (0-30%), carcinogenicity studies (0-25%) or combined chronic and carcinogenicity (0-24%) (BASF DocID 2015/1171114). The incidences for survivors alone and did not show statistical significance.
The dose-response data in females do not provide clear evidence for a substance-related effect. In males, the number of animals with c-cell adenomas did not give evidence for a treatment-relationship. Taking into account the high incidental occurrence of thyroid c-cell adenoma in rats, the fact, that all incidences were well covered by historical controls and the absence of clear dose-response relationship, the observed picture is not indicating an evidence for a treatment-related increased incidence of thyroid c-cell adenoma in females. Therefore, the slightly increased incidence of c-cell adenomas in top dose females is considered incidental and not substance-related.

There was no convincing evidence for any other substance-related effects.

Conclusion:
A clear substance-related adverse effect (reduced body weight gain) was seen in females at the top dose level of 500 ppm. Increased incidences of thickening of the duodenal wall confirmed by pathologically observed thickening of the duodenal mucosa is seen in males and females at the top dose of 500 ppm. There was no evidence of a substance-related oncogenic response. The slightly increased incidence of c-cell adenoma of the thyroid in females at 500 ppm is considered to be an incidental finding.

The NOAEL is 150 ppm (7 and10 mg/kg bw/day in males and females) based on reduced body weight and food consumption and increased duodenal mucosa thickening at 500 ppm (23 and 32 mg/kg bw/day in males and females).

Note: The mouse carcinogenicity study report (BASF DocID 2001/1000021) were already submitted and reviewed in the course of the initial registration of dimoxystrobin. The amendment (BASF DocID 2011/1040675) provides the results of additional statistical evaluations of microscopic findings in all protocol organs. All relevant results of the amendment are included in the summary of the mouse carcinogenicity study below.

Dimoxystrobin (BAS 505 F) – Carcinogenicity study in B6C3F1/Rj mice. Administration in the diet for 18 months (BASF DocID 2001/1000021 and 2011/1040675)

Report:	CA 5.5/1 █████ 2011a BAS 505 F - Carcinogenicity study in B6C3F1/Rj mice - Administration in the diet for 18 months 2011/1040675
Guidelines:	OECD 451, EPA 870.4200, JMAFF, EEC 87/302 B
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Guidelines:	Equivalent to OECD guideline 452 (adopted 1981)
Deviations:	No
GLP:	Yes
Acceptance:	The study was considered acceptable in the EU registration process 2003.

Materials and Methods:

Dimoxystrobin (Batch/purity: N 15: 98.4%) was administered to groups of 50 male and 50 female B6C3F1/Rj mice in the diet for 18 months. Animals were individually housed. Levels of test substance intake for males were 4.0, 31.8, and 177.4 mg/kg bw/day in the 25, 200, and 1000 ppm groups. For females levels of test substance intake were 5.4, 19.8, and 103.7 in the 25, 100, and 500 ppm group.

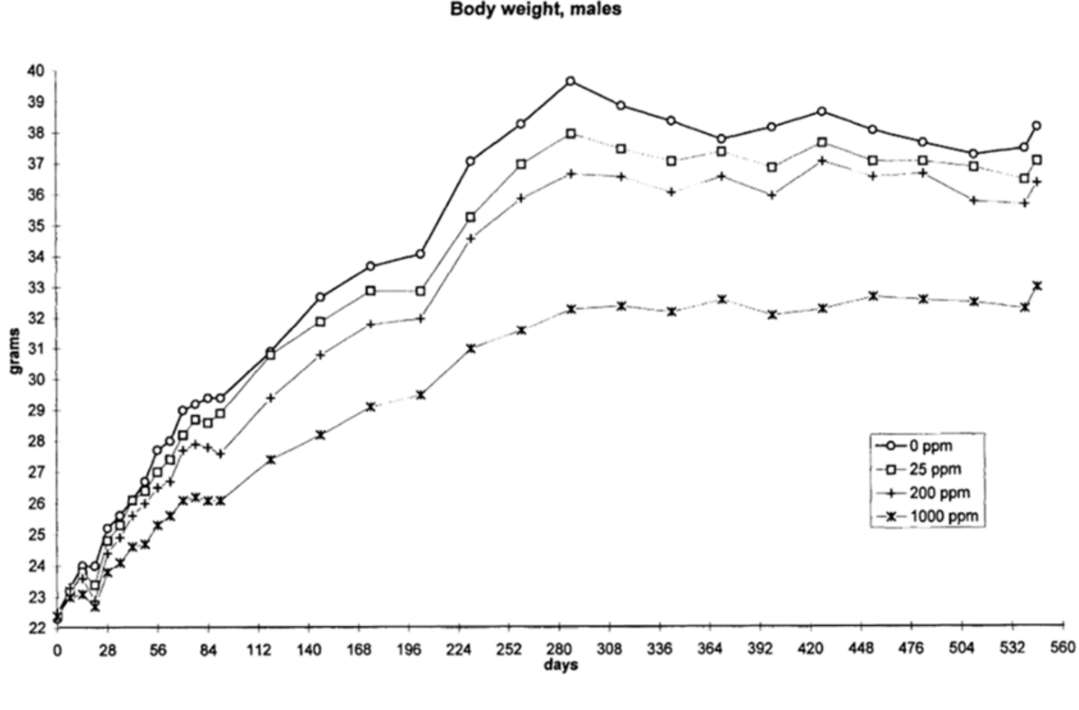
The stability of the test substance, the homogeneous distribution, stability and correct concentration of the test substance in the diet were confirmed by analysis.

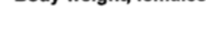
Food consumption and body weight were determined once a week during the first 13 weeks, thereafter at 4-week intervals, and towards the end of the study. A check of the general state of health of the animals was made at least daily. Additionally, the animals were examined in detail and palpated once a week. Blood smears were prepared after 12 months and 18 months and evaluated in control and high dose groups.

After 18 months of treatment, the animals were subjected to gross-pathological assessment and the weights of 10 organs determined (from 10 mice/sex/group). All control and high dose animals of each sex (and intercurrent decedents at 25 and 200 ppm, but not at 100 ppm) were subject to an extensive histopathological examination. In addition stomach, duodenum, liver, lungs and kidneys were examined from all animals in other dose groups; and all gross lesions were examined in all affected animals from all groups.

Results:
There was no test substance related increase in mortality or clinical signs of toxicity in this study. At least 96% of each sex per dose group survived to termination. There was a substance-related reduction in body weight gain at the top dose in males (by 34% at 1 year and at termination) and in females (by 13-15% at 1 year and at termination). A substance related reduction in body weight gain was also seen at 200 ppm in males (by 9-12% at 1 year and at termination) but no substance related effect was seen in mid dose females. A slight reduction in body weight and body weight gain was also seen in low dose males throughout the study but the magnitude of the effect was generally too small to be considered an adverse effect. Figure 5.5-3 shows body weight developments in male and female mice during the entire study period.
There was no effect on food consumption and on food efficiency. There was no effect on white blood cell differential count or on white and red blood cell morphology.

Figure 5.5-3: Body weights of mice administered dimoxystrobin for 18 months





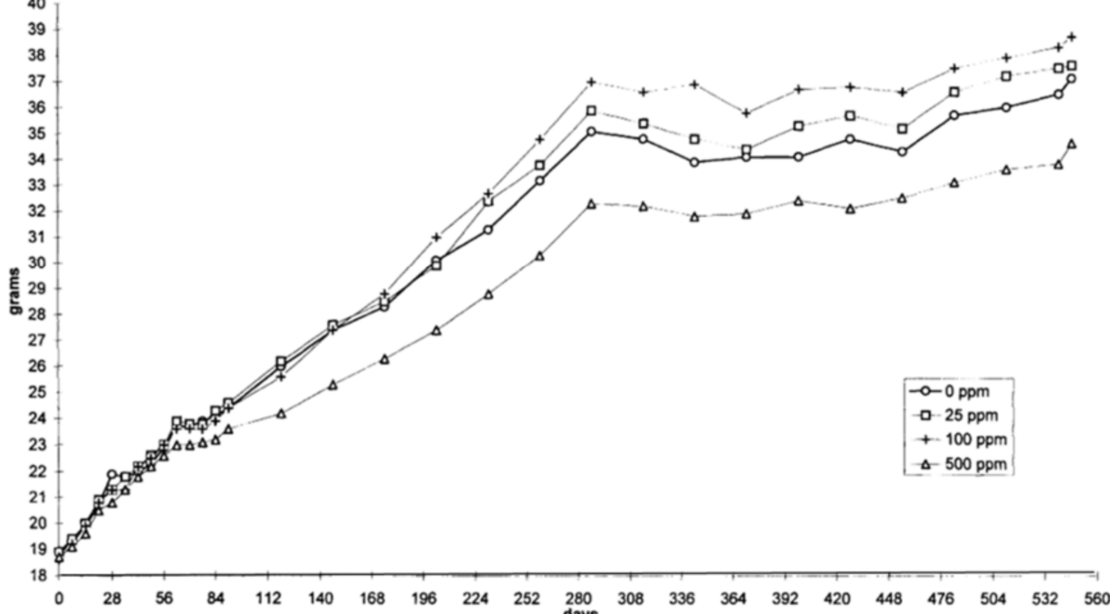


Table 5.5-11: Selected mean absolute and relative organ weights of mice administered dimoxystrobin for 18 months

Sex			Males				Females			
Organ weight [mg]	Dose [ppm] males	Dose [ppm] females	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ% #	Absolute weight	Δ%	Relative weight [% of b.w.]	Δ% #
Terminal weight [g]	0	0	35.57				33.80			
	25	25	34.05	(-4.3)			32.73	(-3.2)		
	200	100	33.56	(-5.7)			34.73	(2.8)		
	1000	500	28.96**	(-18.6)			33.16	(-1.9)		
Liver [mg]	0	0	1255		3.541		1244		3.766	
	25	25	1227	(-2.2)	3.611	(2.0)	1228	(-1.3)	3.848	(2.2)
	200	100	1328	(5.8)	3.995	(12.8)	1282	(3.1)	3.791	(0.7)
	1000	500	1228	(-2.2)	4.263**	(20.4)	1208	(-2.9)	3.697	(-1.8)
Kidney [mg]	0	0	630		1.784		399		1.205	
	25	25	634	(0.6)	1.877	(5.2)	414	(3.8)	1.311	(8.8)
	200	100	676	(7.3)	2.033*	(14.0)	399	(0)	1.175	(-2.5)
	1000	500	587	(-6.8)	2.029**	(13.7)	396	(-0.8)	1.214	(0.7)
Testes [mg]	0		223		0.631					
	25		224	(0.4)	0.661	(4.8)				
	200		220	(-1.3)	0.66	(4.6)				
	1000		225	(0.9)	0.784**	(24.2)				
Ovaries [mg]		0					17.7		0.053	
		25					24.0	(35.6)	0.079	(49.1)
		100					57.2	(223)	0.161	(204)
		500					45.7	(158)	0.154	(191)
Uterus [mg]		0					1001		2.991	
		25					652	(-34.9)	2.146	(-28.3)
		100					807	(-19.4)	2.436	(-18.6)
		500					832	(-16.9)	2.557	(-14.5)
Epididymides [mg]	0		89.0		0.253					
	25		91.2	(2.5)	0.269	(6.3)				
	200		91.0	(2.2)	0.275	(8.7)				
	1000		88.2	(-0.9)	0.306**	(20.9)				
Heart [g]	0	0	196		0.554		143		0.433	
	25	25	191	(-2.6)	0.564	(1.8)	147	(2.8)	0.464	(7.2)
	200	100	199	(1.5)	0.593	(7.0)	153	(7.0)	0.450	(3.9)
	1000	500	185	(-5.6)	0.642**	(15.9)	148	(3.5)	0.456	(5.3)
Spleen [mg]	0	0	61.0		0.174		100		0.301	
	25	25	63.7	(4.4)	0.186	(6.9)	114	(14.0)	0.369	(22.6)
	200	100	65.8	(7.9)	0.197	(13.2)	147	(47.0)	0.455	(51.2)
	1000	500	66.6	(9.2)	0.231**	(32.8)	98	(-2.0)	0.302	(0.3)
Brain [mg]	0	0	484		1.374		496		1.508	
	25	25	487	(0.6)	1.443	(5.0)	499	(0.6)	1.585	(5.1)
	200	100	485	(-0.2)	1.455	(5.9)	500	(0.8)	1.480	(-1.9)
	1000	500	489	(1.0)	1.702**	(23.9)	510	(2.8)	1.576	(4.5)
Adrenal glands [mg]	0	0	4.6		0.013		8.2		0.025	
	25	25	4.8	(4.3)	0.014	(7.7)	9.8**	(19.5)	0.031*	(24)
	200	100	4.9	(6.5)	0.015	(15.4)	8.6	(4.9)	0.025	(0)
	1000	500	5.1	(10.9)	0.018**	(38.5)	7.5	(-8.5)	0.023	(-8)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may be not calculated exactly due to rounding of figures

Statistically significant increases in all investigated organ weights relative to body weight in top dose males are considered to be primarily due to reduced body weight compared to controls (see Table 5.5-11).

Absolute and relative adrenal weights were increased in low dose, but not in the mid and high dose females. No dose-response could be determined. Furthermore, the mean adrenal weights were in the range of historical control data (absolute adrenal weights 8.2-11.3 mg, relative adrenal weights 0.022-0.029%; BASF DocID 2015/1171115). Therefore, this effect was regarded incidental.

The obvious, but not statistically significant increases in ovary weights in the mid and high dose females were attributed to single animals. Excluding only female #307 (with the highest ovary weight of this mid dose group) from the mean organ weight calculation in the mid dose group results in a mean of 43.3 mg (compared to 57.2 mg, as shown in the Table 5.5-11), which is well covered by mean historical control data (17.7-49.6 mg; BASF DocID 2015/1171115). This animal #307 had a large unilateral cyst (diameter: 4.0 mm, 0.175 g), which is also occurring in control animals. In the 500 ppm dose group the mean value is already covered by the historical control range of ovary weights (17.7-49.6 mg; BASF DocID 2015/1171115), however also here one single female (#356 with 210 mg) had an extraordinary high value. Excluding female #356 which had large bilateral cysts (with a diameter of 2.0 mm on the left side and a diameter of 8.0 mm on the right side) from the mean organ weight calculation reduces the mean ovary weights to 25.9 mg (compared to 45.7 mg as shown in the Table 5.5-11). Taking into account the historical control data and the unspecific pathological findings in individual animals showing high ovary weights, these findings are considered incidental and not treatment-related.

Table 5.5-12: Selected macroscopic findings in mice administered dimoxystrobin for at least 18 months

Dose	Males				Females			
(ppm)	0	25	200	1000	0	25	100	500
Duodenum	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
- Thickening of wall	0	0	0	12	0	0	0	3
Glandular stomach	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
- Erosion/ ulcer (gross lesion)	4	1	4	16	9	8	6	14

Bold: No. of animals/organs examined

On gross examination slight-severe thickening of the duodenal wall was seen in the top dose animals of both sexes, but not in other dose groups (see Table 5.5-12). Macroscopically, in the glandular stomach, the number of animals with erosions or ulcers was higher in top dose animals both in males and females compared to control animals.

Also microscopically, a thickening of the duodenal mucosa was diagnosed in 18 males of the 1000 ppm dose group and in 2 females of the 500 ppm dose group, being statistically significant only in males (see Table 5.5-13). The histological structure of affected animals was reported to be nearly comparable with the controls. Only the intestinal villi were elongated and partly slightly broadened. In most of these animals the finding was reported to be minimal or slight, thus indicating an adaptive process.

There was a slight increase in duodenal adenoma and adenocarcinoma in male mice. Three top dose males showed an adenoma in the duodenum. Adenocarcinomas of the duodenum were noted in three top dose males and in one female of the top dose group. Focal hyperplasia occurred in 3 males of the 1000 ppm dose group and 6 females of the 500 ppm group. The occurrence of focal hyperplasia was statistically significant only in females. Focal hyperplasia of the duodenal mucosa were regarded as a pre-neoplastic lesion [see Table 5.5-13].

Table 5.5-13: Selected microscopic findings in mice administered dimoxystrobin for at least 18 months

Dose	Males				Females			
(ppm)	0	25	200	1000	0	25	100	500
Duodenum	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
- Thickening of mucosa	0	0	0	18**	0	0	0	2
- Focal hyperplasia	0	0	0	3	0	0	0	6*
- Adenoma	0	0	0	3	0	0	0	0
- Adenocarcinoma	0	0	0	3	0	0	0	1
Glandular stomach	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
- Erosion/ ulcer	7	1	5	17*	9	9	3	14

Bold: No. of animals/organs examined
$*p \leq 0.05$, $**p \leq 0.01$

The incidence of erosion/ulcer of the glandular stomach was increased at the top dose for each sex both macroscopically and microscopically, although dimoxystrobin does not have an irritating potential.

There were no further treatment-related neoplastic findings (findings occurred either singly or were biologically equally distributed over the control group and the treatment groups).

Conclusion:
The NOAEL in this study was 25 ppm (corresponding to 4.0 mg/kg bw) for males (based on reduced body weight gain at 200 ppm) and 100 ppm (corresponding to 19.8 mg/kg bw) for females (based on reduced body weight gain and pathological changes in the duodenum and stomach at 500 ppm). Substance-related neoplasia and pre-neoplastic changes were observed in the duodenum at 500 ppm (females, corresponding to 103.7 mg/kg bw) and 1000 ppm (males; corresponding to 177.4 mg/kg bw).

Mode of action of duodenum toxicity

A number of mechanistic studies to elucidate the mode of action responsible for thickening of the duodenal mucosa leading to duodenal tumors in mice and the threshold level of dimoxystrobin activity have been presented in the original dossier. A clear correlation between a decreased iron uptake and lower serum iron levels and the effects in the duodenum was shown for rats, as well as there was some (but no conclusive) evidence for iron - complexation properties of dimoxystrobin. Further mechanistic studies have been performed after evaluation of the original dossier. Comprehensive summaries of the peer-reviewed studies, as well as of the new mechanistic studies, are presented in chapter MCA 5.8.2.

Based on the S-phase response studies (already peer-reviewed) conducted in the duodenum of dimoxystrobin-treated male mice, it can be concluded, that dimoxystrobin causes an increased cell proliferation (S-phase response) in the duodenum already 2 days after start of dietary administration of 1000 ppm in male mice. The cell proliferation was **fully reversible** after a 1-week recovery period (after 2-days administration) or a 2-week recovery period (after a 2-week administration). The threshold for cell proliferation was found to be 200 ppm (50 mg/kg bw).

In order to further elucidate the mode of action, leading to duodenum tumors in mice, duodenum slices of the archived duodenum tissue of the S-phase response studies were investigated for iron content (BASF DocID 2012/1221002). There is evidence, that treatment with the test substance led to lower levels of iron (stained as Fe^{2+} and Fe^{3+}) in the duodenum of B6C3F1 mice after 2 and 4 weeks of treatment. As a local mode of action – more precisely a direct interaction of dimoxystrobin with the duodenum receptors (either DMT1, Dcytb or ferroportin) – was hypothesized, an immunohistochemical staining of these receptors was intended, but due to technical reasons, no successful staining could be obtained in the tissues of this study.

Instead, a gene array experiment was conducted in duodenum obtained from male mice - of the same strain as used in the carcinogenicity study - treated with high doses of dimoxystrobin for 7 days. In this study, male mice were administered 4000 and 8000 ppm dimoxystrobin and serum and liver non-hem iron levels, hemoglobin concentrations were measured. Further duodenum pathology and a gene array (using Affymetrics chips) were performed. The administration of dimoxystrobin to male B6C3F1 mice for one week revealed a thickening of the duodenal mucosa. There was no clear difference between the two test groups (4000 and 8000 ppm). The thickening of the mucosa was well in accordance with the observed increase in duodenal weights. This finding is regarded to be related to the treatment with the test substance.

A downward trend in hemoglobin levels and serum iron concentrations was observed in treated animals of both dose groups. Liver non-hem iron levels were significantly increased in the 8000 ppm group compared to the 4000 ppm group, but not compared to the controls.

The gene array experiments showed that oral dimoxystrobin treatment led to marked changes in iron metabolism genes in the duodenum. In general there was a down-regulation of genes involved in iron absorption seen. In particular, both Dcytb and ferroportin mRNA were decreased in duodenum which would be expected to reduce the iron absorption. Since ferroportin controls the amount of iron which enters the body the effects of dimoxystrobin on iron metabolism could be explained by this observation alone. A decrease was also observed in the transcription factor Hif2α which has been shown to directly regulate the transcription of both Dcytb and ferroportin genes.
The data suggest that dimoxystrobin induces iron deficiency in duodenum possibly showing maximum effect at 4000 ppm. This is supported by the data showing increased levels of TfR1 (transferrin receptor) and decreased levels of ferritin. Supportive to the reduced iron storage found in the bone marrow of mice in the 90-day repeated-dose toxicity study, mRNA of ferritin, an iron storage protein, was slightly down regulated in this study (1.6 and 1.3-fold for the 4000 and 8000 ppm dose group). To investigate mucosal ferritin at the protein level a Western blot on duodenal lysates was performed from the control mice and the mice treated with 4000 and 8000 ppm dimoxystrobin. H-Ferritin protein levels were decreased in both treated groups compared to controls.

Based on this gene array experiment including duodenal pathology and hematology investigations, it is suggested, that dimoxystrobin treatment interacts with the duodenal receptors involved in iron uptake or metabolism. Figure 5.5-4 shows a schematic of hem and non-hem iron absorption in duodenal enterocytes.

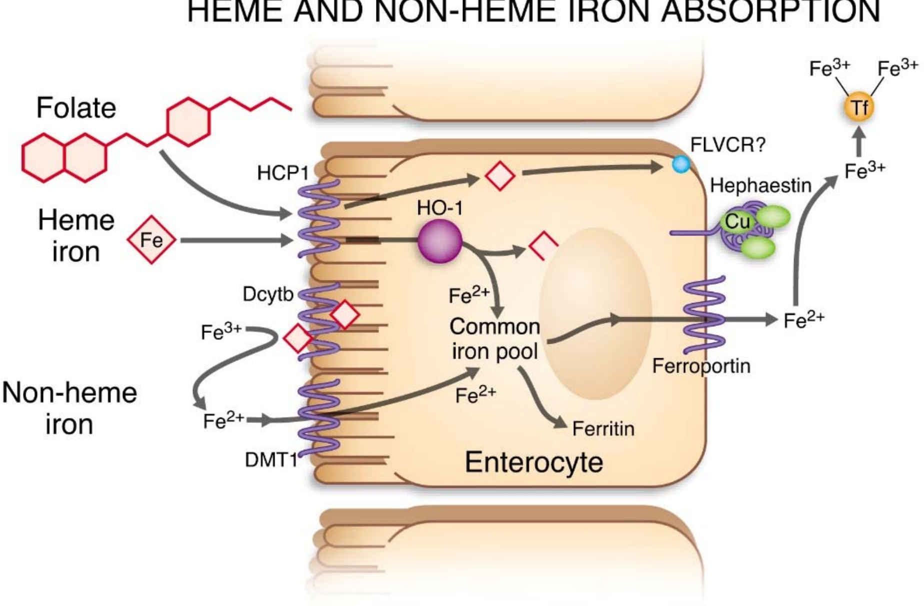


Figure 5.5-4: Heme and non-heme iron absorption in duodenal enterocytes

Dietary iron may be present in the form of hem iron or non-hem iron. Hem iron is more effectively absorbed than the non-hem form, and appears to be absorbed intact by duodenal cells. Non-heme iron may be present in the diet as either the reduced form Fe^{2+} (ferrous iron), or the oxidized form Fe^{3+} (ferric iron). Fe^{3+} in the lumen is reduced to Fe^{2+} through the action of the ferric reductase Dcytb. Fe^{2+} is subsequently absorbed into the enterocytes by the non-specific transporter DMT1, is exported across the basolateral membrane of the enterocyte by ferroportin, oxidized by the transmembrane ferroxidase hephaestin to Fe^{3+} and bound to transferrin for subsequent transport throughout the body. The rate of iron absorption by enterocytes is controlled by the activity of the transporters DMT1 and ferroportin in the relevant membranes.
When iron requirements are increased or body iron stores are low, reduced hepatic production of the peptide hormone hepcidin leads to increased iron absorption through the internalization and degradation of ferroportin, resulting in reduced iron export from enterocytes. Levels of duodenal Dcytb and DMT1 are also affected, most likely in response to altered iron levels in enterocytes caused by the action of hepcidin on iron efflux through ferroportin. “Ferroportin (or IREG1) is an iron-regulated protein involved in the transfer of iron from the intestine to the circulation across the basolateral membrane and controls the amount of iron which enters the body” (McKie, 2000; BASF DocID 2000/1024125). A reduction in this key iron transporter would lead to a decrease in iron absorption and consequently result in the observed serum iron levels. Furthermore, Dcytb was highly down regulated (2.7 and 3.2-fold for the 4000 and 8000 ppm dose group). “Dcytb is the apical ferric reductase converting ferric iron (Fe^{3+}) into ferrous iron (Fe^{2+}), which is the transportable form of iron by DMT1 (or DCT1 for divalent cation transporter)” (McKie, 2001; BASF DocID 2001/1032283). Dietary iron is mostly present as ferric (Fe^{3+}). It could be shown that iron deficiency strongly stimulates reductase activity. In accordance with these findings, transcription factor Hif2, which controls the transcription of Dcytb and ferroportin, was also down regulated.

Overall, treatment of male mice with dimoxystrobin resulted in an increase in the weight of duodenum suggesting a direct effect on the duodenal mucosa causing mucosal cells to proliferate. As the effects on duodenal weights and duodenal histopathology occur almost immediately after dimoxystrobin treatment a local effect at the receptor level is assumed. The gene array experiments showed that dimoxystrobin treatment led to marked changes in iron metabolism genes in the duodenum. In general, there was a down-regulation in genes (namely Dcytb and Ferroportin, supported by a down-regulation of Hif2α, which has been shown to directly regulate the transcription of both Dcytb and ferroportin genes), which are involved in the iron absorption. The data suggest that dimoxystrobin induces iron deficiency in duodenum. There is a clear threshold for duodenal cell proliferation observed at 50 mg/kg bw and the induction of the cell proliferation is reversible.

Conclusion on mode of action:

Taken together the mechanistic studies conducted to elucidate the mode of action responsible for duodenal tumors in mice and for thickening of the duodenal mucosa in mice and rats support the hypothesized mode of action:

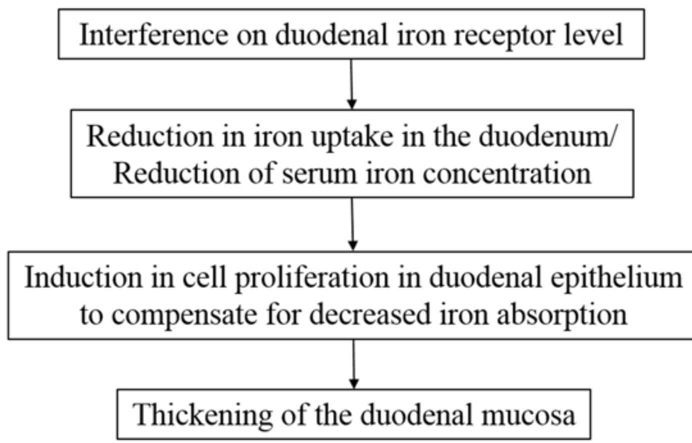


Figure 5.5-5: Flow-scheme of hypothesized mechanism of dimoxystrobin related duodenum toxicity

Dimoxystrobin interferes with different cellular duodenal iron receptors involved in the uptake and metabolism of iron in the duodenal enterocytes. By a reversible interaction with receptors responsible for iron uptake into the enterocytes, dimoxystrobin most probably leads to decreases in serum iron levels in mice. A downward trend in serum iron concentration was detected in mice after administration of dimoxystrobin for 7 days at 4000 and 8000 ppm.
In rats decreased serum iron levels could be detected in a repeated dose toxicity study at dose levels ≥ 250 ppm after dietary administration of dimoxystrobin for 7 days.
The iron-deficiency leads to an adaptive response in order to enhance iron uptake by increasing the surface area by lengthening and broadening of villi. DNA synthesis is induced in the enterocytes resulting in an increased cell proliferation and with this a thickening of the duodenal mucosa and an increase in duodenal weights. This effect of duodenal thickening was seen in several repeated-dose studies in mice and rats after treatment with dimoxystrobin.
The ultimate effects seen in mice after long-term treatment with dimoxystrobin are increased incidences of duodenal adenoma and adenocarcinoma at high dose levels.

It is concluded that the mechanistic data for dimoxystrobin indicates an interaction of dimoxystrobin with iron uptake at the duodenal receptor level, supporting the non-genotoxic, threshold-based mode of action for tumor formation in the mouse only.

There is no evidence in literature for the induction of small bowel adenocarcinoma (SBA) in the duodenum by iron deficiency in humans. Therefore, this mode of action leading to adenoma and adenocarcinoma in the intestine of mice is probably not relevant to the human being. In humans the lack of iron is quite frequent, whereas epithelial duodenum neoplasia are rare. A comprehensive detailed assessment on the relevance of iron deficit for the development of duodenal cancer (small bowel adenocarcinoma, SBA) in humans can be found in chapter MCA 5.9 of this dossier (BASF DocID 2010/1228652).

Conclusion:

Dimoxystrobin caused duodenal tumours in high dose mice only (1000 ppm, approximately 177.4 mg/kg bw/day in males and 500 ppm, approximately 103.7 mg/kg bw/day in females). Three top dose males showed an adenoma in the duodenum. Adenocarcinomas of the duodenum were noted in three top dose males and in one female of the top dose group. No neoplastic lesions were observed in the mid and low dose animals. A clear NOAEL of 200/100 ppm (corresponding to 31.8 and 19.8 mg/kg bw for males and females) could be detected for duodenal effects (duodenal mucosa thickening) caused by dimoxystrobin in mice and a threshold of 200 ppm (about 50 mg/kg bw) was identified for the induction of cell-proliferation in the duodenum. No other types of tumours occurred in any other species administered dimoxystrobin in long-term toxicity and carcinogenicity studies.

Additional considerations (weight of evidence):
Only a small number of animals showed duodenal tumours in the carcinogenicity study in mice (adenoma in 3/50 males, adenocarcinoma in 3/50 males and 1/50 females). The occurrence of tumors in the high dose groups was not statistically significant. As spontaneously occurring intestinal tumors are uncommon in this strain of mice, these findings were considered to be treatment related (exceeding the historical control data). As indicated above only one tumor type was evident in the carcinogenicity studies and only one animal species was affected. Furthermore, there is no epidemiological evidence for the induction of small bowel adenocarcinoma (SBA) in the duodenum by iron deficiency in humans (see evaluation in Chapter M-CA 5.9 of this dossier). Therefore, the finding of increased incidences of adenocarcinoma in the duodenum of mice following dimoxystrobin-induced reversible local interaction with duodenum receptors and iron-deficiency at high doses with clear thresholds may thus be considered irrelevant for humans at the expected human exposures, when handling dimoxystrobin-containing products.

References

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CA 5.6 Reproductive Toxicity

Studies evaluated in the draft assessment report (DAR, July 2003):

Dimoxystrobin (BAS 505 F) has been tested in a two-generation study and a modified one-generation study in rats and in prenatal toxicity studies in rats and rabbits. All studies are scientifically valid. The studies listed in Table 5.6-1 have been evaluated and peer reviewed during the previous Annex I inclusion process.

Table 5.6-1: Summary of reproduction toxicity studies with Dimoxystrobin already evaluated

Study	NOAEL (mg/kg bw/d)	Critical effects	Reference
2-generation study Wistar rats 0, 50, 150, 500, 1200 ppm	Reproduction performance and fertility: 1200 ppm (136 mg/kg bw/day); Systemic toxicity (parents): 150 ppm (17 mg/kg bw/day) Developmental toxicity: 50 ppm (5 mg/kg bw/day in adults*; however estimated actual doses in pups are 12 mg/kg bw/day*)	Parental toxicity 500 - 1200 ppm: reduced food consumption, impaired bw and bw gain; Pup toxicity 500 - 1200 ppm: reduced no. of liveborn, increased no. of stillborn, reduced no. of implants, reduced no. of delivered pups (findings inconsistent between both generations), impaired bw and bw gain, delays in development landmarks, decreased weights of thymus and spleen, yellowish liver discoloration, cardiomegaly (in PND 21 pups only) Pup toxicity 150 ppm: Impaired bw and bw gain, decreased weight of thymus, yellowish liver discoloration, cardiomegaly (in PND 21 pups only)	BASF DocID 2000/1016869
Modified one-generation study Wistar rats 0, 150, 500, 1200 ppm	BMD calculations for body weight effects in F1 adult females and F2 pups: $BMDL_{05}$ (females, PND 21): 25.5-45.3 mg/kg bw, (using measured substance intakes during lactation); $BMDL_{05}$ (pups, PND 21): 39.8 mg/kg bw (using estimated substance intakes for PND 21)	Parental toxicity 500 - 1200 ppm: reduced food consumption, impaired bw gain, microcytic hypochromic anemia 150 ppm: Microcytic hypochromic anemia (slight) Pup toxicity 500 - 1200 ppm: impaired bw gain, microcytic hypochromic anemia, increased relative heart weights, cardiomegaly, yellowish liver discoloration, milky fluid in abdomen, pale kidneys (in PND21 pups only) 150 ppm: microcytic hypochromic anemia (slight), increased reticulocytes	BASF DocID 2000/1016870

Study	NOAEL (mg/kg bw/d)	Critical effects	Reference
Prenatal toxicity Wistar rats 0, 60, 120, 300 mg/kg bw/day, day 6 - 19 post coitum	Maternal toxicity: 60 mg/kg bw Developmental toxicity: 300 mg/kg bw.	Maternal toxicity at 120 and 300 mg/kg bw/day: Reduced food consumption, reduction in body weight gain and in corrected body weight gain No developmental toxicity Not teratogenic	BASF DocID 1999/11680
Prenatal toxicity Himalayan rabbit 0, 25, 50, 100 mg/kg bw/day, day 7 - 28 post insemination	Maternal toxicity: 5 mg/kg bw Developmental toxicity: *50 mg/kg bw**	Maternal toxicity 25 - 100 mg/kg bw/day: Mortality (100 mg/kg bw), no defaecation (50 and 100 mg/kg bw), diarrhoea, reduced food consumption, impaired bw gain Developmental toxicity *100 mg/kg bw:* Reduced gravid uterus weight, increased resorption rate and increased post implantation loss due to severe maternal toxicity (100 mg/kg bw), *increased no. of fetuses with fused sternebrae (a skeletal variation) within HCD** Not teratogenic	BASF DocID 2000/1016867
Prenatal toxicity Himalayan rabbit 0, 5, 20, 75 mg/kg bw/day, day 7 - 28 post insemination		Maternal toxicity 20, 75 mg/kg bw: Mortality and no defaecation (75 mg/kg bw), diarrhoea, reduced food consumption, impaired bw gain Developmental toxicity 75 mg/kg bw: Reduced gravid uterus weight, increased resorption rate, increased post implantation loss due to severe maternal toxicity, increased no. of fetuses with fused sternebrae (a skeletal variation) within HCD Not teratogenic	BASF DocID 2001/1016351

The information *in italics* represent most recent assessments based on further data
*The developmental NOAEL of the first rabbit study was previously considered to be 25 mg/kg bw, based on increased incidences of fetuses with fused sternebrae seen in the mid dose of 50 mg/kg bw. However using more appropriate historical control data, the dose of 50 mg/kg bw (from the first rabbit study) can be considered to be the overall $NOAEL_{development}$ for rabbit.

Summary (as taken from the Draft Assessment Report)

The reproduction toxicity of dimoxystrobin was investigated in a 2-generation reproduction toxicity study in rats supplemented by a modified one-generation toxicity study to determine potential haematological effects. Although this was a detailed investigation, there was no systemic investigation of possible histopathological effects on the duodenum of adults and no histopathological investigation of the pups.

Prenatal developmental toxicity studies were performed in rats and rabbits. In the multigeneration study in rats, no effects on reproductive performance or fertility were detected up to the highest dose level of 1200 ppm. In this investigation, and the modified one-generation study, the dose-dependent general (parental) toxicity was evidenced in particular by impairment of food consumption, body weight and body weight gain as well as a regenerative microcytic hypochromic anaemia (reduced haemoglobin concentrations and mean corpuscular indices associated with increased microcytosis and reticulocytes). There was a reduced number of liveborn and increased number of stillborn F1 pups, reduced number of implants and delivered F2 pups, lower pup body weights and impaired body weight gains. Pups also showed delays in development landmarks, decreased weights of thymus and spleen, yellowish liver discoloration, cardiomegaly and regenerative microcytic hypochromic anaemia. The study investigators proposed that these effects on liver and heart of pups were related to the regenerative microcytic hypochromic anaemia. Effects on the pups were seen at the same dose levels as effects on the parents. Notably, the LOAEL for the haematological findings in parents and pups was 150 ppm (18 mg/kg bw for adults, conversion not calculated for pups). Although haematological effects on pups were clearly greater than that of parents at the top dose tested, it is not clear if pups were more sensitive because older pups would have been consuming the test diet directly. At the LOAEL, the body weight of pups was reduced during lactation in the absence of an effect on body weight of lactating dams, but the overall effect on pup weight prior to weaning was slight. NO classification for developmental toxicity is considered appropriate based on these results.

In the prenatal toxicity study in rats, no developmental toxicity was observed, even at the highest dose tested. This dose level was, however, clearly toxic to the dams, as demonstrated by a 7% reduction in corrected body weight gain. Two prenatal toxicity studies in rabbits were performed. Whereas no NOAEL for maternal toxicity could be determined in the first study, the second study demonstrated a maternal NOAEL of 5 mg/kg bw based on disturbances of intestinal function, reduced food consumption and body weight gain. There was no evidence for dimoxystrobin being teratogenic. However increased resorptions were seen at a dose level which was highly toxic to does (a few maternal deaths occurred). An increased incidence of a skeletal variation, fused sternebrae, was also seen at lower dose levels in the presence of clear maternal toxicity. Since developmental toxicity was seen only in the presence of maternal toxicity and the increased incidence of fused sternebrae was within the historical control range, it is not appropriate to classify dimoxystrobin as a developmental toxicant on the basis of these rabbit studies.

As is typically the case, there are limitation to the NOAELs proposed for general parental toxicity (non-reproductive effects) in these reproductive toxicity studies because of limitations to the extent of investigations of parental toxicity. For example in he developmental toxicity studies the extent of gross pathological examination is not stated and there was no investigation of duodenal histology or blood effects.

Based on the available data, the following endpoints were determined during the previous Annex I listing of dimoxystrobin:

Target / critical effect - Reproduction:	No adverse effects on reproductive performance. Developmental toxicity (slight microcytic hypochromic anemia, reduced body weight gain, reduced thymus weight, discoloured liver, cardiomegaly) in presence of parental toxicity (slight microcytic hypochromic anemia)
Lowest relevant reproductive NOAEL / NOEL:	Reproductive performance: 136 mg/kg bw/day Parental: 17 mg/kg bw/day
Target / critical effect - Developmental toxicity:	Rabbit: Increased incidence of skeletal variation at maternally toxic dose
Lowest relevant developmental NOAEL / NOEL:	Maternal toxicity: 5 mg/kg bw/day (rabbits) Developmental effects: 20 mg/kg bw/day (rabbits)

Studies submitted in this supplementary dossier (not yet peer-reviewed):
An enhanced one-generation reproduction toxicity study in Wistar rats was conducted according to OECD TG 416 (2001) in order to identify a NOAEL for hematology and serum iron levels in adults and offspring and is included as new information in the dossier (BASF DocID 2011/1211676). A detailed study summary can be found in this chapter.

Table 5.6-2: Summary of reproduction toxicity studies with Dimoxystrobin not evaluated yet

Study	NOAEL (mg/kg bw/d)	Critical effects	Reference
Enhanced* one-generation study Wistar rats 0, 10, 20, 50 ppm	Parental rats NOAEL, systemic toxicity: ≥ 50 ppm (about 4.3 mg/kg bw/day) NOEL, fertility and reproductive performance: ≥ 50 ppm (about 4.3 mg/kg bw/day) Progeny NOEL, developmental toxicity: ≥ 50 ppm (about 4.3 mg/kg bw/day)	Systemic toxicity: No critical effects were observed. None of the hematological and clinical chemical endpoints revealed an indication of a treatment-related anemia up to the highest dose tested. Fertility: No critical effects were observed. Developmental toxicity: No critical effects were observed.	BASF DocID 2011/1211676

*Additional hematological parameter were included in adults and offspring and serum iron was determined.

Dimoxystrobin is classified with R63 (corresponding to Repr. 2. according to GHS), as discussed at ECB in March and November 2005. Neither the RMS, nor EFSA had proposed an R63 based on the available data set. As the notifier also disagrees with this classification and new studies have been generated, which are presented and discussed in this dossier, since the decision was taken at the ECB, a detailed evaluation on all reproductive and developmental toxicity studies with dimoxystrobin are presented in this chapter. A justification is made on why a classification with Repr. 2 (H361d) is not warranted.

Using more appropriate historical control data, including estimates on actual pup dosing and calculating benchmark doses for effects on body weights in adults and offspring a re-evaluation of the reproduction and the developmental toxicity studies was considered needed. Based on the new evaluation, including the results of the new enhanced 1-generation study, the following list of endpoints is proposed:

Reproductive Toxicity

Reproduction target / critical effect	Parental toxicity: reduced food consumption and body weight, microcytic hypochromic anemia Reproductive toxicity: no adverse effects observed in rat 2-generation study Offspring toxicity: microcytic hypochromic anemia, reduced pup body weights, cardiomegaly (PND 21)
Relevant parental NOAEL	17 mg/kg bw/day $BMDL_{05}$ (F1 female adults): 25.5 – 45.3 mg/kg bw, during lactation
Relevant reproductive NOAEL	136 mg/kg bw/day
Relevant offspring NOAEL	5 mg/kg bw/day (based on adult data, about 12 mg/kg estimated value in pups) $BMDL_{05}$ (F2 pups) 39.8 mg/kg bw, PND21 calculated

Developmental Toxicity

Developmental target / critical effect	Rabbit: Maternal toxicity: mortality, abortion, reduced bod weight gain and food consumption, diarrhea Developmental toxicity: Increased resorptions and post implantation loss, increased incidence of skeletal variation (within HCD) at maternally toxic dose
Relevant maternal NOAEL	Rabbit: 5 mg/kg bw/day
Relevant developmental NOAEL	Rabbit: 50 mg/kg bw/day

CA 5.6.1 Generational studies

Note: The rat 2-generation and modified 1-generation toxicity study reports (2000/1016869 and 2000/1016870) were already submitted and reviewed in the course of the initial registration of dimoxystrobin.

BASF DocID 2000/1016869): Two-generation reproduction study with dimoxystrobin in rats (Study presented in the original Annex I Dossier)
BASF DocID 2015/1172904 (Benchmark dose calculation of body weight effects in parental females and offspring)

Executive summary:
In a 2-generation reproduction toxicity study, dimoxystrobin (Batch: N 15; Purity 98.4%) was administered in the diet to groups of 25 male and 25 female Wistar rats (Chbb = THOM (SPF)) at dietary concentrations of 0, 50, 150, 500 and 1200 ppm (0, 5, 17, 55 and 136 mg/kg bw) throughout 2 generations.

No treatment-related mortality was observed in any of the male and female parental animals throughout the study. Food consumption and body weight development were impaired in the 500 and 1200 ppm dose F0 and F1 female parental animals and F1 male parental animals. In F0 male parental animals, food consumption and body weight development was impaired in the 1200 ppm dose group. There were no treatment-related clinical, gross, or histopathological observations indicating general, systemic toxicity in both parental generations at 50 and 150 ppm.

Treatment with dimoxystrobin up to the concentration of 1200 ppm had no effects on the estrous cycle, the number, morphology and motility of sperm as well as on male or female fertility. Male and female fertility indices ranged between 80 and 100% without any relation to dose. Dimoxystrobin treatment did not affect the reproductive performance as was evident from the absence of effects on the pre-coital interval or gestation lengths as well as gestation (96 to 100%) or live birth indices (95 to 99%). Some high dose effects were not consistent between the first and the second generation in the 2-generation study with dimoxystrobin and were well covered by data compiled from historical control data. Overall, all the effects on reproductive and developmental parameters can be regarded as incidental and not treatment-related.
Gross- and histopathological examination of the reproductive organs of apparently infertile males and females did not reveal any common cause for the lack of reproductive success and thus were considered to be unrelated to treatment. Finally, ovarian follicle counts did not reveal any treatment-related differences between control and high dose groups.

Survival of pups was not affected by treatment as viability and lactation indices were in the range of 92 to 100% and 97 to 100%, respectively. Body weight development of the 1200 and 500 ppm dose F1 and additionally the 150 ppm F2 pups was significantly impaired. Pup body weight effects were absent at birth and developed over time during lactation being most prominent at PND 14 and 21 after start of self-feeding of the pups. Calculated dimoxystrobin doses of the pups are considerably higher compared to the dams at the same dietary dose levels.

Other pup parameters like sex ratio, clinical observations and organ weights findings did not reveal any treatment-related effects. The observed effects on absolute and relative thymus and spleen weights were secondary to the lower terminal pup body weights. Gross necropsy findings comprised pale yellowish discoloration of the liver, cardiomegaly, milky fluid in the abdomen and/or thorax after organ evisceration, and hypoplasia of thymus, which was related to the reduced pup body weights. The findings on hearts, and liver were considered to be secondary to a microcytic hypochromic anemia. The milky fluids reported in the abdomen and the breast cavity are considered to be secondary to the heart-insufficiency (cardiomegaly) induced by the chronic microcytic anemia. A decompensated right heart insufficiency is known to lead to fluid retention in the body as observed by edema in the legs as well as ascites in the big body cavities (abdomen and breast cavity).
Cardiomegaly was reported in the PND21 pups only and was not see in PND4 pups macroscopically. Furthermore, parental F1 animals did not show cardiomegaly. There is information in the literature indicating that young animals undergo cardiac remodeling secondary to anemia. Cardiomegaly is a transient effect and not an irreversible structural malformation.

The delay in vaginal opening and preputial separation in selected F1 females was assessed as being a consequence of the reduced body weights and not as a sign of general delay in sexual maturation. The sexual maturation of F1 offspring was, therefore, not directly affected by treatment.

Based on the effects on body weight development and food consumption the parental NOAEL for general, systemic toxicity in this study was identified at 150 ppm (17 mg/kg bw/day). In the absence of any effects on fertility and reproductive performance the NOAEL for reproductive performance and fertility was at least 1200 ppm (136 mg/kg bw/day). The NOAEL for developmental toxicity was 150 ppm (17 mg/kg bw/day in adults; about 30 mg/kg bw/day estimated value in pups) in the F1 and 50 ppm (5 mg/kg bw/day in adults; about 12 mg/kg bw/day estimated value in pups) in the F2 progeny based on impairments in pup body weight.
When doing benchmark dose calculations the no effect levels (= PODs) for body weight effects between dams (25.5-45.3 mg/kg bw during lactation) and pups (about 39.8 mg/kg bw; PND 21) are essentially comparable.

Materials and Methods:
Dimoxystrobin (batch/purity: N 15: 98.4%) was administered to groups of 25 male and 25 female young Wistar rats (F0 parental generation) as a constant homogeneous addition to the food in different concentrations (0, 50, 150, 500 and 1200 ppm). At least 74 days after the beginning of treatment, F0 animals were mated (1:1) to produce a litter (F1). Litters were standardised to 8 pups on day 4. Mating pairs were from the same dose group and F1 animals selected for breeding were continued in the same dosing group as their parents. Groups of 25 males and 25 females selected from F1 pups as F1 parental generation were offered diets containing 0, 50, 150, 500 and 1200 ppm of the test substance post weaning, and the breeding program was repeated to produce F2 litter. The study was terminated with the terminal sacrifice of the F2 weanlings and F1 adult animals.

Results:

A. TEST SUBSTANCE – ANALYSIS AND INTAKE

The stability of the test substance in the diet was demonstrated for a period of 49 days at room temperature in a comparable batch.
The homogeneity of the test substance preparations at concentrations of 50 and 1200 ppm was proven before the start and towards the end of the present study.
The concentration control analyses of all concentrations in the samples taken before the start and towards the end of the study revealed that the values were within the expected range of the target concentrations.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related clinical observations were detected in the male and female F0 parental animals in any group during the administration period.
There were no particular substance-related clinical findings in F0 females during the gestation period for F1 litter. After mating, one control F0 female (#201) had no sperm in vaginal smear and did not deliver F1 pups. Two sperm positive females of the 50 ppm test group (#246, 247), three of the 500 ppm test group (#276, 289, 293) and one of the 1200 ppm test group (#316) did not deliver any F1 pups. The isolated occurrence and the absence of a dose-response relationship indicated a spontaneous origin of these findings.
The F0 dams revealed no treatment-related clinical finding during the lactation period. Only some spontaneous clinical findings occurred in a few rats of all test groups. The absence of a dose-response indicated a spontaneous origin of these findings.

No test substance-related clinical signs were detected in male or female F1 parental animals. There were no particular substance-related clinical findings in F1 parental females during the gestation period for F2 litters. Of the sperm positive F1 females one control (#602), two of the 50 ppm test group (#656, 659), and two of the 1200 ppm test group (#706, 714) did not deliver any F1 pups. Insufficient nesting activity was observed for one 150 ppm female (#669). The isolated occurrence and the absence of a dose-response relationship indicated a spontaneous origin of these findings.
The F1 dams revealed no treatment-related clinical finding during the lactation period.

2. Mortality

No treatment-related mortality was observed in any of the male and female F0 parental animals throughout the study.
One low dose F0 generation dam (#234) died during delivery of F1 pups, without showing any remarkable clinical findings before the unscheduled death. Pathological examinations confirmed the death of this rat was spontaneous and not treatment-related.

No mortality was observed in F1 parental animals.

C. PARENTAL BODY WEIGHT AND BODY WEIGHT GAIN

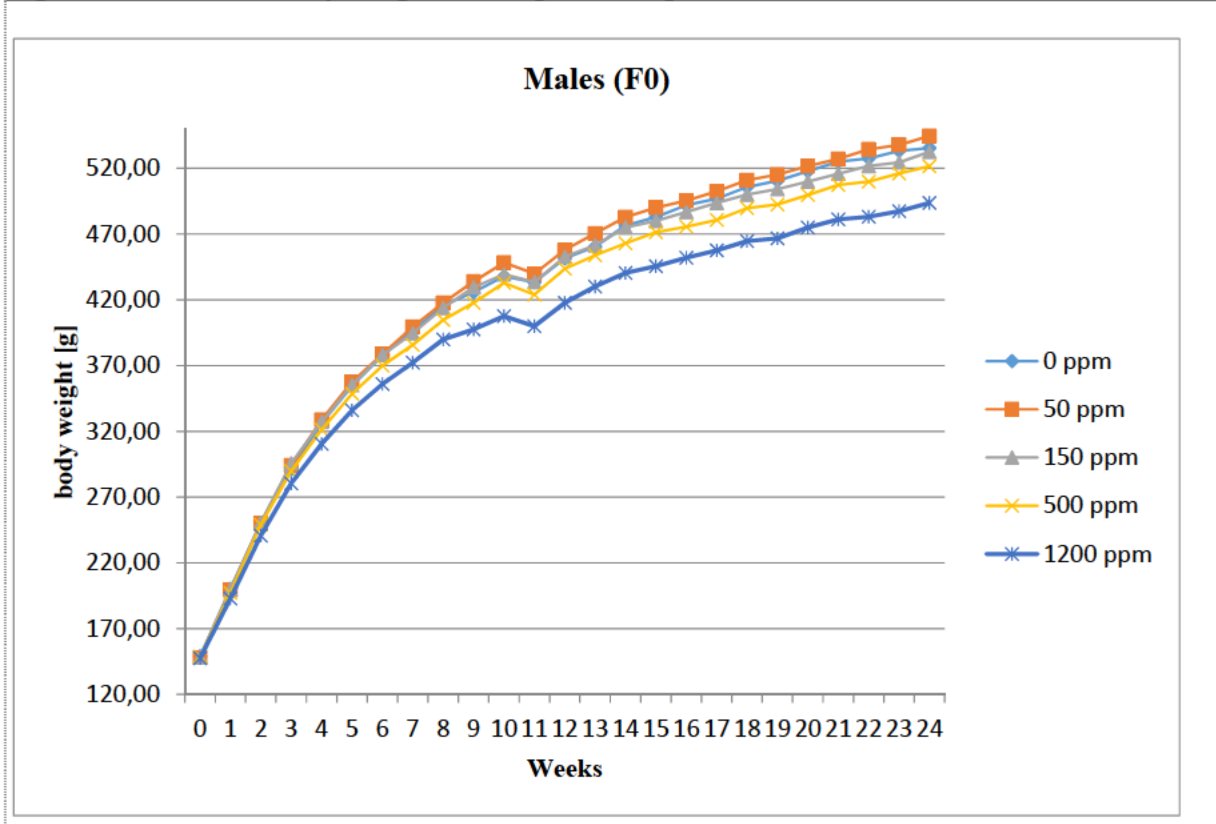
Body weight development was impaired in 500 ppm and 1200 ppm female F0 and F1 animals as well as in 1200 ppm male F0 animals and 500 ppm and 1200 ppm F1 males.

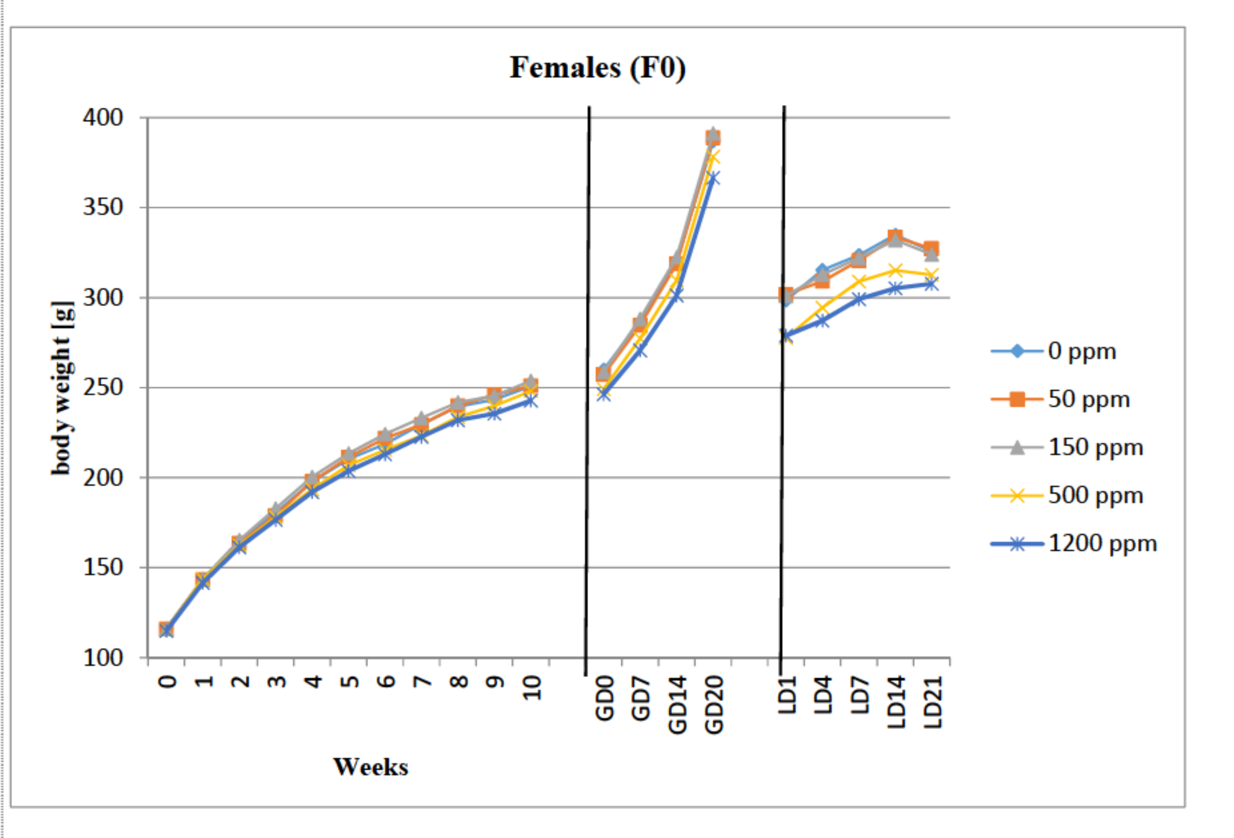
F0 parental animals:

Mean body weights of F0 parental males were impaired at 1200 ppm by test substance administration during the entire study period [see Figure 5.6.1-1].
Statistically significantly reduced body weight in the high dose (1200 ppm) males was observed from study week 4 until the end of the study, about 8% below controls at termination of the study. This was in-line with the reduced food consumption in this dose group. Mean body weight gains of this dose group were also clearly impaired (about 10% below control).

Mean body weights and mean body weight gains of high dose (1200 ppm) F0 parental females were comparable to controls during premating. During gestation and lactation, as well as after weaning, however, mean body weights were below controls, reaching statistically significance during some intervals [see Figure 5.6.1-1]. Throughout gestation and lactation, as well as after weaning, mean body weights of high dose females were decreased (-5%, -6%, -7%). Moreover, the mean body weights of the 500 ppm dose group females were slightly below controls during lactation and post-weaning phase. The impairments in the body weight of the 500 and 1200 ppm dams were regarded as treatment-related. As concurrent impairments in food consumption also occurred in these rats, the impairments in body weight were considered to be treatment-related.

Figure 5.6.1-1: Body weight development of parental F0 rats



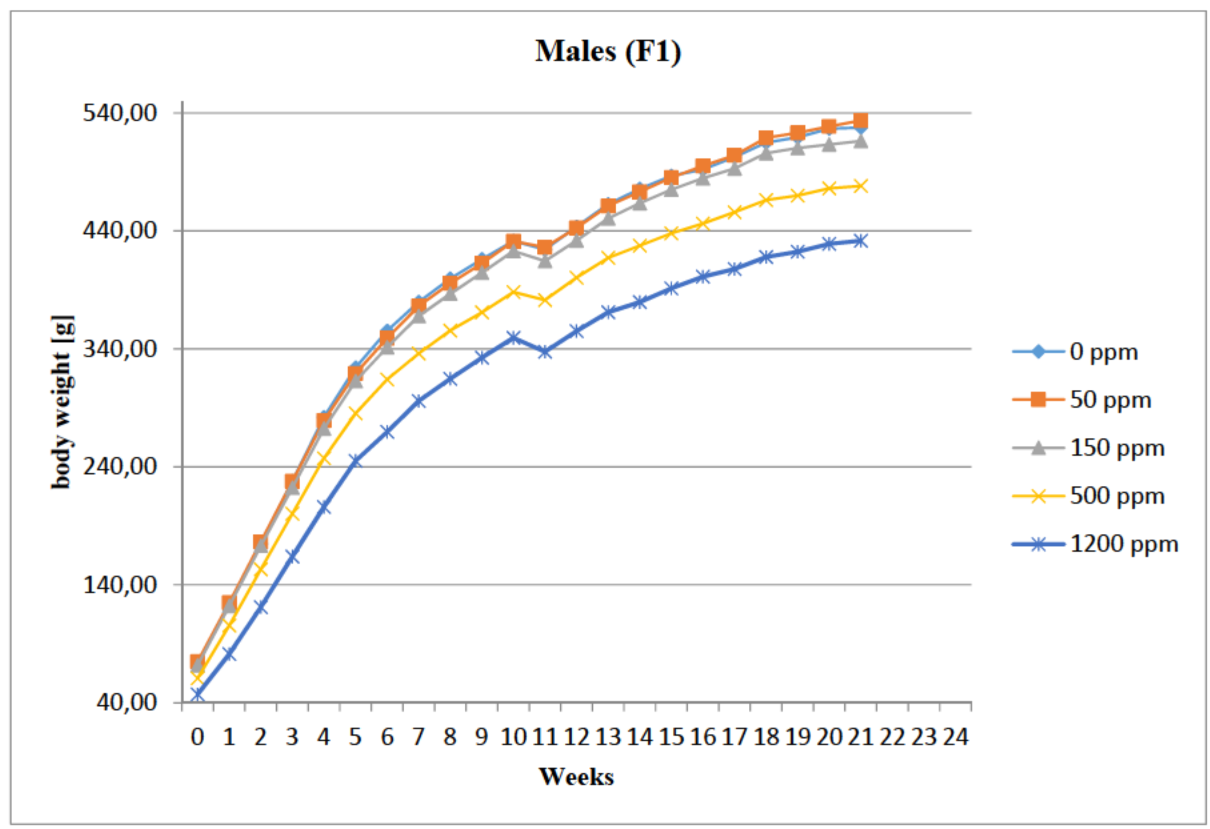


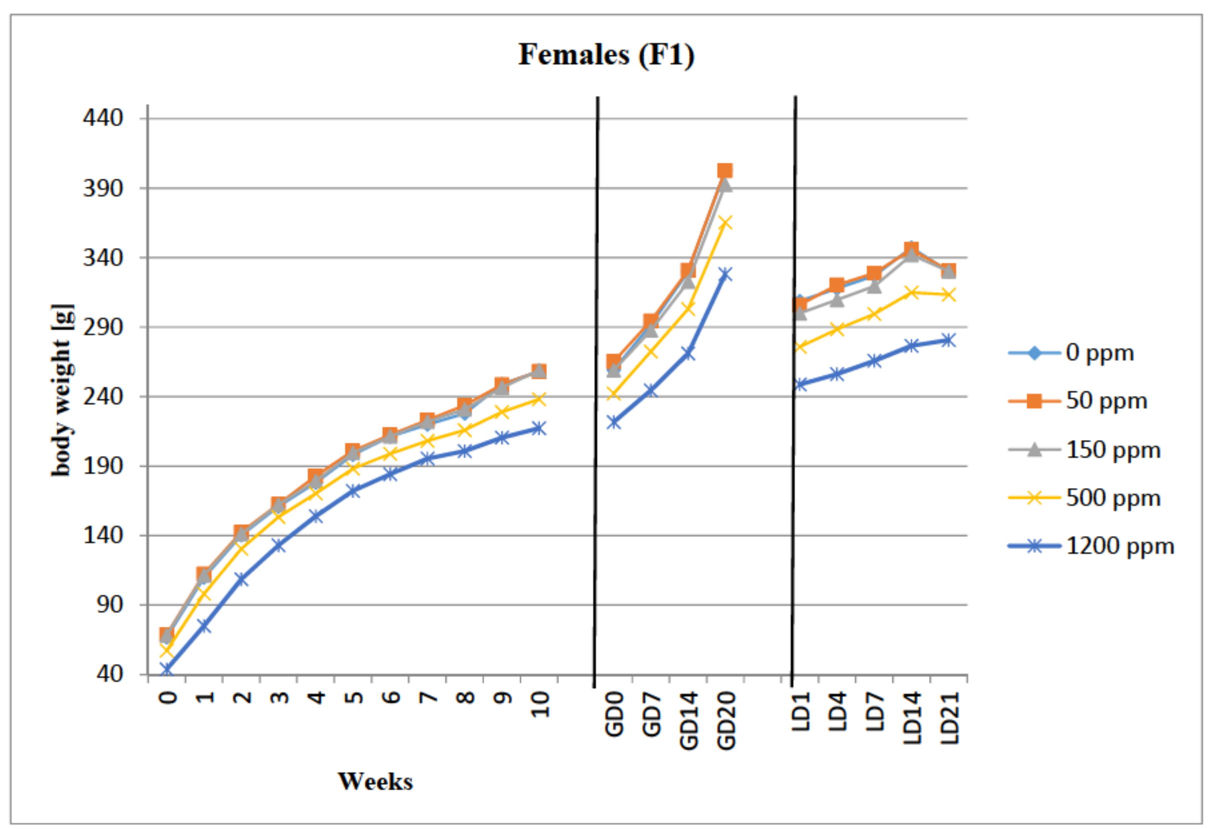
F1 parental animals:

Mean body weights of the 500 ppm and 1200 ppm parental F1 males were statistically significantly impaired during the entire pre-mating period [see Figure 5.6.1-2]. Before sacrifice mean body weighs were about 9% and 18% below controls. Mean body weight gains were similarily affected, but attaining only sporadically statistical significance. This was in-line with the reduced food consumption in this dose group.

Mean body weights of the 500 and 1200 ppm F1 parental females were reduced during various phases of the entire study [see Figure 5.6.1-2]. Throughout pre-mating, gestation and lactation mean body weights of high dose females were decreased (-16%, -19%, -15%). Body weights of the 500 ppm females were also reduced during pre-mating, gestation and lactation (about -8%, -9% and -5% below controls). The impairments in the body weight of the 500 and 1200 ppm dams were regarded as treatment-related. As concurrent impairments in food consumption also occurred in these rats, the impairments in body weight were considered to be treatment-related.

Figure 5.6.1-2: Body weight development of parental F_1 rats





Body weight development in the 50 and 150 ppm parental male and females were not affected by treatment.

D. PARENTAL FOOD CONSUMPTION AND COMPOUND INTAKE

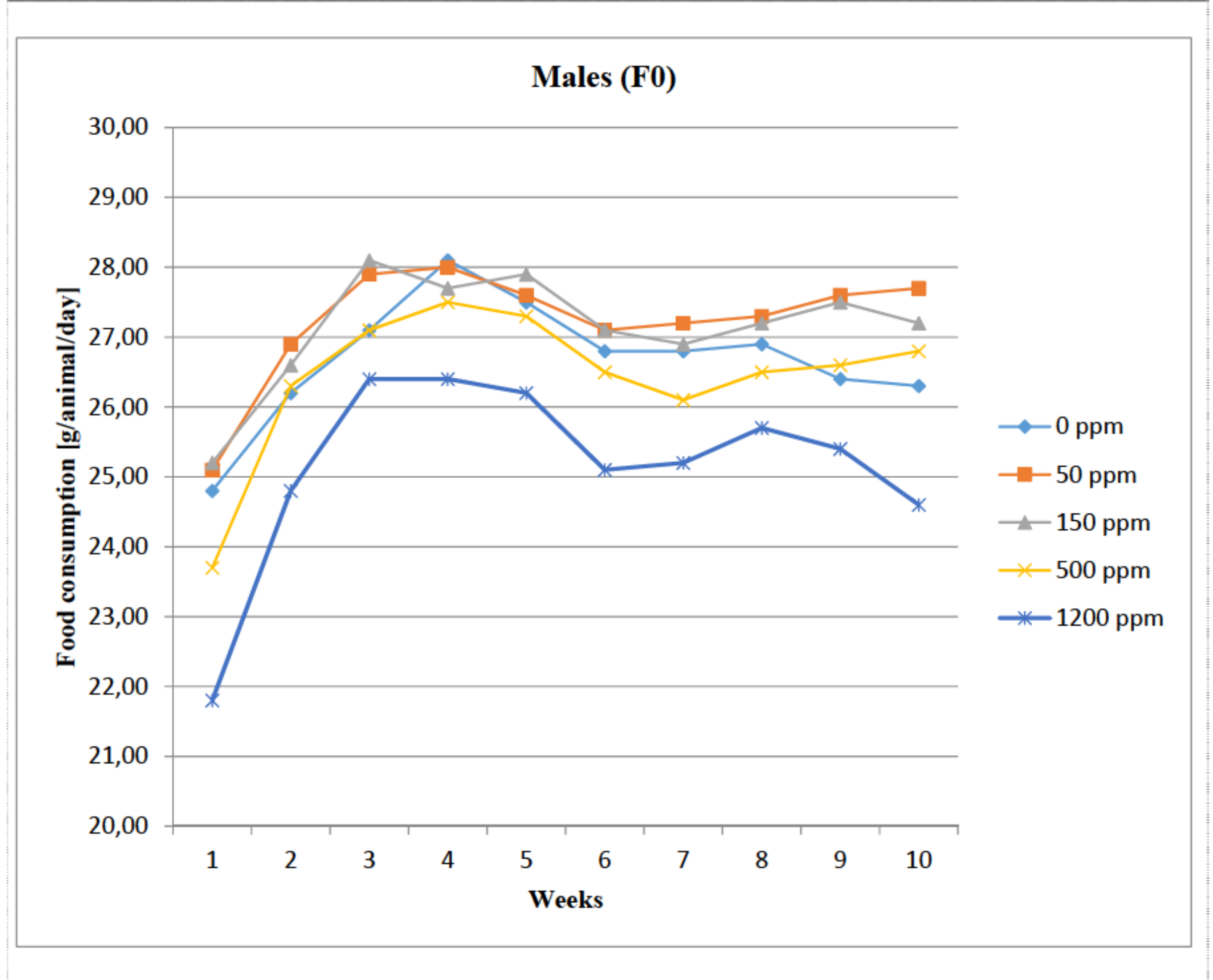
Treatment-related effects on food consumption were restricted to high dose (1200 ppm) F0 males and females and 500 and 1200 ppm F1 males and females.

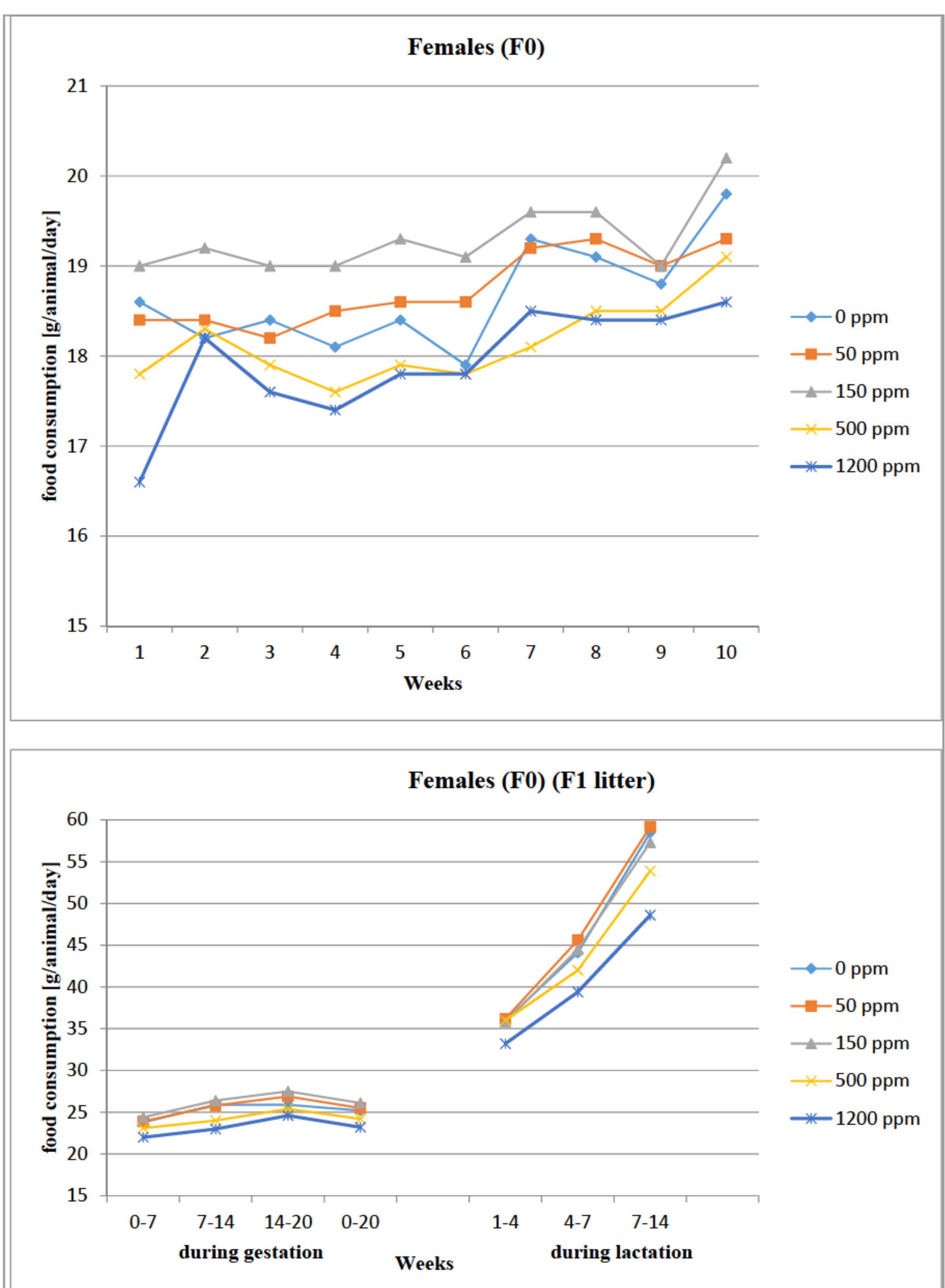
F0 parental animals:

Food consumption was reduced to about 4% in high dose (1200 ppm) F0 parental male animals during the entire premating period (statistically significant at weeks 7-8 and 9-10) [see Figure 5.6.1-3].

During pre-mating food consumption of the high dose (1200 ppm) F0 parental females was only slightly below the corresponding controls (about 4%) [see Figure 5.6.1-3]. During gestation and lactation however food consumption in this group was about 8-13% lower compared to the control group, which also had an effect on the body weight of dams and was thus considered substance-related.

Figure 5.6.1-3: Food consumption of parental F0 males and females





Food consumption of all other treated F0 males and females was not affected by treatment. The impairments of food consumption of the high dose F0 parental animals were considered to be treatment-related, due to concurrent impairments in body weight/body weight gain in these rats.

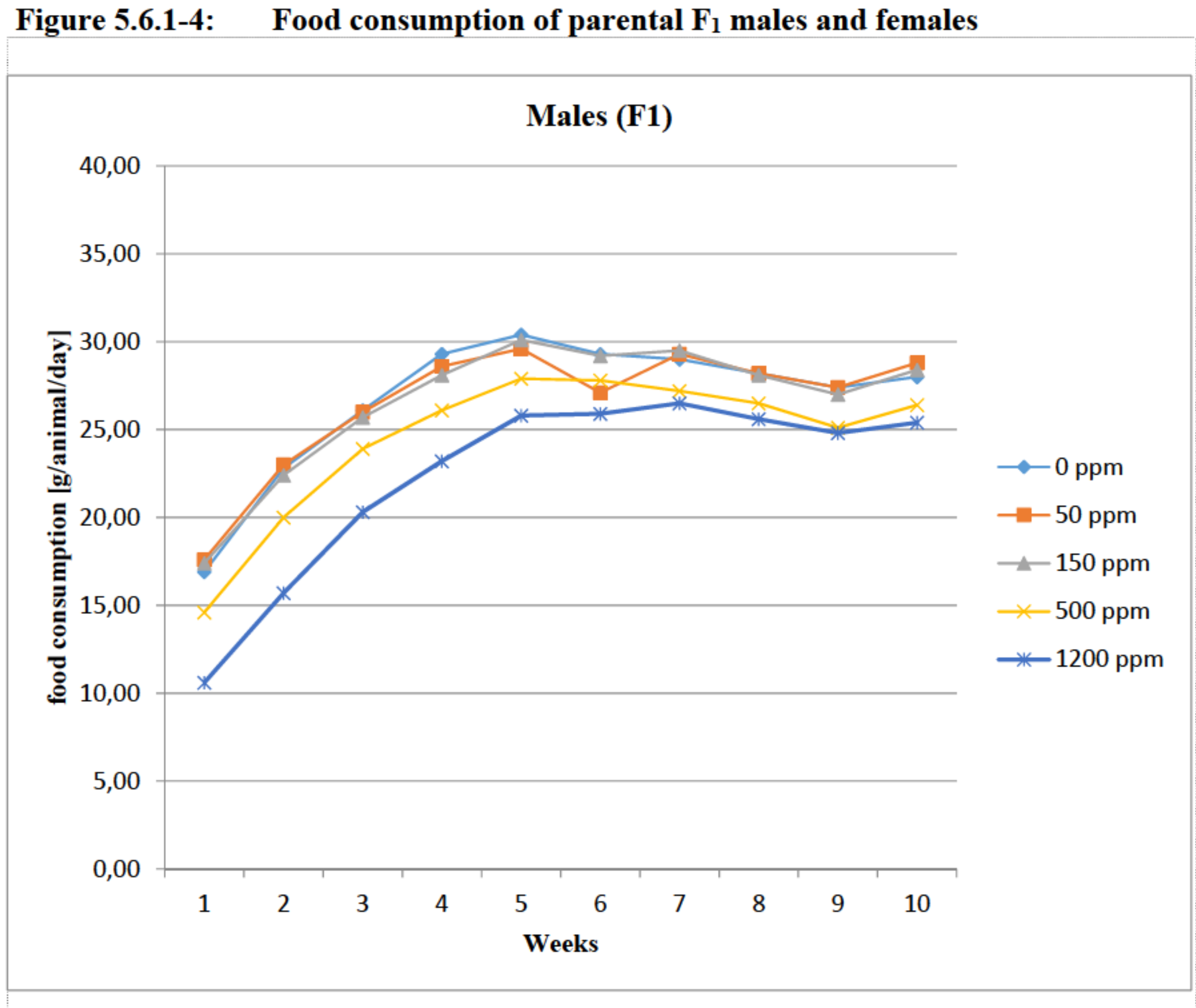
F_1 parental animals:

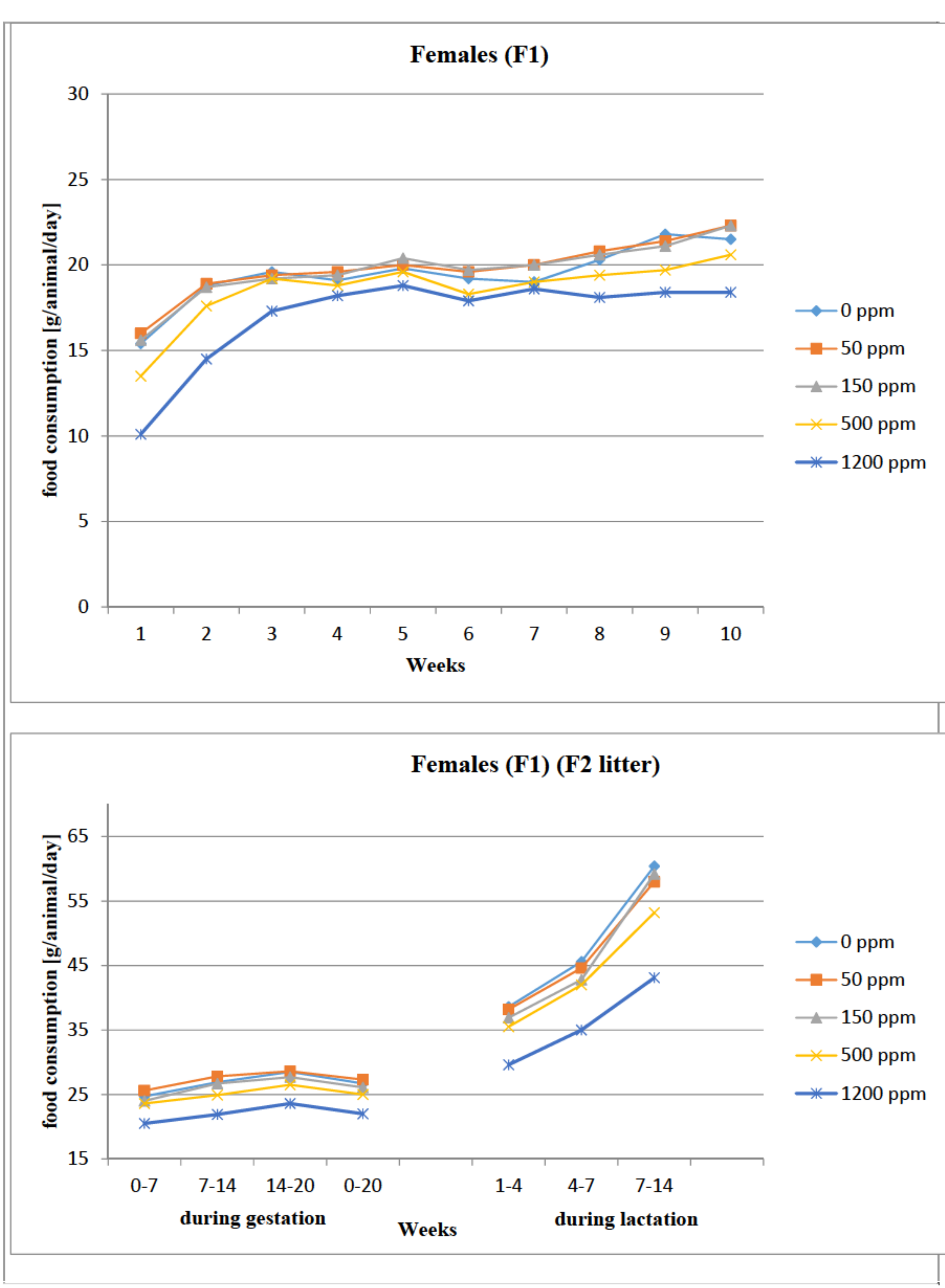
Food consumption was statistically significant reduced in the 500 (during most weeks) and 1200 ppm parental F1 male animals during pre-mating [see Figure 5.6.1-4]. Food consumption over the entire pre-mating period was impaired by about 8 or 16% compared to the concurrent control. This is in-line with the impaired body weights at 500 and 1200 ppm.

Food consumption in 500 and 1200 ppm F1 parental females was reduced during the whole premating period, but less pronounced compared to males [see Figure 5.6.1-4]. Food consumption in these dose groups was further impaired during gestation and lactation attaining statistical significance during several intervals. Compared to the corresponding controls food consumption at 500 ppm was about 4, 6, and 10%, that of the 1200 ppm dose group about 12, 18, and 26% lower over the entire pre-mating, gestation and lactation phase.

Food consumption of all treated 50 ppm and 150 ppm F1 males and females was not affected by treatment.

Figure 5.6.1-4: Food consumption of parental F_1 males and females





The impairments of food consumption of the F1 parental animals of the 500 and 1200 ppm dose groups were considered to be treatment-related, due to concurrent impairments in body weight in these rats.

E. ESTROUS CYCLE DETERMINATIONS

Estrous cycles as determined during 3 weeks prior to mating were very regular in all dose groups in F0 and F1 females. The mean cycle length was 3.9, 3.8, 3.9, 3.9, and 3.8 days in F0 and 4.9, 5.0, 5.0, 4.4, and 4.7 days in F1 females at 0, 50, 150, 500, and 1200 ppm, respectively.

F. MATING AND GESTATION DATA

1. Male reproductive performance

Male reproductive performance was not affected by treatment. The effects on mating and fertility displayed no dose-response relationship and were within the normal variation of this strain of rats used.

Nearly all F0 males, except one control animal, mated and induced pregnancy in 23, 23, 25, 22, and 24 females at 0, 50, 150, 500, and 1200 ppm, respectively [see Table 5.6.1-1]. One control F0 male (#1), two 50 ppm males (#46, 47), three 500 ppm males (#76, 89, 93), and one 1200 ppm male (#116) failed to generate F1 pups. The sporadic occurrence of infertility did not show any dose-response and is therefore regarded as being incidental.

Nearly all F1 males mated and induced pregnancy in 23, 20, 23, 25, and 22 females at 0, 50, 150, 500, and 1200 ppm, respectively [see Table 5.6.1-1]. No indication for a successful mating was observed for one male each of the test groups. For the vast majority of F1 parental males, fertility could be confirmed. The fertility index varied between 80 and 100% without showing a relation to dosing. Two control F1 males (#411, 418), five 50 ppm males (#4427 429, 438, 444, 449), two 150 ppm males (#461, 470), three 1200 ppm males (#514, 518, 523) failed to generate F1 pups. The sporadic occurrence of infertility did not show any dose-response and is therefore regarded as being incidental.

Table 5.6.1-1: Reproduction parameters of male rats treated with dimoxystrobin

Parental generation	F0					F1				
Dose [mg/kg]	0	50	150	500	1200	0	50	150	500	1200
Animals per dose	25	25	25	25	25	25	25	25	25	25
Male fertility										
- placed with females	24	25	25	25	25	25	25	25	25	25
- mated [n]	23	25	25	25	25	24	24	24	25	24
- mating index [%]	96	100	100	100	100	96	96	96	100	96
- pregnant [n]	23	23	25	22	24	23	20	23	25	22
- Fertility index [%]	96	92	100	88	96	92	80	92	100	88

For only one of the male rats (#1) corroborative histopathological findings occurred in testes and epididymides, which might explain the observed infertility.
The apparently infertile F1 males did not show histopathological findings that could explain infertility, with the exception of males #444 and #514 showing an altered size of testes and epididymides.

2. Sperm analysis

Sperm analysis (number of homogenization resistant testicular spermatids or caudal sperm, % abnormal and normal sperm and motility) did not indicate any effects of treatment in F0 and F1 males [see Table 5.6.1-2]. All values were within the range of historical data.

Table 5.6.1-2: Sperm parameters of males administered dimoxystrobin

Parental generation	F0					F1				
Dose [ppm]	0	50	150	500	1200	0	50	150	500	1200
Sperm count [10^6/g]										
Testis	86	107	103	104	89	96	110	113	121	95
Cauda epididymis	514	551	545	543	462	668	573	598	612	635
Normal sperm [%]	98.3	97.2	96.9	98.4	93.0	96.6	94.7	97.7	98.0	96.3
Abnormal sperm [%]	1.7	2.8	3.1	1.6	7.0	3.4	5.3	2.3	2.0	3.7
Sperm motility [%]	90	90	92	88	83	92	88	89	92	89

3. Female reproductive performance

Female reproductive performance was not affected by treatment.

All, except one control F0 females mated and 24, 23, 25, 22, and 24 females became pregnant at 0, 50, 150, 500 and 1200 ppm, respectively [see Table 5.6.1-3]. Two 50 ppm females (#246, 247), three 500 ppm females (#276, 298, 293), and one 1200 ppm female (#316) did not become pregnant. There appeared no histopathological findings which could explain the apparent infertility. Therefore, and due to the lack of a substance-relationship, this was assessed as being incidental.
All, except one female of the control, 50 ppm, 150 ppm, 1200 ppm dose groups each, F1 females mated and 23, 20, 23, 25, and 22 females became pregnant at 0, 50, 150, 500 and 1200 ppm, respectively [see Table 5.6.1-4]. One control F1 female (#602), four 50 ppm males (#629, 641, 642, 644), one 150 ppm males (#656), two 1200 ppm males (#706, 714) did not become pregnant. Due to the lack of substance-relationship and no corroborative findings in histopathology, this was assessed as being incidental.

The pre-coital interval was in the range of 2.6 to 3.1 days in F0 females and of 2.2 to 2.7 days in F1 females and displayed no relation to treatment level. Likewise, duration of gestation was similar in F0 (21.8 to 21.9 days) and F1 females (21.6 to 21.9 days) [see Table 5.6.1-3 and Table 5.6.1-4].

The number of F0 females with stillborn pups and thus the total number of stillborn F1 pups were slightly increased in the parental F0 generation (F1 litters) at 1200 ppm [see Table 5.6.1-3], but fully within the historical control data range. Consequently, the live birth index was slightly, but statistically significantly reduced at 1200 ppm (95%), which however was fully within the historical control data (HCD 90-99%).
The assessment, that the increased incidences of females with stillborn pups is incidental is further supported by control data generated in a further 2-generation toxicity study (Study No. 94041), which was performed with the same rat strain at the same lab in the same timeframe. In this study the number of females with stillborn pups (13) and the number of stillborn pups (35) were actually highest in the control animals, fully covering the increased numbers in the 2-generation study with dimoxystrobin, and thus demonstrating the biological variability of these parameters

The fact that the mean number of delivered pups was not affected by treatment across the groups gives further evidence that dimoxystrobin has no effect on reproduction in this generation.

Evaluating the second generation of this 2-generation study, the mean number of implantations was statistically significantly decreased compared to controls at 1200 ppm in the F1 dams (F2 litters) [see Table 5.6.1-4]. The evaluations of implantation sites was not a standard parameter in studies at that time. Therefore, no historical control data from this lab are available and can be considered. But the decreased number of implantations in the high dose group was in the range of that published in literature (HCD 11.5–18.3; Hood, 2006). The mean number of delivered F2 pups (11.8; see Table 5.6.1-4) was statistically significantly reduced in the high dose, but was covered by historical control data (HCD 11.1-16.4). The mean number of pups per litter alive on PND 0 was not statistically significantly lower than controls and there was no increase in stillbirths in this generation.
The number of stillborn F2 pups was comparable between control and treated groups. There was no effect on the live birth index. It ranged from 97 to 99% in F2 pups. The values were well within the range of biological variation (90-99%).

The gestation index in F0 females was 100% in all groups, indicating that all pregnant females delivered live F1 pups [see Table 5.6.1-3]. The gestation index in F1 females ranged between 96 and 100% [see Table 5.6.1-4]. This is considered to be incidental due to the isolated occurrence and lack of a dose-response relationship.

Table 5.6.1-3: Reproduction and gestational parameters of female F0 rats treated with dimoxystrobin

Parental generation	F0				
Dose [ppm]	0	50	150	500	1200
Animals per dose	25	25	25	25	25
Female fertility					
- placed with males					
- mated [n]	24	25	25	25	25
- mating index [%]	96	100	100	100	100
- pregnant [n]	24	23	25	22	24
- **Fertility index** [%]	100	92	100	88	96
Pre coital interval [days]	3.1	2.6	2.8	2.6	2.7
Duration of gestation [days]	21.9	21.9	21.8	21.9	21.9
Implantation sites, total [n]	361	338	395	338	363
- dto per dam [n]	15.0	15.4	15.8	15.4	15.1
Post implantation loss [n]	44	39	54	21	28
- dto per dam [n]	1.8	1.8	2.2	1.0	1.2
- dto per litter [mean %]	11.7	11.4	14.4	5.5	7.3
Females with liveborn	24	23	25	22	24
- **Gestation index** [%]	100	100	100	100	100
- with stillborn pups [n] *(HCD 3-13)*	2	6	4	3	11**
- with all stillborn [n]	0	0	0	0	0
Pups delivered [n]	317	303	341	317	335
- per dam [mean n]	13.2	13.2	13.6	14.4	14.0
- liveborn [n]	315	296	336	314	318**
- stillborn [n] *(HCD 4-35)*	2	7	5	3	17**
- **Live birth index** [%] *(HCD 90-99%)*	99	98	99	99	95

* p ≤ 0.05; ** p ≤ 0.01 (Dunnet-test, two sided or Fisher's exact test, one sided)
Values may not calculate exactly due to rounding of values

Table 5.6.1-4: Reproduction and gestational parameters of female F1 rats treated with dimoxystrobin

Parental generation	F0				
Dose [ppm]	0	50	150	500	1200
Animals per dose	25	25	25	25	25
Female fertility					
- placed with males					
- mated [n]	24	24	24	25	24
- mating index [%]	96	96	96	100	96
- pregnant [n]	23	20	23	25	22
- **Fertility index** [%]	96	83	96	100	92
Pre coital interval [days]	2.2	2.5	2.3	2.6	2.7
Duration of gestation [days]	21.9	21.7	21.6	21.6	21.7
Implantation sites, total [n]	378	308	364	349	383
- dto per dam [n]	16.4	15.4	16.5	14.0	12.9**
(HCD 11.5–18.3)[a)]					
Post implantation loss [n]	36	33	36	35	23
- dto per dam [n]	1.6	1.6	1.6	1.4	1.0
- dto per litter [mean %]	9.2	11.1	13.4	10.7	8.4
Females with liveborn	23	20	22	25	22
- **Gestation index** [%]	100	100	96	100	100
- with stillborn pups [n]	4	7	4	4	5
- with all stillborn [n]	0	0	0	0	0
Pups delivered [n]	342	275	330	314	260
- per dam [mean n]	14.9	13.8	15.0	12.6	11.8*
(HCD 11.1-16.4)					
- liveborn [n]	338	266	322	310	253
- stillborn [n]	4	9	8	4	7
- **Live birth index** [%]	99	97	98	99	97

* p ≤ 0.05; ** p ≤ 0.01 (Dunnet-test, two sided or Fisher's exact test, one sided)
Values may not calculate exactly due to rounding of values
[a)]HCD derived from publlished literature (Hood, 2006), since evaluations of implantation sites was not a standard parameter in studies at that time.

The high dose effects described above were not consistent between the first and the second generation in the 2-generation study with dimoxystrobin and can be conclusively explained by data compiled from historical control data. Overall, all the effects on reproductive and developmental parameters can be regarded as incidental and not treatment-related.

G. PUP DATA

1. Survival

Survival of pups was not affected by the treatment in either generation.

The viability index (survival days 0 to 4 pre cull) ranged between 93% and 96% in F1 pups [see Table 5.6.1-5] and between 92 and 97% in F2 pups [see Table 5.6.1-6] and was fully covered by historical control data (83-99%).

The total number of F2 pups that died, was statistically significantly increased at 150 and 1200 ppm [see Table 5.6.1-6], but fully within the historical control data range. In the high dose group this was predominantly caused by just one litter (eight pups of this dam (#707) died/were found dead on day 1 after delivery, which can also happen sporadically in control animals of this rat strain). This increased number of died pups in the high dose did not affect the viability index, which was with 92% still within the historical control range (83 - 99%). Therefore, the effect was considered to be spontaneous.
This can be illustrated by the results of a further 2-generation toxicity study (Study No. 96172) performed with the same rat strain at the same lab. In this study the number of pups died were actually highest in the control (31) and low dose animals (40) in the F1 generation and not affected in the second generation, showing the huge biological variability of this parameter.

The statistically significantly increased number of cannibalized pups in the 500 ppm dose group is considered to be spontaneous in nature as this was predominantly caused by just one dam (#298) and was not dose-related.

Table 5.6.1-5: Pup survival, sex-ratio and body weights (F1 pups)

Pup generation	F1				
Dose [mg/kg]	0	50	150	500	1200
Number of litters	24	23	25	22	24
- with liveborn pups	24	23	25	22	24
- with stillborn pups	2	6	4	3	11**
Pups liveborn [n]	315	296	336	314	318**
Pups died [n] *(HCD 4-31)*	9	8	11	78	17
Pups cannibalized [n]	4	2	5	12*	7
Pups culled day 4 [n]	117	111	133	126	110
Pups day 4 - pre-cull [n]	302	285	321	293	296
- **Viability index** [%] *(HCD 83-99%)*	96	96	96	93	93
Pups day 4 - post cull [n]	185	171	187	164	183
Pups day 21 [n]	185	171	187	164	183
- **Lactation index** [%	100	98	99	98	98
Sex ratio [% live males]					
- Day 0	47.6	50.3	48.2	50.0	50.6
- Day 21	49.7	50.3	50.3	49.4	50.3
Male pup weight [g]					
- day 1 [g]	6.6	6.6	6.5	6.3	6.3
- day 4 - pre cull [g]	9.5	9.4	9.8	8.4	8.3*
- day 4 - post cull [g]	9.5	9.4	9.5	8.4	8.3*
- day 7 [g]	15.2	15.1	15.3	13.3*	12.2**
- day 14 [g]	32.6	32.2	31.9	28.0**	23.8**
- day 21 [g]	54.0	54.4	52.6	44.4**	34.7**
Male body weight gain					
- day 4 to 21 [g]	44.5	45.0	43.1	36.0**	26.4**
[%]		1.1	-3.1	-19.1	-40.7
Female pup weight [g]					
- day 1 [g]	6.3	6.2	6.3	6.0	6.0
- day 4 - pre cull [g]	9.1	8.9	9.1	8.0*	7.9*
- day 4 - post cull [g]	9.1	9.0	9.1	8.0*	7.9*
- day 7 [g]	14.7	14.6	14.9	12.9*	11.7**
- day 14 [g]	31.4	31.5	31.6	27.4**	22.9**
- day 21 [g]	51.4	51.6	51.1	43.2**	33.2**
Female body weight gain					
- day 4 to 21 [g]	42.3	42.7	42.0	35.2**	25.3**
[Δ%]		0.9	-0.7	-16.8	-40.2

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test, two sided or Fisher's exact test, one sided)
Values may not calculate exactly due to rounding of values

Table 5.6.1-6: Pup survival, sex-ratio and body weights (F2 pups)

Pup generation	F2				
Dose [mg/kg]					
Number of litters	23	20	22	25	22
- with liveborn pups	23	20	22	25	22
- with stillborn pups	4	7	4	4	5
Pups liveborn [n]	338	266	330	314	260
Pups died [n]	5	10	14*	10	19**
(HCD 4-31)					
Pups cannibalized [n]	4	5	4	7	4
Pups culled day 4 [n]	145	104	132	110	65
Pups day 4 - pre-cull [n]	329	253	308	295	232**
- **Viability index** [%]	97	95	96	95	92
Pups day 4 - post cull [n]	182	147	171	183	165
Pups day 21 [n]	182	147	171	183	165
- **Lactation index** [%	99	99	97	99	99
Sex ratio [% live males]					
- Day 0	53.3	50.4	50.6	53.3	48.6
- Day 21	51.6	48.3	53.2	51.4	48.5
Male pup weight [g]					
- day 1 [g]	6.5	6.5	6.3	6.5	6.5
- day 4 - pre cull [g]	9.4	9.6	8.5	9.3	8.7
- day 4 - post cull [g]	9.3	9.7	8.6	9.4	8.7
- day 7 [g]	15.0	15.5	13.3*	14.4	12.1**
- day 14 [g]	32.3	33.0	29.5*	29.2*	22.8**
- day 21 [g]	52.3	53.2	47.8*	45.0**	33.5**
Male body weight gain					
- day 4 to 21 [g]	43.0	43.6	39.2*	35.6**	24.8**
[Δ%]		1.4	-8.8	-17.2	-42.3
Female pup weight [g]					
- day 1 [g]	6.1	6.3	6.0	6.2	6.2
- day 4 - pre cull [g]	9.0	9.4	8.3	8.9	8.3
- day 4 - post cull [g]	9.0	9.4	8.3	8.9	8.3
- day 7 [g]	14.6	15.1	13.1	13.8	11.5**
- day 14 [g]	31.4	32.2	29.0	28.2**	21.8**
- day 21 [g]	50.0	51.1	46.4*	43.2**	31.9**
Female body weight gain					
- day 4 to 21 [g]	41.0	41.7	38.1*	34.3**	23.5**
[Δ%]		1.7	-7.1	-16.3	-42.7

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnet-test, two sided or Fisher's exact test, one sided)
Values may not calculate exactly due to rounding of values

The lactation index (survival day 4 post cull to 21) was not affected by treatment. The lactation indices ranged for F1 pups between 98 and 100% [see Table 5.6.1-5], for F2 pups between 97 and 99% [see Table 5.6.1-6].

2. Sex ratio

The sex ratios at day 0 and 21 were not affected by treatment in both generations. All differences were within the historical control range and not indicative of a treatment-related effect [see Table 5.6.1-5 and Table 5.6.1-6].

3. Pup clinical observations

The F1 pups did not show any clinical signs up to weaning which could be attributed to treatment. Only some spontaneous clinical findings occurred in single F1 pups of all test groups including control (kinky tail in control pup #6 of dam #207, hydrocephaly in 50 ppm pup #2 of dam #242, anophthalmia in 1200 ppm pup #7 of dam #311, traumatic lesion of its right hindlimb in high dose pup #10 of dam #312, filiformed tails in 150 ppm pup #4 of dam #257 and 500 ppm pup #17 of dam #280).
With the exception of one low dose pup (#1) of dam #635, which showed hydrocephaly, there were no further F2 generation pups, which did show any clinical signs up to weaning.

4. Body weight

Significant effects on body weight were essentially absent at birth in the offspring animals. Therefore, an in utero effect can be excluded. In line with maternal body weight development, mean pup body weights of F1 pups in the 500 and 1200 ppm dose test groups were statistically significantly reduced compared to controls from postnatal day (PND) 4 onward.
Body weight effects in the offspring became more pronounced in the later phase of lactation, especially in the last week of lactation (time points PND 14 and 21), when the pups start self-feeding (around PND12; Hood, 2011; Tyl et al., 2008 (BASF DocID 2008/1102837)). Table 5.6.1-7 shows the comparison of maternal body weights (F0 generation) with pup body weights (F1 generation).

Table 5.6.1-7: Maternal (F0) and pup (F1) body weights during lactation

F0 maternal	**ppm**				
day	**0**	**50**	**150**	**500**	**1200**
1	298.5	301.7	300.9	277.6* (-7.0)	278.9* (-6.6)
4	315.3	309	312.8	294.4** (-6.6)	287.2** (-8.9)
7	323.6	320.6	322.1	308.9 (-4.5)	299.2** (-7.5)
14	334.9	333.6	332	315.2* (-5.9)	305.2** (-8.9)
21	325.7	327.3	324	312.7 (-4.0)	307.7* (-5.5)
F1 litters	**ppm**				
day	**0**	**50**	**150**	**500**	**1200**
1	6.4	6.4	6.4	6.2 (-3.1)	6.1 (-4.7)
4 preculling	9.2	9.1	9.3	8.2 (-10.9)	8.1* (-12.0)
4 postculling	9.3	9.2	9.3	8.2* (-11.8)	8.1* (-12.9)
7	14.9	14.8	15.1	13.1* (-12.1)	11.9** (-20.1)
14	32	31.8	31.7	27.7** (-13.4)	23.4** (-26.9)
21	52.6	52.9	51.8	43.8** (-16.7)	34.0** (-35.4)

*p ≤ 0.05; ** p ≤ 0.01
In brackets (% vs. control)

It is to be expected that pups show more severe effects on body weights since they receive higher daily doses of dimoxystrobin compared to the dams at the same dietary doses. This is due to the fact that dietary exposure was continuous throughout the 2-generation study (and the supplementary one-generation study, see below), and dietary concentrations were not adjusted during gestation or lactation. Therefore, actual maternal dose levels during the lactation phase are increased due to a physiological higher need for food. This leads in turn to a considerably higher dosing of the pups due to an assumed higher concentration in milk and additionally at the later lactational phase via self-feeding starting at around PND 12 (Hood, 2011; Tyl et al., 2008 (BASF DocID 2008/1102837)). A comparison of the actually measured maternal (F0 and F1) dimoxystrobin doses is shown in Table 5.6.1-8, including the estimated mg/kg bw/day exposures for the F1 and F2 pups during the last week of lactation only, which consides estimated direct exposure of pups via treated feed. Assuming the presence of dimoxystrobin in milk even increases the doses of offspring animals.

Table 5.6.1-8: Approximate mg/kg bw/day compound exposure to parental (F0 and F1) animals and estimated mg/kg bw/day exposure to F1 and F2 pups during the last week of lactation (excluding amount transferred in milk) in the 2-generation study

	Compound exposure (mg/kg bw/day)				
ppm in diet	**0**	**50**	**150**	**500**	**1200**
F0 male (premating)	0	4.7	14.1	46.4	108.8
F0 female (premating)	0	5.1	15.6	49.9	118.9
F0 Female (gestation)	0	4.5	13.6	43.6	102.5
F0 Female (lactation)[a)]	0	7.6	22.1	74.5	168.2
F1 pups[b)]	0	9.8	29.7	96.3	227.7
F1 pups (not corrected)[c)]	0	6.1	17.9	59.1	135.4
F1 Male (premating)	0	5.9	18.2	61.8	156.4
F1 Female (premating)	0	6.2	18.6	63.7	159
F1 Female (gestation)	0	4.6	13.6	46.1	107.8
F1 Female (lactation)[a)]	0	7.4	22.4	75.4	168
F2 pups[b)]	0	12.1	36.8	125.5	315.4
F2 pups (not corrected)[c)]	0	6	18	60.8	138

a) Excludes final week of lactation because of pup self-feeding

b) 2.0 fold factor for estimated pup dietary consumption on a mg/kg bw/day basis as adults through self feeding behavior in the last week of lactation based on pre-weaning pup consumption of radiolabelled microsphere recorded by Hanley and Watanabe (1985; BASF DocID 1985/1002252) (weaning at PND 28), plus estimated compound consumption during late lactation supported by the dietary 2,4-D rangefinding TK study (Saghir et al., 2013; BASF DocID 2013/1419940). This factor was applied to the compound intake based on mean premating adult male and female feed consumption.

c) Not corrected values for pup dietary test substance intake based on approximate compound exposure to females during gestation and lactation (mean values)

The estimated values for pups substance intake (in the last week of lactation) is considerably higher in pups compared to female adults (a dose of 227.7 mg/kg bw is estimated for F1 pups, while females consume only 168.2 mg/kg bw over the lactation period in the 1200 ppm dose group). This difference is even more pronounced in the F2 pups of the 1200 ppm dose group with an estimated test substance intake of 315.4 mg/kg bw/day compared to 168 mg/kg bw/day in the respective high dose females (Table 5.6.1-8).

These are important considerations for evaluating relative sensitivities between parental animals and offspring and demonstrate that dietary concentrations are not directly comparable between dams (or adults) and pups, and any comparisons of findings to evaluate potential pup sensitivity or relative severity of effects in the pups should be assessed to the extent possible on a mg/kg bw/day basis and not on the ppm in the diet.

A similar picture with regard to pup body weights is seen in the second generation of this 2-generation toxicity study [see Table 5.6.1-9].

Table 5.6.1-9: Maternal (F1) and pup body weights (F2 litters) during lactation

F1 maternal	Body weight [g]				
day	**0 ppm**	**50 ppm**	**150 ppm**	**500 ppm**	**1200 ppm**
1	308.7	306.0	299.9	275.8** (-10.7)	248.7** (-19.4)
4	317.9	320.3	309.6	288.4** (-9.3)	256.2** (-19.4)
7	327	328.9	319.4	299.5** (-8.4)	265.7** (-18.7)
14	347.2	345.9	342	314.9** (-9.3)	276.6** (-20.3)
21	330	330.6	330.2	313.4 (-5.0)	280.7** (-14.9)
F2 litters	**Body weight [g]**				
day	**0 ppm**	**50 ppm**	**150 ppm**	**500 ppm**	**1200 ppm**
1	6.4	6.4	6 (-6.3)	6.2 (-3.1)	6.2 (-3.1)
4 preculling	9.2	9.5	8.4 (-8.7)	9.2 (0)	8.5 (-7.6)
4 postculling	9.2	9.6	8.5 (-7.6)	9.2 (0)	8.5 (-7.6)
7	14.8	15.3	13.2* (-10.8)	14.2 (-4.1)	11.8** (-20.3)
14	31.9	32.7	29.2* (-8.5)	28.8** (-9.7)	22.3** (-30.1)
21	51.2	52.3	47.1* (-8.0)	44.3** (-13.5)	32.7** (-36.1)

*$p \leq 0.05$; ** $p \leq 0.01$
In brackets (% vs. control)

Also here a clearly increased trend in pup body weight effects especially after PND 14 can be observed with no effects at birth. The NOAEL for pup body weight effects was 50 ppm, while the NOAEL for maternal body weight effects was 150 ppm. Although this picture seems to indicate a higher susceptibility of the pups compared to the adults, it needs to be considered, that again the actual doses between pups and adults are considerably different at the same dietary concentrations (see Table 5.6.1-8). Even, when comparing the measured substance intakes between the first and the second generation of this study, it is evident, that F1 parents during premating and F2 pups had considerably higher substance intakes compared to the respective values of the F0/F1 generation: top dose F0 females had a compound intake of 118.9 mg/kg bw during premating, while the respective compound intake of F1 females was 159 mg/kg bw.

When doing benchmark dose (BMD) calculations of the maternal and offspring PODs (points of departures) for body weight effects at lactation day 21, the female (F1) $BMDL_{05}$ is 25.5-45.3 mg/kg bw (based measured substance intake data during different phases of lactation) and the pup (F2) $BMDL_{05}$ is 39.8 mg/kg bw (using the same modelling assumptions) (see Table 5.6.1-10; BASF DocID 2015/1172904). This further demonstrates, that the no effect levels (= PODs) between dams and pups is essentially comparable, when more accurate doses are used for estimation.

Table 5.6.1-10: BMD calculations for dam body weigh on lactation day 0, 1, 7, 14 and 21 and pup body weight on day 21

Endpoint	Model	BMD	BMDL
Body weight on lactation day 1	Exponential (M2)	33.4	28.6
Body weight on lactation day 4	Exponential (M2)	33.1	28.4
Body weight on lactation day 7	Exponential (M4)	39.7	25.5
Body weight on lactation day 14	Linear	46.9	41.5
Body weight on lactation day 21*	Linear	54.7	45.3
Pup body weight on day 21	Linear	43.6	39.8

Exponential (M2) Y[dose] = a * exp{sign * b * dose}
Exponential(M4) Y[dose] = a * [c-(c-1) * exp{-b * dose}]
Linear Y[dose] = beta_0 + beta_1*dose

*BMD calculation uses mean intakes between LD 1-14

Therefore, the apparently more severe effects in pup body weights compared to parental animals on PND 14 and PND 21 are considered to be linked to the higher compound intake of pups and not to a higher susceptibility. Further, any amount of the compound occurring in milk is contributing to the overall dose of the pups and is not included in the above assumptions.

The absence of effects on pup weights at birth in both the F1 and F2 generation pups of the two-generation study and also in the F1 pups of the one-generation study (see study summary of the modified one-generation study with dimoxystrobin (BASF DocID 2000/1016870) below) is consistent with the absence of effects on fetal body weight in the dimoxystrobin rat developmental study (BASF DocID 1999/11680), which had a higher mg/kg bw/day dose (up to 300 mg/kg bw/day) than did the reproductive toxicity studies (summaries of these studies can be found below in this chapter).

Additionally, reduced serum iron levels and anemia as observed in dimoxystrobin-treated animals (see MCA 5.8.2 for mechanistic studies on serum iron levels and study summary of the modified one-generation study with dimoxystrobin (BASF DocID 2000/1016870) below) is assumed to lead to reduced iron levels in milk of treated-dams, which contributes to impaired body weight development in pups (Roth and Smith, 1988; BASF DocID 1988/1003436).

5. Organ weights

There were several increased and/or decreased mean absolute and relative F1 and F2 pup organ weights in the 500 and 1200 ppm pups [see Table 5.6.1-11]. A treatment relationship is considered likely, if the same effect (increase or decrease) was noted for the absolute or for the relative organ weight and a dose-relationship was evident. The distinct decrease in the thymus, spleen and brain weights in the 500 and 1200 ppm pups were assessed as being a direct consequence of the significant delays in mean pup body weight gains at these dose levels.

All other differences in absolute and/or relative F1 and F2 pup organ weights were assessed as being incidental.

Table 5.6.1-11: Organ weights of F_1 and F_2 pups

Generation		F_1 (Males & Females combined)				F_2 (Males & Females combined)			
	Dose [mg/kg]	Absolute weight [g]	Δ%	Relative weight [% of b.w.]	Δ%	Absolute weight [mg]	Δ%	Relative weight [% of b.w.]	Δ%
Terminal weight	0	52.6				51.2			
	50	52.9	(0.6)			52.3	2.1		
	150	51.8	(-1.5)			47.1*	-8.0		
	500	43.8**	(-16.7)			44.3**	-13.5		
	1200	34.0**	(-35.4)			32.7**	-36.1		
Brain	0	1.439		2.747		1.458		2.873	
	50	1.451	1.0	2.734	-0.5	1.473	1.0	2.869	-0.1
	150	1.458	1.3	2.800	1.9	1.434	-1.6	3.101**	7.9
	500	1.420	-1.3	3.228**	17.5	1.448	-0.7	3.278**	14.1
	1200	1.351**	-6.1	3.970**	44.5	1.340**	-8.1	4.188**	45.8
Thymus	0	0.197		0.375		0.181		0.354	
	50	0.191	-3.0	0.356	-5.1	0.182	0.6	0.349	-1.4
	150	0.182	-7.6	0.347	-7.5	0.161*	-11.0	0.343	-3.1
	500	0.140**	-28.9	0.316**	-15.7	0.149**	-17.7	0.336	-5.1
	1200	0.083**	-57.9	0.238**	-36.5	0.087**	-51.9	0.264**	-25.4
Spleen	0	0.235		0.445		0.223		0.435	
	50	0.234	-0.4	0.435	-2.2	0.220	-1.3	0.422	-3.0
	150	0.224	-4.7	0.427	-4.0	0.198*	-11.2	0.420	-3.4
	500	0.174**	-26.0	0.393**	-11.7	0.180**	-19.3	0.400*	-8.0
	1200	0.112**	-52.3	0.322**	-27.6	0.101**	-54.7	0.313**	-28.0

* $p \le 0.05$, ** $p \le 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))
Values may not calculate exactly due to rounding of figures

6. Pup necropsy findings

The most obvious necropsy observations in F1 pups, which were considered to be treatment-related were

- Pale yellowish discoloration of the liver at 500 and 1200 ppm
- Cardiomegaly at 500 and 1200 ppm
- Milky fluid in the abdomen and/or thorax after organ evisceration at the high dose

Hypoplasia of thymus at the high dose related to decreased body weights and reduced thymus weights. An overview over the findings is given in Table 5.6.1-12.

The above mentioned findings on liver and heart are considered to be a consequence of an iron-deficiency microcytic hypochromic anemia (Cluzeaud et al., 1981; BASF DocID 1981/1001522; Tanne et al., 1994; BASF DocID 1994/1005569; Crowe et al., 1995; BASF DocID 1995/1008574; Rothenbacher and Sherman, 1980; BASF DocID 1980/1001747; Roth and Smith, 1988; BASF DocID 1988/1003436), which was seen in several other repeated-dose toxicity studies with dimoxystrobin and furthermore in the modified one-generation reproduction toxicity study in Wistar rats. The milky fluids reported in the abdomen and the breast cavity are considered to be secondary to the heart-insufficiency (cardiomegaly) induced by the chronic microcytic anemia. The same effects are also seen in the F2 pups.

Cardiomegaly was reported in the PND21 pups only and was not see in PND4 pups macroscopically. Furthermore, parental F1 animals did not show cardiomegaly or other effects on the heart [see Table 5.6.1-13]. There is information in the literature indicating that young animals undergo cardiac remodelling secondary to anemia (Cluzeaud et al., 1981; BASF DocID 1981/1001522; Tanne et al., 1994; BASF DocID 1994/1005569; Crowe et al., 1995; BASF DocID 1995/1008574). Thus cardiomegaly is a transient effect and not an irreversible structural malformation.

Furthermore, a few of the large number of examined F1 and F2 pups showed some spontaneous findings at necropsy.

Table 5.6.1-12: Incidence of gross necropsy observations in F_1 and F_2 pups

Dose [mg/kg]	0	50	150	500	1200
			F_1 pups		
Litters evaluated	23	23	25	21	24
Pups evaluated	263	250	286	255	278
- Live	261	243	281	252	261
- Stillborn	2	7	5	3	17
Milky fluid in abdomen	0	0	0	0	3 (2)
Hypoplasia of thymus	0	0	0	0	30 (13**)
Cardiomegaly	0	0	2 (1)	5 (3)	56 (19**)
Liver: pale-yellowish	0	0	2 (1)	6 (3)	42 (15**)
Total pup necropsy observations	9 (8)	12 (8)	11 (8)	19 (9)	67 (20**)
- % affected pups/litter	4.3	4.4	3.5	7.5	23.9**
			F_2 pups		
Litters evaluated	23	20	22	25	22
Pups evaluated	338	270	326	307	256
- Live	334	261	318	303	249
- Stillborn	4	9	8	4	7
Milky fluid in abdomen	0	0	0	0	8 (1)
Hypoplasia of thymus	0	0	0	0	6 (3)
Cardiomegaly	0	0	0	7 (5*)	52 (12**)
Liver: pale-yellowish	0	0	1 (1)	1 (1)	41 (9**)
Total pup necropsy observations	10 (9)	6 (5)	19 (9)	15 (10)	70 (16*)
- % affected pups/litter	2.9	2.8	5.7	4.9	25.5**

* $p \leq 0.05$, ** $p \leq 0.01$ (Wilcoxon-test, one-sided)
( ) values in brackets give litter incidence

Table 5.6.1-13: Overview on cardiac effects in the 2-generation toxicity study

Dose [ppm]	50	150	500	1200
F0 parental	No effect	No effect	No effect	No effect
F1 pups	No effect	No effect	**Cardiomegaly** (only in PND21 pups, not PND4)	**Cardiomegaly** (only in PND21 pups, not PND4)
F1 parental	No effect	No effect	Heart dilation in 1 male animal*	No effect
F2 pups	No effect	No effect	**Cardiomegaly** (only in PND21 pups, not PND4)	**Cardiomegaly** (only in PND21 pups, not PND4)

*incidental finding due to single occurrence and no dose-relationship

7. Sexual maturation

Male and female F1 pups selected to become F1 parental animals were examined for sexual maturation. No treatment-related effects on sexual maturation were observed [see Table 5.6.1-14 and Table 5.6.1-15].

Table 5.6.1-14: Sexual maturation of F1 female pups

Sex & parameter	Vaginal opening				
Dose [ppm]	0	50	150	500	1200
Animals per dose	25	25	25	25	25
- Days to criterion	34.9	36.0	35.8	36.4*	40.6**

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett-test, two-sided; Fisher's exact test, two-sided)

Table 5.6.1-15: Sexual maturation of F1 male pups

Sex & parameter	Preputial Separation				
Dose [ppm]	0	50	150	500	1200
Animals per dose	25	25	25	25	21
- Days to criterion	43.4	43.7	43.6	45.1	47.8**

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett-test, two-sided; Fisher's exact test, two-sided)

In females vaginal opening was not affected by treatment in the 50 and 150 ppm dose groups [see Table 5.6.1-14]. In the 500 and 1200 ppm dose females the mean age for vaginal opening was slightly but statistically significantly delayed. This effect was related to the impaired body weights/body weight gains at the respective dose groups.

In males preputial separation was not affected by treatment in the 50 and 150 ppm dose groups [see Table 5.6.1-15]. In the 500 and 1200 ppm dose males the mean age for preputial separation was slightly, for the 1200 ppm dose group statistically significantly delayed. This effect is considered to rather reflect the general retardation of the young animals due to impaired body weights/body weight gains at the respective dose groups in males and females. Lower male body weights at PND 21 strongly correlates to a delayed onset of puberty (Melching-Kollmuß et al., 2014; BASF DocID 2014/1326033). There is broad evidence in the literature that lower body weights of the offspring, as well as the decreased food consumption or lower body weights of dams during pregnancy, correlate with the age at preputial separation of its male offspring and may cause delayed onset of puberty in male and female rats (Carney et al., 2004; BASF DocID 2004/1041034; Chernoff et al., 2009; BASF DocID 2009/1132002).

The fact that both parameters preputial separation in males and vaginal opening in females are delayed further supports the assumption that the delayed onset of puberty is a secondary effect of lower offspring body weights and not a direct endocrine mediated effect.

H. Parental terminal investigations

1. Organ weights

Organ weight determination in parental animals revealed a number of significant changes of absolute and/or relative organ weights [see Table 5.6.1-16].

Alterations of absolute and/or relative organ weights in F0 and F1 parental rats were attributed to decreased body weights. None of the organs with weight changes in the adults showed correlating histopathology.

Table 5.6.1-16: Major findings in parental F0 and F1 males and females[a)]

Dose [mg/kg]	0	50	150	500	1200
	F0 males				
Organs weights	No effect	No effect	↑testes (relative)	↑kidney, brain, testes, epididymides, cauda epididymides (relative)	↓ liver, spleen, thymus (absolute); ↑ kidney, brain, testes, epididymides, cauda epididymides (relative)
Necropsy findings	No effect	No effect	No effect	No effect	No effect
Histopathology	No effect	No effect	No effect	No effect	No effect
	F0 females				
Organs weights	No effect	No effect	No effect	↓ adrenal (absolute); ↑kidney, brain (relative)	↓ pituitary (absolute); ↑liver, kidney, brain (relative)
Necropsy findings	No effect	No effect	No effect	No effect	No effect
Histopathology	No effect	No effect	No effect	No effect	No effect
	F1 males				
Organs weights	No effect	No effect		↓ liver, kidney, testes, spleen; ↑thymus (absolute); ↑ epididymides, cauda epididymis, thymus and brain (relative)	↓ liver, kidney, spleen, testes, prostate, brain, adrenal (absolute); ↑ kidneys, testes, epididymides, cauda epididymis, seminal vesicle, thymus, brain (relative)
Necropsy findings	No effect	No effect	No effect	No effect	No effect
Histopathology	No effect	No effect	No effect	No effect	No effect
	F1 females				
Organs weights	No effect	No effect	↑brain (relative)	↓ kidney, adrenal (absolute); ↑ liver, kidney, ovary, spleen, brain (relative)	↓ kidney, ovary, brain, adrenal, pituitary (absolute); ↑ liver, kidney, spleen, brain (relative)
Necropsy findings	No effect	No effect	No effect	No effect	No effect
Histopathology	No effect	No effect	No effect	No effect	No effect

a) Increases (↑) and decreases (↓) relative to control.
b) Changes in organ weights attributed to body weight decreases; there were no histopathological correlates for organ weight findings. Organ weights listed are statistically significantly different from control.
c) Gross lesions few and sporadic across exposure groups; regarded as of spontaneous origin and not exposure related.
d) Histopathological lesions few and spread across exposure groups; regarded as of spontaneous origin and not exposure-related.

2. Differential ovarian follicle count

The results of the differential ovarian follicle count (DOFC) – comprising the numbers of primordial, growing and antral follicles, as well as the combined incidence of primordial plus growing follicles and corpora lutea – did not reveal significant deviations between controls and the high dose groups of F0 and F1 generation animals [see Table 5.6.1-17 and Table 5.6.1-18].

The increased mean numbers of primordial, growing and antral follicles, as well as the combined incidence of primordial plus growing follicles and corpora lutea were higher in the F0 high dose group than in the control. However, the deviations from the control were regarded to be within the biological variability and they were not indicative for a treatment related or adverse alteration.

In the F1 high dose group the results of the differential ovarian follicle count did result in a slight although significant decrease in the number of growing follicles and of corpora lutea. Overall, the mean numbers of primordial, as well as the combined incidence of primordial plus growing follicles was higher in the high dose group than in the control, whereas the mean number of growing and antral follicles and corpora lutea was lower. However, although the decrease in the number of growing follicles and of corpora lutea was significant, both the significant and the numerical deviations from the control were regarded to be within the biological variability.

Table 5.6.1-17: Ovarian follicle count in F_0 maternal females

Group	Primordial	Growing	Primordial + growing	Antral	Corpora lutea
	Absolute number				
Control	2355	1021	3376	159	705
1200 ppm	2874	1284	4158	208	878
	Mean number				
Control	94	41	135	6.4	28
1200 ppm	115	51	166	8.3	35

*** p ≤ 0.05, ** p ≤ 0.01 (Wilcoxon-test, one-sided)**

Table 5.6.1-18: Ovarian follicle count in F_1 maternal females

Group	Primordial	Growing	Primordial + growing	Antral	Corpora lutea
	Absolute number				
Control	2923	1212	4135	165	650
500 ppm	3237	1345	4582	216	660
1200 ppm	3178	1063	4241	146	570
	Mean number				
Control	117	48	165	6.6	26
500 ppm	129	54	183	8.6	26
1200 ppm	127	43*	170	5.8	23*

* p ≤ 0.05, ** p ≤ 0.01 (Wilcoxon-test, one-sided)

III. CONCLUSIONS

In conclusion, dimoxystrobin causes decreased body weight developments in pups and dams. There are no effects on pup body weights seen at birth, the effects only develop over time during lactation, being most pronounced at PND 14 and 21.

Under the conditions of the present 2-generation reproduction toxicity study the NOAEL for general, systemic toxicity is 150 ppm (about 17 mg/kg bw/day) for the F0 and F1 parental rats, based on impairments in food consumption and body weight/body weight gain observed at the LOAEL of 500 ppm in the F1 parental animals.

The NOAEL for fertility and reproductive performance for the F0 and F1 parental rats is 1200 ppm (about 136 mg/kg bw/day). At necropsy, the F1 and the F2 pups show increased incidences of liver discoloration and cardiomegalies at the top dose of 1200 ppm. These findings are correlated to the microcytic hypochromic anemia caused by dimoxystrobin. The cardiomegalies occur only at PND 21, not at PND 4 and not in the F1 adults, indicating a transient effect. The NOAEL for developmental toxicity in the F1 progeny is 150 ppm (17 mg/kg bw/day in adult animals; about 30 mg/kg bw/day estimated value for pups) and in F2 progeny 50 ppm (about 5 mg/kg bw/d in adults; about 12 mg/kg bw/day estimated value for pups) based on impairments in pup body weight.

The apparently more severe effects in F2 pups compared to F1 adults is related to a considerably higher compound intake of the pups compared to the dams at the same dietary dose levels. Comparing more accurately calculated doses between pups and dams, effects on pup body weight development occurs at higher (F1 litters) or roughly the same doses (F2 litters) than in dams. The $BMDL_{05}$ for F1 dams is 25.5 – 45.3 mg/kg bw (using measured substance intakes during lactation) and the $BMDL_{05}$ for pups is 39.8 mg/kg bw (using estimated pup intakes at PND21). Thus the no-effect doses of F2 pups and F1 dams are essentially comparable.

To further investigate the effects of dimoxystrobin on hematological parameters a modified one-generation toxicity study had been performed.

BASF DocID 2000/1016870): Modified one-generation study with dimoxystrobin in rats (Study presented in the original Annex I Dossier)

Executive summary:

Dimoxystrobin was administered to groups of 10 male and 10 female healthy young Wistar rats (F0 parental generation) as a constant homogeneous addition to the food in different concentrations (0, 150, 500 and 1200 ppm). This study was conducted in order to further elucidate the mode of action for the observed cardiomegalies and liver discolorations in offspring, and therefore only administered expected "effect doses".At least 47 days after the beginning of treatment, F0 animals were mated to produce a litter (F1). Mating pairs were from the same dose group. The study was terminated with the terminal sacrifice of the F1 weanlings and F0 adult animals. Test diets containing dimoxystrobin were offered continuously throughout the study.
The administration of 150, 500 and 1200 ppm of the test compound to male and female rats in this modified one-generation reproduction toxicity study caused a dose-dependent regenerative microcytic hypochromic anemia in the parental animals and the F1 pups at all doses. The anemia was characterized by reduced haemoglobin concentrations and mean corpuscular indices as well as increased microcytosis and reticulocytes.
Furthermore, 500 and 1200 ppm induced other substance-induced adverse effects, primarily on parental food consumption, parental and pup body weight data and pup necropsy findings, which are in-line with the observed anemia.

The slight effects on hematological parameters observed in the 150 ppm parental animals indicate a beginning anemia, which is treatment-related, but not considered to represent an adverse outcome, thus the lowest NOAEL for parental toxicity is considered to be 150 ppm (about 17 mg/kg bw). The apparently more severe effects on hematology in pups compared to dams can be related to a considerably higher compound intake at the same dietary dose levels. When more accurate calculated doses between pups and dams are used for estimation, the no effect levels are essentially comparable.

Materials and Methods:
Dimoxystrobin (batch/purity: N 15: 98.4%) was administered to groups of 10 male and 10 female young Wistar rats (F0 parental generation) as a constant homogeneous addition to the food in different concentrations (0, 150, 500 and 1200 ppm). At least 47 days after the beginning of treatment, F0 animals were mated (1:1) to produce a litter (F1). Litters were standardised to 8 pups on day 4. Mating pairs were from the same dose group. The study was terminated with the terminal sacrifice of the F1 weanlings and F0 adult animals. Test diets containing dimoxystrobin were offered continuously throughout the study.

Results:

A. TEST SUBSTANCE – ANALYSIS AND INTAKE

The stability of the test substance in the diet was demonstrated for a period of 49 days at room temperature in a comparable batch (N 5).
The homogeneity of the test substance preparations at concentrations of 150 and 1200 ppm was proven before the start and towards the end of the present study.
The concentration control analyses of all concentrations in the samples taken before the start and towards the end of the study revealed that the values were within 10% of the target concentrations.
Table 5.6.1-19 shows the approximate mg/kg bw/day compound exposure to parental animals and estimated mg/kg bw/day exposure to pups during last week of lactation (excluding amount, if any, transferred in milk) in the modified one-generation study.

Table 5.6.1-19: Approximate mg/kg bw/day compound exposure to parental animals and estimated mg/kg bw/day exposure to pups during last week of lactation (excluding amount, if any, transferred in milk) in the modified one-generation study

ppm in diet	0	150	500	1200
Male (pre-mating)	0	17.5	56.6	128.4
Female (pre-mating)	0	18.3	58.2	131.9
Female (gestation)	0	14.5	47.4	109.3
Female (lactation)[a)]	0	23.3	82.8	170.5
Pups [b)]	0	36	114	260
Pups (not corrected)[c)]	0	18	57	130

a) Excludes final week of lactation because of pup self-feeding

b) 2.0 fold factor for estimated pup dietary consumption on a mg/kg bw/day basis as adults through self-feeding behaviour in the last week of lactation based on pre-weaning pup consumption of radiolabelled microsphere recorded by Hanley and Watanabe (1985; BASF DocID 1985/1002252) (weaning at PND 28), plus estimated compound consumption during late lactation supported by the dietary 2,4-D range-finding TK study (Saghir et al., 2013; BASF DocID 2013/1419940). This factor was applied to the compound intake based on mean premating adult male and female feed consumption.

c) Not corrected values for pup dietary test substance intake based on approximate compound exposure to females during gestation and lactation (mean values)

B. OBSERVATIONS

1. Clinical signs of toxicity

No abnormal clinical signs of toxicity were observed.

2. Mortality

There were no substance-related or spontaneous mortalities in any of the male and female F0 parental animals in any groups.

3. Detailed clinical observations

Summary clinical observations for males and females (except gestation/lactation period)

No clinical signs, which might be attributed to the test substance, were detected in the male or female F0 generation parental animals. The 3 doses (150; 500 and 1200 ppm) administered in the diet did not lead to disturbances of the general behaviour in any of the F0 parental animals. In one mid dose male (No. 27) a skin lesion in the region of shoulder occurred between study weeks 3 to 4. Moreover, in one low dose female (No. 118) a skin lesion in the mammary line was detected during study week 14, which was confirmed during the gross examinations at necropsy. These transient skin findings in single rats without any relation to dosing or sex are considered to be spontaneous in nature.

Summary clinical observations for females during gestation of F1 litters

There were no particular substance-related clinical findings in F0 females during the gestation period for F1 litter. After mating, one low dose F0 female (No. 112) had no sperm in vaginal smear (no day 0 p.c.) and did not deliver F1 pups. Furthermore, four sperm positive females of test group 00 (Nos. 103, 104, 109 and 110 - 0 ppm), one female rat of test group 01 (No. 115 - 150 ppm) and one female animal of test group 02 (No.124 - 500 ppm) did not deliver any F1 pups.

Mid dose female No. 126 showed transient alopecia at the left flank on days 7 - 10 p.c., which is considered to be a spontaneous finding.

Summary clinical observations for females during lactation of F1 litters

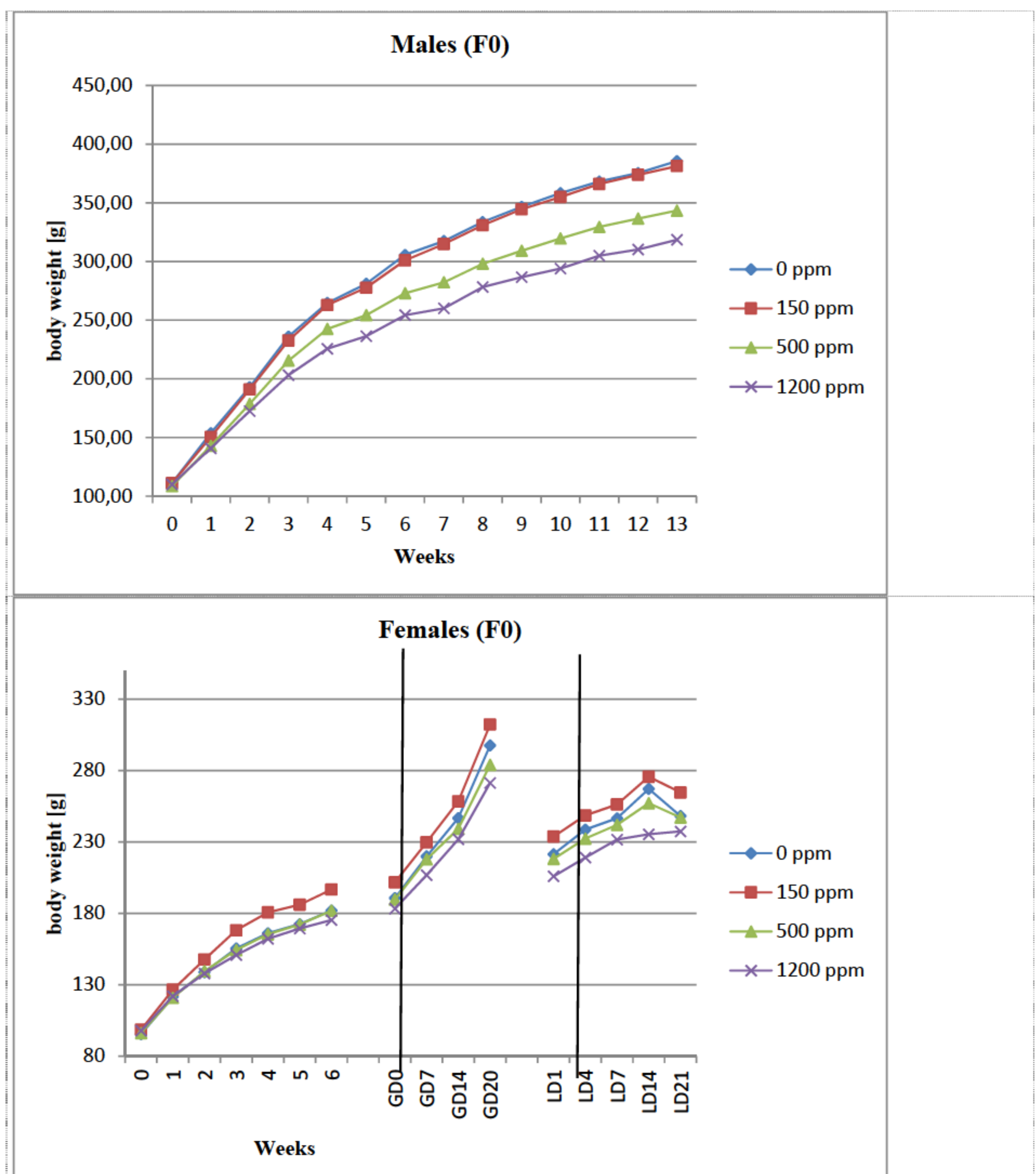
The F0 dams revealed no substance-related clinical finding during the lactation period of the F1 pups.

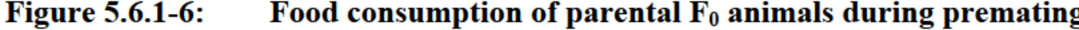
C. BODY WEIGHT AND BODY WEIGHT GAIN

Mean body weights of the mid (500 ppm) and high dose (1200 ppm) males were influenced by the test substance administration during the entire study period [see Figure 5.6.1-5]. From week one until the end of the study the differences attained statistical significance in comparison to that of the control males. At termination of the study (week 13), the mean body weight of the 500 ppm males was about 11 %, that of the 1200 ppm males about 17% below the corresponding control value. This is in-line with the concurrent reductions in food consumption in these groups. Mean body weight gain of the mid and high dose F0 males were also clearly impaired (with or without attaining statistical significance); if calculated for study weeks 0 - 13, the mean weight gain in the mid dose group was about 14 %, that of the high dose group about 24% below the respective control value. The mean body weights and mean body weight gains of the 150 ppm F0 males were substantially similar to control values.

Mean body weights of the substance-treated F0 females were substantially similar to controls during the premating period, but the weight gain of the high dose females (1200 ppm) was about 11% below the corresponding control value if calculated for weeks 0 – 6 (without attaining statistical significance) [see Figure 5.6.1-5].
During gestation the mean body weights and body weight gains of the high dose F0 parental females were also impaired. The mean body weight of the 1200 ppm dams on day 20 p.c. was about 9%, the body weight gain from days 0 - 20 p.c. was about 18% below the corresponding control value.
During the lactation period the mean body weights of the 1200 ppm females were also below the corresponding control values (up to about 12% on day 14 p.p.), the differences, however, did not always reach statistical significance. The mean body weight gains of the substance-treated females did not show a consistent trend throughout the lactation period; particularly at the high dose, the weight gains were sometimes distinctly above, sometime distinctly below the corresponding control values. Mean body weights and mean body weight gains of the 150 and 500 ppm females were not affected by the test substance administration during premating, gestation or lactation, particularly if the normal range of biological variation is taken into consideration.

Figure 5.6.1-5: Body weight development of parental F_0 animals





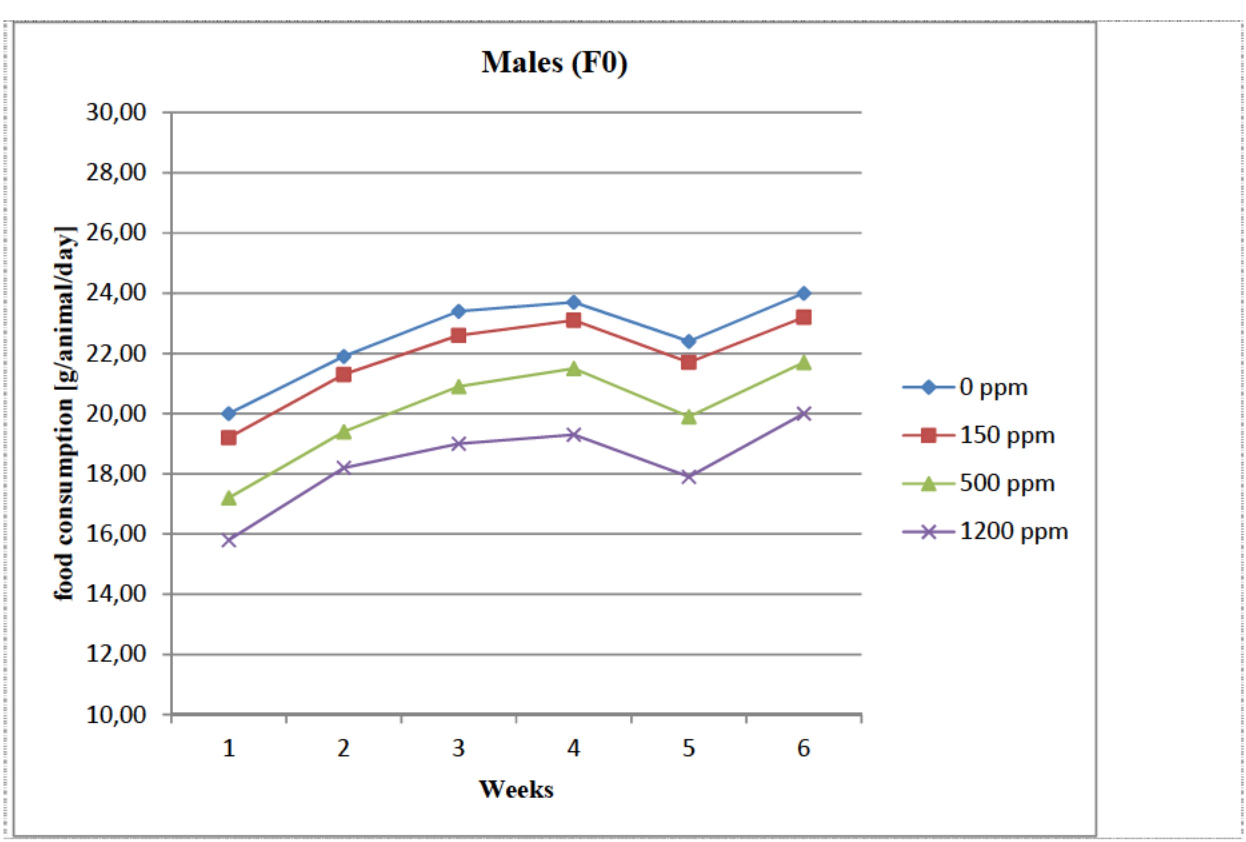
D. FOOD CONSUMPTION

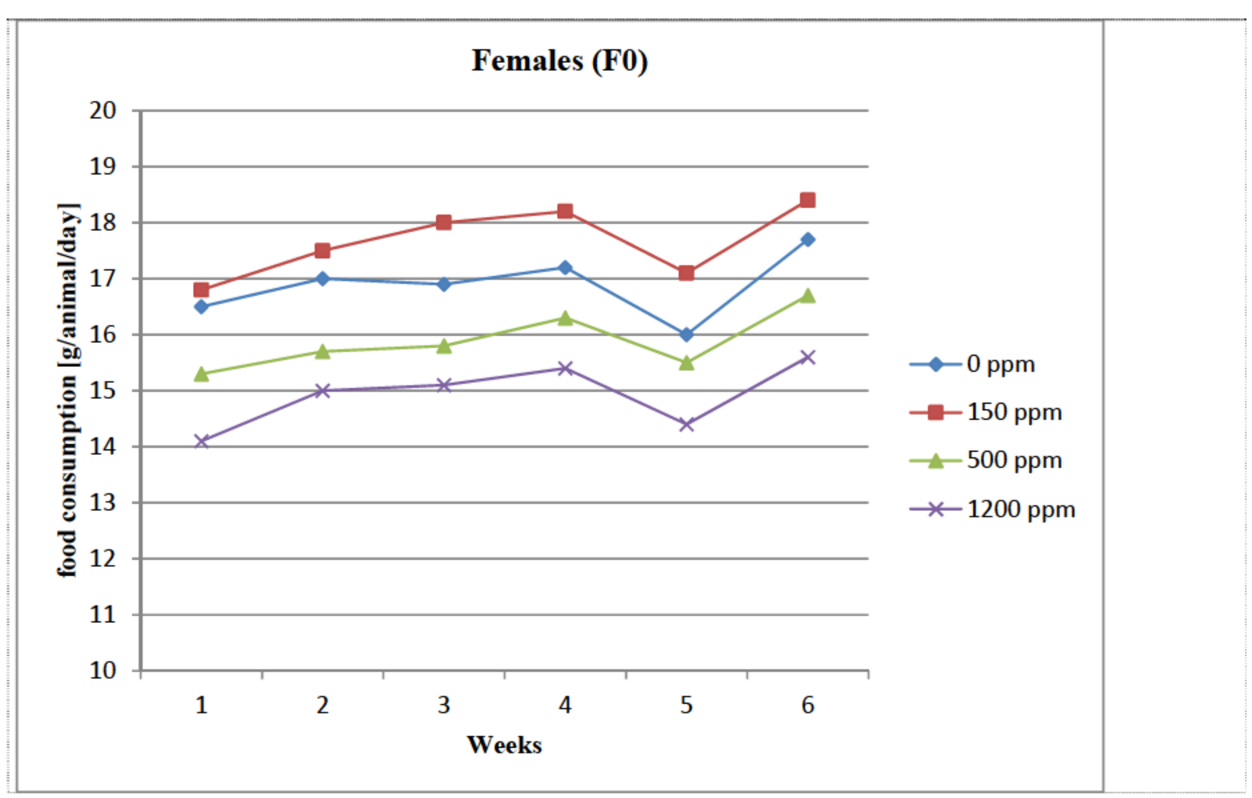
In the F0 parental male animals of the mid and high dose group (500 or 1200 ppm) the food consumption was statistically significantly reduced during the premating period (study weeks 0 - 6); if calculated for the entire premating period the food intake was about 11% (500 ppm) or 19% (1200 ppm), respectively, below the corresponding control value [see Figure 5.6.1-6]. This is considered to reflect a substance-related effect as body weight data of the mid and high dose males were concomitantly affected. The food intake of the low dose males, however, was similar to control values.

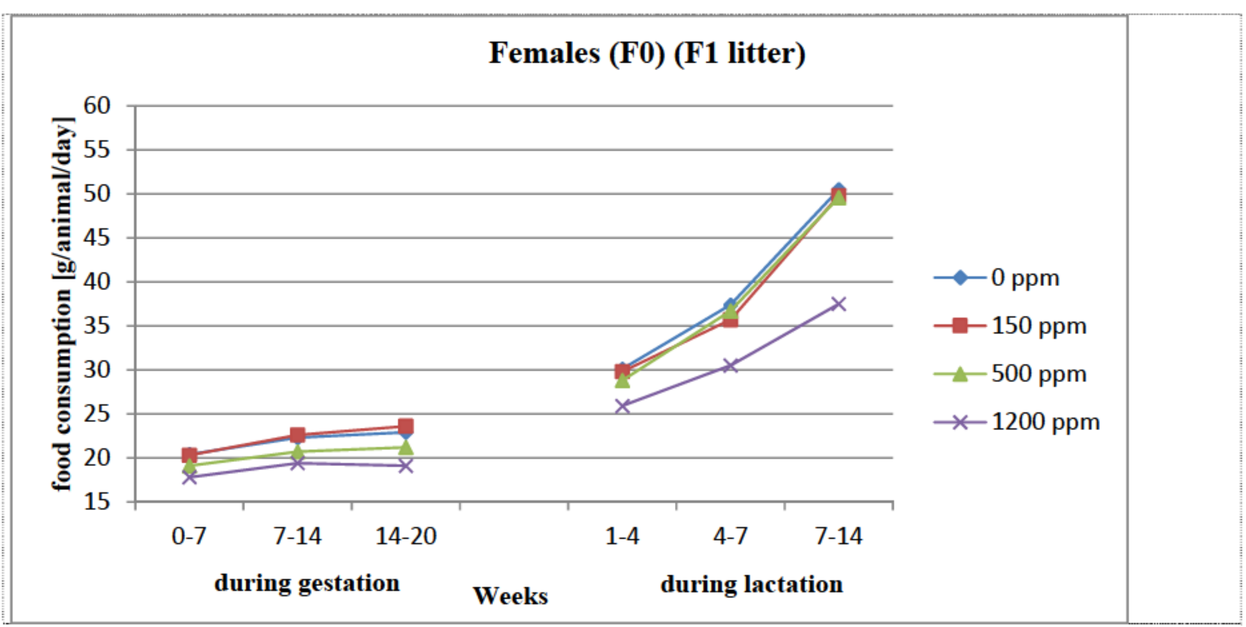
The food consumption of the high dose F0 parental females was statistically significantly reduced during the premating period (study weeks 0 - 6); if calculated for the entire premating period, the food intake was about 12% below the corresponding control value [see Figure 5.6.1-6].

During gestation and lactation of the F1 litter, food consumption of the 1200 ppm F0 dams was also statistically significantly impaired and on average about 14 - 20% lower than in the concurrent control group. As the impairments in the dams' food intake at the high dose had also some concurrent effects on the dams' body weight data, these effects are considered as substance - related. The food consumption of the 150 and 500 ppm female F0 parental rats during premating, gestation and lactation did not show any statistically significant differences and was generally comparable to that of the controls taking the normal range of biological variation into consideration.

Figure 5.6.1-6: Food consumption of parental F_0 animals during premating




Females (F0)
food consumption [g/animal/day]
20
19
18
17
16
15
14
13
12
11
10
1
2
3
4
5
6
Weeks
0 ppm
150 ppm
500 ppm
1200 ppm


Females (F0) (F1 litter)
food consumption [g/animal/day]
60
55
50
45
40
35
30
25
20
15
0-7
7-14
14-20
1-4
4-7
7-14
during gestation
Weeks
during lactation
0 ppm
150 ppm
500 ppm
1200 ppm

D. REPRODUCTION PARAMETERS

1. Male reproduction data

Male mating index
For nearly all F0 parental males, which were placed with females to generate F1 pups, mating was confirmed, except for low dose male No. 12. Thus the male mating index reached 100% in the control group and test groups 02 and 03 (500 and 1200 ppm), while it was 90% at 150 ppm.

Male fertility index
Fertility could be proven for most F0 parental males within the scheduled mating interval for F1 litter. The male fertility index varied between 60% and 100% without showing any relation to dosing [see Table 5.6.1-20]. Actually, four control males (males Nos. 3, 4, 9 and 10 mated with female Nos. 103, 104, 109 and 110) and two males of test group 01 (males Nos. 12 and 15 mated with female Nos. 112 and 115 - 150 ppm) did not generate F1 pups. Moreover, one male of test group 02 (male No. 24 mated with female No. 124 - 500 ppm) did generate F1 progeny, but all implants of the corresponding dam died already in utero. The sporadic occurrence of infertility without any relation to dosing in several male rats of the different test group including the controls is assessed as being incidental and spontaneous in nature. Moreover, there occurred no substance-induced impairments on the fertility in the previous extensive two-generation reproduction toxicity study.

Table 5.6.1-20: Male fertility index for F0 males (%)

	Test group 00 (0 ppm)	Test group 01 (150 ppm)	Test group 02 (500 ppm)	Test group 03 (1200 ppm)
Concerning F1 litters	60	80	100*	100

*= The female mating partner of mid dose male No. 24 had only dead implants in utero at necropsy and did not deliver pups.

2. Female reproduction data

Female mating index
The female mating index calculated after the mating period for F1 litter was 90% for the low dose group and 100% for all remaining groups. In one low dose F0 female rat (No. 112) no sperm was detected within the 14-day mating period; this female rat did not give birth to a litter/had no implants in utero.
The mean duration until sperm was detected (day 0 p.c.) varied between 2.7 and 3.7 days without any relation to dosing. These values reflect the normal range of biological variation inherent in the strain of rats used for this study.

Female fertility index

During the mating interval, all sperm positive rats delivered pups except four females of test group 00 and one female of test group 01, which did not become pregnant. One female of test group 02 did not deliver pups, but showed two implants in utero. As all females of test group 03 were pregnant, the fertility index varied between 60% (test group 00), 90% (test group 01) and 100% (test group 02 and 03) [see Table 5.6.1-21].

The occurrence of infertility in several female rats of test groups 0 and 1 (0 and 150 ppm) is assessed as being incidental and spontaneous in nature, also because no substance-induced impairments on the fertility occurred in the previous extensive two generation reproduction toxicity study.

The mean duration of gestation was very similar in all groups and the variation was negligible (between 21.7 and 22.0 days).

The gestation index was 100% for test group 00, test group 01 and test group 03, indicating that all pregnant females delivered live F1 pups.

In the test group 02 the gestation index reached 90% due to the fact, that one pregnant female delivered no pups, but had only dead implants in utero. Implantation was not affected by the treatment since the mean number of implantation sites was comparable between all test groups if the normal range of biological variation is taken into consideration. Furthermore, there were no indications for a substance-induced increase in intrauterine embryo-/feto-lethality since the postimplantation loss values were unaffected by treatment and did not show any dose-response relationship.

Table 5.6.1-21: Female fertility index for F0 females (%)

	Test group 00 (0 ppm)	Test group 01 (150 ppm)	Test group 02 (500 ppm)	Test group 03 (1200 ppm)
Concerning F1 litters	60	90	100	100

3. F1 generation litter/pups

Litter data

The mean number of delivered pups/dam and the rate of liveborn and stillborn pups were not affected by the administration of the test substance considering the overall low litter number, the lack of a dose response and the normal biological variation.

Pup viability/mortality

There were no substance-related differences between the control and the substance-treated groups concerning mortality and viability of the F1 pup generation. The viability indices, as indicators for perinatal mortality varied between 93% and 100%, are fully in the range of biological variation and thus reflect the normal range of biological variation inherent in the strain of rats used for this study. This includes the statistically significantly lower viability index at 150 ppm.

The lactation indices as indicators for pup mortality between days 4 - 21 p.p. ranged between 100% (control group, test groups 01 and 03), and 99% (test group 02) and therefore did not show any differences of biological relevance between the substance-treated groups and the control group.

Sex ratio
The sex distribution and sex ratios of live F1 pups on the day of birth and on day 21 p.p. did not show any substantial differences between controls and treated groups; all differences observed are regarded to be spontaneous in nature.

Pup body weight data
In line with the 2-generation reproduction toxicity study significant effects on body weights of pups were essentially absent at birth in the offspring animals. Mean body weights of F1 pups in test groups 03 (1200 ppm) were statistically significantly reduced from day 7 p.p. onwards until scheduled sacrifice on day 21 p.p. [see Table 5.6.1-22]. Body weight effects became more pronounced in the later phase of lactation, especially in the last week of lactation (time points PND 14 and 21), when the pups start self-feeding (around PND12; Hood, 2011; Tyl et al., 2008 (BASF DocID 2008/1102837)). On day 21 p.p. mean body weights of the F1 pups of test group 03 (1200 ppm) were about 38% below the concurrent control value if both sexes were combined. Mean body weights of F1 pups in test group 02 (500 ppm) were slightly reduced on day 21 p.p. (about 12% below the concurrent control group). Table 5.6.1-22 shows the comparison of maternal body weights (F0 generation) with pup body weights (F1 generation). The impairments in pup body weights at 500 and 1200 ppm are considered to be a consequence of test substance administration.
The F1 pups of the 150 ppm group did not show any statistically significant or biologically relevant differences for body weights/body weight gains.

Table 5.6.1-22: Maternal and pup body weights during lactation

Maternal	**Body weight [g]**			
day	**0 ppm**	**150 ppm**	**500 ppm**	**1200 ppm**
1	221.5	233.8	218.2 (-1.5)	206 (-7.0)
4	238.7	248.6	232.4 (-2.6)	219.1 (-8.2)
7	246.6	256.3	242 (-1.9)	231.7 (-6.0)
14	267.2	275.7	257.2 (-3.7)	235.4** (-11.9)
21	248.2	264.7	247.2 (-0.4)	237.4 (-4.4)
Litters	**Body weight [g]**			
day	**0 ppm**	**150 ppm**	**500 ppm**	**1200 ppm**
1	5.8	5.6	5.1 (-12.1)	5.3 (-8.6)
4 preculling	8.6	8.4	9.1 (5.8)	7.4 (-14)
4 postculling	8.7	8.4	9.1 (4.6)	7.5 (-13.8)
7	14	13.9	13.7 (-2.7)	10.8** (-22.9)
14	29.1	29.6	27.1 (-6.9)	20.2** (-30.6)
21	47.1	46.3	41.4 (-12.1)	29** (-38.4)

*p ≤ 0.05; ** p ≤ 0.01
In brackets (% vs. control)

The overall picture of pup body weight effects is in-line with the results of the 2-generation toxicity study.

Pup clinical observations
The F1 generation pups did not show any clinical signs up to weaning which could be attributed to the treatment.
Only one spontaneous clinical finding occurred in one low dose F1 pup (filiformed tail).

Pup organ weights
The mean absolute heart weights of the F1 pups of test groups 01 - 03 (150, 500 or 1200 ppm) did not show any substance-induced differences in comparison to the control values.

The mean relative heart weights of the pups of test groups 02 and 03 (500 and 1200 ppm) were statistically significantly increased (if both sexes are combined), whereas the relative mean heart weights at 150 ppm were similar to control values. If calculated for both sexes, the mean relative heart weight was increased above control values to about 20% (mid dose) or 57% (high dose) [see Table 5.6.1-23].
The increased mean relative heart weights of the PND21 F1 pups in test groups 02 - 03 (500 and 1200 ppm) are considered to be substance-related and directly in-line with the cardiomegaly observed macroscopically at the high dose (1200 ppm).

Table 5.6.1-23: Overview on cardiac effects in the modified one-generation study

Dose [ppm]	**150**	**500**	**1200**
Parental	No effect	Absolute heart weight slightly decreased (m);	Absolute heart weight slightly decreased (m) (related to decreased bw); ↑Relative heart weights due to decreased bw (not treatment-related) Gross lesions: not effects on hearts
Pups PND 21	No effect	↑Relative heart weight (20%) (males statistically significantly increased)	↑Relative heart weight (57%) (males and females statistically significantly increased)
Pups Necropsy findings PND21	No effect	-	Cardiomegaly (only in PND21 pups, not PND4)

Pup necropsy observations
The most obvious necropsy observations in the F1 pups, which are considered to be substance-induced, were increased occurrences of:

- pale yellowish discoloration of the liver at 500 and 1200 ppm
- cardiomegaly at 1200 ppm
- milky fluid in abdomen and/or thorax after organ evisceration at 500 and 1200 ppm
- pale discoloration of kidney(s) at 500 and 1200 ppm

It is assumed, that the aforementioned liver, kidney and heart findings are a consequence of a microcytic hypochromic anemia, which occurs in this study. The milky fluids reported in the abdomen and the breast cavity are considered to be secondary to the heart-insufficiency (cardiomegaly) induced by the chronic microcytic anemia.
In-line with the 2-generation study cardiomegaly was evident in the PND21 pups only and not seen in PND4 pups. Cardiomegaly occurs secondary to the microcytic hypochromic iron-deficiency anemia (see below) after the start of direct food intake of the pups.

A few of the large number of examined F1 pups showed some spontaneous findings at necropsy or in the additional examinations carried out later (e.g. incisors sloped, post mortem autolysis, dilated renal pelvis, hydroureter, filiformed tail) scattered throughout the test groups. These findings occurred without a clear relation to dosing and/or do also occur sporadically in the strain of rats used for the present study.

E. CLINICAL PATHOLOGY

1. Hematology

After 4 weeks of test substance administration (before mating period) decreased haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were found in the peripheral blood of the high dose males (1200 ppm). Increased red blood cell counts, anisocytosis, microcytosis and hypochromasia were also detected in these males. Moreover, in the mid dose males (500 ppm) MCV and MCH were reduced and microcytosis was increased. In the females, decreased MCH and MCHC values and slightly increased microcytosis and hypochromasia were found in the high dose animals at this time interval [see Table 5.6.1-24].
After 3 months of test substance administration (shortly before sacrifice) slightly increased microcytosis was seen in the high dose males (1200 ppm), only. In the peripheral blood of the high dose dams, decreased haemoglobin, MCV, MCH and MCHC values and increased red blood cells, platelets, reticulocytes, microcytosis and hypochromasia were found. In the dams of the mid dose group (500 ppm) decreases in MCH and MCHC as well as increases in microcytosis were detected. Slight effects were detected in the low dose group of 150 ppm.

On day 21 after birth the following hematology changes were observed in the high dose male and female F1 pups (1200 ppm): decreases in red blood cells, haemoglobin, haematocrit, MCV and MCH; increases in platelets, reticulocytes, microcytosis and anisochromasia. Moreover, in the high dose male pups MCHC was increased. In the male and female pups of the mid dose group (500 ppm) haemoglobin, haematocrit, MCV and MCH were decreased and reticulocytes, microcytosis, anisochromasia and normoblasts were increased. Significantly reduced erythrocytes were also measured in the blood of the mid dose male pups. In the low dose male pups (150 ppm) MCV was reduced and microcytosis and anisochromasia were increased. In the low dose female pups reticulocytes and microcytosis were elevated.

An overview on the hematological changes in this study for parental animals and PND21 pups can be found in Table 5.6.1-25.

Table 5.6.1-24: Summary of hematology parameters in the modified one-generation study

Dose [ppm]	Hematological parameters						
Males, day 29	**RBC (TERA/L)**	**HGB (MMOL/L)**	**HCT (L/L)**	**MCV (FL)**	**MCH (FMOL)**	**MCHC (MMOL/L)**	**PLT (GIGA/L)**
0	7.71	9.3	0.411	53.3	1.21	22.69	819
150	7.49	9.1	0.399	53.4	1.22	22.79	800
500	7.87	9.1	0.404	51.4**	1.16**	22.47	856
1200	8.2**	8.5***	0.387	47.2***	1.04***	22.04**	896
Males, day 98							
0	8.84	9.5	0.484	54.7	1.08	19.68	709
150	8.6	9.5	0.474	55.2	1.11	20.09**	700
500	9	9.6	0.489	54.3	1.06	19.55	706
1200	8.97	9.4	0.479	53.4	1.05	19.63	723
Females, day 29							
0	7.68	9.4	0.409	53.3	1.22	22.94	740
150	7.58	9.3	0.403	53.1	1.22	22.59	768
500	7.72	9.2	0.403	52.2	1.19	22.78	728
1200	7.96	9	0.405	51	1.14**	22.28**	841
Females, day 100							
0	8.5	10.4	0.498	58.8	1.22	20.76	779
150	8.31	9.5**	0.471	56.7	1.14*	20.07*	763
500	9.09	10	0.505	55.7	1.11*	19.90***	793
1200	9.54*	9.4***	0.491	52.0*	1.00**	19.21***	981**
Male pups, PND 21							
0	4.64	5.4	0.305	65.9	1.17	17.67	825
150	4.67	5.1	0.286	61.4**	1.08	17.62	821
500	4.28*	3.9***	0.223***	52.0***	0.91***	17.48	1065
1200	3.10***	3.0***	0.152***	47.1***	0.97**	20.59*	1227**
Female pups, PND 21							
0	4.47	5.1	0.281	63	1.14	18.08	759
150	4.78	5.1	0.291	61	1.07	17.6	690
500	4.37	4.0***	0.230**	52.3***	0.91***	17.39	1084
1200	3.02**	2.7***	0.139**	44.5***	0.91***	20.65	1471*

*$p \leq 0.05$; ** $p \leq 0.02$; *** $p \leq 0.002$

Table 5.6.1-25: Overview of hematological changes in the modified one-generation study

Dose [ppm]	150	500	1200
Adult males	No effect	**Premating:** ↓ MCH (-4%), MCV (-4%); ↑ microcytosis	**Premating:** ↓ Hgb (-9%), MCV (-11%), MCH (-14%), MCHC (-3%); ↑ RBC (+6%), anisocytosis, microcytosis and hypochromasia, reticulocutes (+52%) **Study day 100:** slight↑ microcytosis
Adult females	**Study day 100:** ↓ Hgb (-9%), MCH (-7%), MCHC (-3%); ↑ microcytosis	**Study day 100:** ↓ MCH (-9%), MCHC (-4%); ↑ microcytosis, reticulocytes (+44%)	**Premating:** ↓ MCV (-3%), MCH (-7%), ↑ RBC (+4%), microcytosis, hypochromasia, reticulocytes (+20%) **Study day 100:** ↓ Hgb (-10%), MCV (-12%), MCH (-18%), MCHC(-7%); ↑ RBC (+12%), microcytosis, hypochromasia, platelets and reticulocytes (+333%)
Pups PND 21	↓MCV (M:-7%) ↑microcytosis and reticulocytes (M:+6%; F:+56%); ↑anisochromasia (males)	↓ Hgb (M:-28%; F:-22%), Hct (M:-27%; F:-18%), MCV (M:-21%; F:-17%), MCH (M:-22%,F: -20%); ↓ RBC (M:-8%); ↑ anoisochromasia, microcytosis, reticulocytes (M:+57%; F:+58%), normoblasts (both sexes)	↓ RBC (M: -33%; F:-32%), Hgb (M:-45%; F:-47%), Hct (M:-50%; F:-51%), MCV (M:-29%; F:-29%), MCH (M:-17%; F:-20%), MCHC (M: -19%); ↑ anisochromasia, microcytosis, platelets and reticulocytes (M:+66%; F:+216%)

The changes in hematological parameters observed are indicative for an iron-deficiency microcytic hypochromic anemia. Dimoxystrobin reduces iron uptake in the duodenum and thus causes lower serum iron levels in rats and a microcytic hypochromic anemia (also seen in subchronic and chronic studies), which is characterized by reduced blood haemoglobin (HGB), reduced mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV). The correlation of anemia and reduced iron levels was clearly shown in a mechanistic study, where a reduction in serum iron levels was accompanied by changes in hematological parameters indicative for anemia (see MCA 5.8.2; BASF DocID 2002/1005354).

Anemia was seen at the same dietary concentrations in pups and parental animals. However, the individual hematology parameters were more pronounced in the PND 21 pups.
This is not considered to be indicative for a higher susceptibility in the pups, because
- the milk of anemic dams contains less iron compared to control dams,
- pups are lacking body iron stores, and
- pups were exposed to higher dimoxystrobin doses (see above; estimated pup intake is 260 mg/kg bw, while parental females consume 170.5 mg/kg bw during lactation; see Table 5.6.1-19).

Milk is generally a poor source of iron, milk of dams suffering from iron deficiency anemia contains less iron than usual (e.g. 34 µg/g dry wt in controls vs. 22 µg/g dry wt in treated dams; Roth and Smith, 1988; BASF DocID 1988/1003436), so that these dams are even less able to transfer sufficient iron to the young via the milk in the early postpartum period (Anaokar and Garry, 1981; BASF DocID 1981/1001521).
Compared to adult animals, that can store excessive amounts of iron in tissues in either two forms, ferritin or hemosiderin, which can be mobilized in an iron deficient state, pups have physiologically only very small iron stores. Therefore, the body iron stores of nursing pups can easily be depleted since their blood volume expands to accommodate the increasing body size (Roth and Smith, 1988; BASF DocID 1988/1003436).

The iron-deficiency anemia is occurring in dams and offspring of the reproduction toxicity studies at the same dose levels ($\geq$ 150 ppm). The susceptibility of the pups to develop an anemia is not higher compared to the adults, as the iron deficiency is the first event to occur after dimoxystrobin treatment and there are new studies to show, that the maternal NOAEL is identical to the offspring NOAEL.
For example a new enhanced one-generation study (study summary can be found below in this chapter; BASF DocID 2011/1211676) was performed at lower dose levels. No treatment-related, adverse changes of hematological parameters as well as transferrin and iron levels were observed in the parental animals and pups up to the highest dose tested (50 ppm; about 4.3 mg/kg bw/day for adults). The same NOAEL of roughly 4 mg/kg bw/day for reduction in serum iron levels was found in a mechanistic study with young rats (see MCA 5.8.2; BASF DocID 2010/1026748), as well as for adult rats (BASF DocID 2002/1014345). There is no evidence, that young animals are more sensitive than adults (with regard to anemia and serum iron levels).

F. PATHOLOGY

1. Organ weights

The mean terminal body weight was significantly decreased in males of mid and high dose groups showing dose response relationship. This was regarded as treatment-related. In females of the high dose group, the mean terminal body weight was slightly decreased (-4.7%), however, this was not significant. In contrast, in females of the low dose group, the mean terminal body weight was slightly although significantly increased. This was, however, regarded as unrelated to treatment.
In males of the mid and high dose groups, the mean weight of heart was slightly although significantly decreased. This was regarded to be related to the decreased mean terminal body weight [see Table 5.6.1-26].
In females of the high dose group, the mean liver weight was slightly although significantly increased. In females of the low dose group, the mean weights of liver and spleen were significantly increased. This was regarded to be related to the increased mean terminal body weight rather than to treatment [see Table 5.6.1-27].
The other mean absolute weight parameters did not show significant differences when compared with the control group.

Due to the significantly decreased mean terminal body weight, the mean relative weights of liver and heart (males, high dose group) were significantly increased. This was not regarded treatment-related.
In females of the high dose group, the mean relative weights of liver and heart were also significantly increased. Although the decrease of the mean terminal body weight in this group was not significant, the increased mean weights of both organs are interpreted to be related to the decreased mean terminal body weight rather than to a treatment-related effect.
The other mean relative weight parameters did not show significant differences when compared with the control group.

A comparison of the cardiac effects in parental animals and pups can be found in Table 5.6.1-23.

Table 5.6.1-26: Absolute and relative organ weights of F1 males (Mean ± SD[#])

Dose group		0	150	500	1200
Terminal bw [g]	absolute	366 ± 27	362 ± 30	**325 ± 26****	299 ± 19
	relative	-	-	-	-
Liver [g]	absolute	9.03 ± 1.02	8.63 ± 0.85	8.25 ± 0.73	8.45 ± 0.39
	relative	2.47 ± 0.18	2.38 ± 0.10	2.54 ± 0.11	**2.83 ± 0.18****
Heart [g]	absolute	1.07 ± 0.10	1.04 ± 0.08	**0.96 ± 0.08***	**0.96 ± 0.08***
	relative	0.292 ± 0.017	0.288 ± 0.019	0.295 ± 0.010	**0.323 ± 0.028****

#: numbers were rounded and thus may not exactly reflect the numbers given in the study report
* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))

Table 5.6.1-27: Absolute and relative organ weights of F1 females (Mean ± SD[#])

Dose group		0	150	500	1200
Terminal bw [g]	absolute	202.8 ± 17.4	224.5 ± 21.0	205.0 ± 17.4	193.2 ± 13.4
	relative	-	-	-	-
Liver [g]	absolute	5.98 ± 1.36	**6.98 ± 0.74***	6.76 ± 1.48	**7.16 ± 1.30***
	relative	2.94 ± 0.56	3.13 ± 0.40	3.29 ± 0.59	**3.69 ± 0.49****
Heart [g]	absolute	0.816 ± 0.120	0.868 ± 0.066	0.874 ± 0.094	0.872 ± 0.043
	relative	0.402 ± 0.043	0.389 ± 0.037	0.427 ± 0.042	**0.452 ± 0.022***

#: numbers were rounded and thus may not exactly reflect the numbers given in the study report
* $p \leq 0.05$, ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test (two-sided))

2. Gross lesions

Thickening of the wall of the duodenum was noted in one male of the high dose group. This adaptive observation was regarded to be treatment-related. A few other gross lesions were noted in the glandular stomach (erosion ulcer in each one female of all groups), mammary gland (mass in a low dose female), and axillary and iliac lymph nodes (enlarged in a low dose group female). They were all regarded incidental and unrelated to treatment.

3. Histopathology

Histopathology was not performed.

III. CONCLUSIONS

The administration of 150, 500 and 1200 ppm of the test compound to male and female rats in this modified one-generation reproduction toxicity study caused a dose-dependent regenerative microcytic hypochromic anemia in the parental animals and the pups. The anemia was characterized by reduced haemoglobin concentrations and mean corpuscular indices as well as increased microcytosis and reticulocytes.
The lowest overall NOAEL for general parental toxicity is 150 ppm (about 17 mg/kg bw/day), as the slight beginning anemia, which was observed at 150 ppm in maternal animals, is regarded treatment-related but not adverse.
The apparently more severe effects on hematological parameter in pups compared to dams can be related to a considerably higher compound intake (260 mg/kg bw in pups vs 170.5 mg/kg bw in parental females) at the same dietary dose levels.

Studies not yet peer-reviewed

A new enhanced one-generation reproduction toxicity study was conducted to further investigate the effects on iron-deficiency anemia and to obtain NOAELs for adults and offspring.

Report:	CA 5.6.1/1 [redacted] et al., 2011a BAS 505 F (Dimoxystrobin) - Enhanced one-generation reproduction toxicity study in Wistar rats - Administration via the diet 2011/1211676
Guidelines:	EPA 870.3800, OECD 416, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In an enhanced one generation toxicity study, dimoxystrobin (BAS 505 F; Batch: OP-No. 13; Purity 98.5%) was administered in the diet to groups of 25 male and 25 female Crl:WI(Han) Wistar rats at nominal dose levels of 0, 10, 20 and 50 ppm. The dietary concentrations of dimoxystrobin were adjusted to 0, 5, 10, 25 ppm during lactation, thereby maintaining constant dose-levels during this period of increased food intake. The overall mean dose of dimoxystrobin administered to the male Wistar rats during the entire study period was approx. 0.8 mg/kg bw/day in the 10 ppm group, approx. 1.6 mg/kg bw/day in the 20 ppm group and approx. 4.0 mg/kg bw/day in the 50 ppm group. For the females mean doses of 0.9, 1.8, and 4.5 mg/kg bw/day were administered to groups 1, 2, and 3, respectively, during the premating period. During gestation and lactation mean doses of 0.8/0.7, 1.6/1.5, and 3.8/3.8 were administered to the test groups 1, 2, and 3, respectively.

No treatment-related mortality was observed throughout the study. No clinical signs or changes of general behavior, which may be attributed to the test substance, were detected in any of the male and female parental animals in any of the generations. Body weight development and food consumption was sporadically different to the control group, but without biological relevance.

Overall, male and female fertility indices ranged between 96 and 100% without any relation to dose. Dimoxystrobin treatment did not affect the reproductive performance as was evident from the absence of effects on the pre-coital interval or gestation lengths as well as gestation (100%) or live birth indices (98 to 100%). The observed numerical differences displayed no dose-response relationship and were thus not indicative of a relation to treatment.

Survival of pups was not affected by treatment as viability indices in the range of 97 to 99% without dose relation were observed. Lactation indices indicating pup mortality between PND 4-21 varied between 49% and 55%. The lowered lactation index was caused by the fact that selected pups were sacrificed for blood sampling on PND 7, 14, and 21.

No treatment-related, adverse changes among hematological parameters as well as transferrin and iron levels were detected in the F_0 generation or in the F_1 generation on day 7, 14, and 21.

Under the conditions of the present enhanced one-generation reproduction toxicity study the NOAEL for the F_0 parental rats for general, systemic toxicity is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.
The NOEL for fertility and reproductive performance for the F_0 parental rats is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.
The NOEL for developmental toxicity in the F_1 progeny is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: BAS 505 F (dimoxystrobin)

Description:	solid / beige
Lot/Batch #:	OP-No. 13
Purity:	98.5% (tolerance +- 1.0%)
Stability of test compound:	The test substance was stable over the study period; (Expiry date: 01.11.2015)

2. Vehicle and/or positive control: rodent diet

3. Test animals:

Species:	Rat, Wistar
Strain:	Crl:WI(Han)
Sex:	Male and female
Age:	F_0 parental animals: 28 ± 1 days at delivery; 36 ± 1 days at beginning of treatment
Weight at dosing:	♂: 127.6 g- 159.8 g, ♀: 95.6 g – 124.0 g
Source:	Charles River Laboratories, Research Models and Services, Germany GmbH
Acclimation period:	8 days
Diet:	Kliba maintenance diet rat/mouse "GLP", Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	drinking water from water bottles, ad libitum

Housing:	individual housing in Makrolon type M III cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area of about 800 cm²), with the following exceptions: • During mating male and female mating pairs were housed together in Makrolon type M III cages overnight • pregnant animals and their litters housed together until PND 21 (end of lactaction) until PND 21 (end of lactation) enrichment: wooden gnawing blocks (Typ NGM E-022 supplied by Abedd® Lab. and Vet. Service GmbH, Vienna, Austria); nesting material: cellulose wadding
Environmental conditions:	
Temperature:	20 - 24°C (central air-conditioned rooms)
Humidity:	30 - 70% (central air-conditioned rooms)
Air changes:	15 times per hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 07-Dec-2010 - 25-Aug-2011
(In-life dates: 15-Dec-2010 (start of administration of F_0 parental animals) to 26-Apr-2011 (sacrifice of F_0 parental animals)

2. Animal assignment and treatment:

Dimoxystrobin was administered in the diet to groups of 25 male and 25 female rats at nominal dose levels of 0, 10 (low dose), 20 (mid dose), and 50 ppm (high dose). The animals used as F_0 parental animals were derived from different litters according to a written statement from the breeder. By this, sibling mating was avoided. The animals were randomly assigned to the test groups by means of computer generated randomization list based on body weights.

After the acclimatization period F_0 parental animals continuously received the test-substance throughout the entire study. Prior to sacrifice food was withdrawn overnight.
At least 73 days after the beginning of treatment, male and female rats of the same dose groups were mated overnight (details see below).
Females were allowed to deliver and rear their pups (F_1 generation pups) until day PND 4 (standardization; see below) or day 21 after parturition. After weaning of F_1 pups the F_0 generation parental animals were sacrificed.

Mating procedure: Males and females were mated overnight at a 1 : 1 ratio for a maximum of 2 weeks. Throughout the mating period, each female was paired with a predetermined male from the same dose group. The animals were paired by placing the male in the cage of the female mating partner from about 4.00 pm until 7.00 - 9.00 am of the following morning. Deviations from the specified times were possible on weekends and public holidays, and were reported in the raw data.
A vaginal smear was prepared after each mating and examined for the presence of sperm. If sperm was detected, pairing of the animals was discontinued. The day on which sperm were detected was denoted "gestation day (GD) 0" and the following day "gestation day (GD) 1".

Standardization of litters: On PND 4, the individual litters were standardized in such a way that, where possible, each litter contained 4 male and 4 female pups (always the first 4 pups/sex and litter were taken for further rearing). If individual litters did not have 4 pups/sex it was proceeded in such a way that the most evenly distributed 8 pups per litter were taken for further rearing (e.g., 5 male and 3 female pups). Standardization of litters was not performed in litters with ≤ 8 pups.

3. Test substance preparation and analysis:
The diets were prepared by mixing weighed amounts of test substance (pulverized in a mortar and sieved) with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer.

The following nominal dose levels were selected for the study:

10	ppm as low dose
20	ppm as intermediate dose
50	ppm as high dose

Dimoxystrobin concentrations in the diet of the F_0 females were reduced to 50% during the lactation period. This dietary adjustment, derived from historical body weight and food consumption data, maintained the dams at constant dose-levels of dimoxystrobin during this period of increased food intake.

The calculation of the group test substance intake per day was carried out according to the following formula:

$$IT_x = \frac{FC_x \text{ x } C}{BW_y}$$

ITx = mean group intake of test substance on day x (mg/kg bw/d); FC_x = mean daily food consumption on day x (g); C = dietary dimoxystrobin concentration (ppm); BW_y = mean body weight on day x (g) (last weighing before day x).

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 42 days

Homogeneity and concentration control analyses were carried out at the beginning and end of the premating phase, during the gestation and lactation periods. For homogeneity analysis two randomly sampled specimen from the top, middle and bottom of the storage containers were sampled and analyzed.

Table 5.6.1-28: Analysis of diet preparations for homogeneity and test-item content

Nominal Dose level [ppm]	Sampling	Concentration [ppm]	Nominal concentration [%]	Mean nominal concentration [%][#]	Relative standard deviation [%]
10	13.12.2010	11.92	119.2	107.8	14.1
10	13.12.2010	9.055	90.6		
10	13.12.2010	11.36	113.6		
20	13.12.2010	19.04	95.2	-	-
50	13.12.2010	49.76	99.5	99.7	1.0
50	13.12.2010	50.38	100.8		
50	13.12.2010	49.44	98.9		
10	28.02.2011	9.220	92.2	101.5	9.1
10	28.02.2011	10.17	101.7		
10	28.02.2011	11.06	110.6		
20	28.02.2011	21.97	109.9	-	-
50	28.02.2011	50.76	101.5	99.4	5.3
50	28.02.2011	51.63	103.3		
50	28.02.2011	46.72	93.4		
5	14.03.2011	5.460	109.2	108.4	7.4
5	14.03.2011	5.800	116.0		
5	14.03.2011	4.999	100.0		
10	14.03.2011	9.180	92.0	-	-
25	14.03.2011	26.55	106.2	103.1	2.9
25	14.03.2011	25.76	103.0		
25	14.03.2011	25.06	100.2		
10	05.04.2011	10.83	108.3	107.7	2.0
10	05.04.2011	10.53	105.3		
10	05.04.2011	10.95	109.5		
20	05.04.2011	21.00	105.0	-	-
50	05.04.2011	53.50	107.0	101.9	7.1
50	05.04.2011	46.84	93.7		
50	05.04.2011	52.50	105.0		

[#] based on mean values of the three individual samples

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
Food consumption (parental animals), body weight and body weight change (parental animals and pups; for the pup weights, the litter means were used), number of mating days, duration of gestation, number of implantation sites, postimplantation loss and % postimplantation loss, number of pups delivered per litter	Simultaneous comparison of all dose groups with the control group using the DUNNETT-test (two-sided) for the hypothesis of equal means
Male and female mating indices, male and female fertility indices, gestation index, females with liveborn pups, females with stillborn pups, females with all stillborn pups, live birth index, pups stillborn, pups died, pups cannibalized, pups sacrificed moribund, viability index, lactation index, number of litters with affected pups at necropsy	Pairwise comparison of each dose group with the control group using FISHER'S EXACT test for the hypothesis of equal proportions
Proportions of affected pups per litter with necropsy observations	Pairwise comparison of each dose group with the control group using the WILCOXON-test (one-sided) for the hypothesis of equal medians

Statistics of clinical pathology

Blood parameters	For parameters with bidirectional changes: Non-parametric one-way analysis using KRUSKAL-WALLIS test. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the hypothesis of equal medians

C. Methods

1. Observations:

The animals, i.e. parental animals and pups, were examined for mortality twice daily on working days and once daily on weekends and public holidays. If animals were in a moribund state, they were sacrificed and necropsied. Observations for evident signs of toxicity were performed at least once daily.
The parturition and lactation behavior of the dams was generally evaluated in the mornings in combination with the daily clinical inspection of the dams. Only special findings (e.g., disability to deliver) were documented on an individual dam basis. Except on weekends and public holidays, the parturition behavior was additionally checked in the afternoons.
The live pups were examined daily for clinical symptoms (including gross morphological findings) during the clinical inspection of the dams. If pups showed particular findings, these were documented with the dam concerned.

2. Body weight:

Body weight of **parental animals** was determined on the first day of the premating period and weekly thereafter at the same time of the day. The following exceptions are notable for female parental animals:

a. The F_0 generation parental females were weighed on the day of positive evidence of sperm (GD 0) and on GD 7, 14, and 20.
b. Females were not weighed during mating until there was a positive evidence of sperm in vaginal smears
c. Females with litter were weighed on the day after parturition (PND 1) and on PND 4, 7, 14, and 21.
d. Females without litter were not weighed during the lactation phase.

Pup body weights were determined on the day after birth (PND 1) and on PND 4 (before standardization), 7, 14, and 21.

3. Food consumption, food efficiency and compound intake:

Food consumption was determined once a week (for a period of 6 days) for parental animals and calculated as mean food consumption in grams per animal and day. The following exceptions are notable for female parental animals:

a. Food consumption of females during pregnancy was determined weekly for GD 0-7, 7-14, and 14-20
b. During the lactation period food consumption of the females was determined for PND 1 - 4, 4 - 7, 7 - 14, and 14 - 21.
c. No food consumption was determined during the mating period and for females without positive evidence of sperm and females without litter.

The mean daily intake of test substance (group means in mg/kg bw/d) was calculated based upon individual values for body weight and food consumption:

$$\text{Substance intake for day } x = \frac{FC_x \times C}{BW_y}$$

FC_x = daily food consumption on day x [g]; C = concentration in ppm; BW_y = body weight on day y (g) (last weighing before day x)

4. Ophtalmoscopy:

Not performed in this study

5. Hematology and clinical chemistry:

Blood was withdrawn from the adult animals from the retro-orbital venous plexus, and from pups after decapitation, following isoflurane anesthesia.
Blood samples were withdrawn from 12 adult F_0 animals at sacrifice and from 1 pup per sex from each litter on PND 7, 14 and 21. The following hematological and clinical chemistry parameters were determined:

Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Iron		Albumin		Alanine aminotransferase (ALT)
✓	Transferrin		Bilirubin (total)		Aspartate aminotransferase (AST)
	Calcium		Cholesterol		Alkaline phosphatase (ALP)
	Chloride		Creatinine		γ-glutamyl transpeptidase (γ-GT)
	Magnesium		Globulin (by calculation)		
	Phosphorus (inorganic)		Glucose		
	Potassium		Protein (total)		
	Sodium		Triglycerides		
			Urea		
Hematology:					
✓	Leukocytes	✓	Reticulocytes	✓	Blood smears
✓	Erythrocytes	✓	Differential blood count		
✓	Hemoglobin	✓	Mean corpuscular volume		
✓	Hematocrit (calculation)	✓	Mean corpuscular hemoglobin (calculation)		
✓	Platelets	✓	Mean corp. hemoglobin conc. (calculation		

6. Male reproduction data

For the males, mating and fertility indices were calculated for F_1 litters according to the following equations:

$$\text{Male mating index [\%]} = \frac{\text{number of males with confirmed mating *}}{\text{number of males placed with females}} \times 100$$

* defined by a female with vaginal sperm or with implants in utero

$$\text{Male fertility index [\%]} = \frac{\text{number of males proving their fertility *}}{\text{number of males placed with females}} \times 100$$

* defined by a female with implants in utero

7. Female reproduction and delivery data

For the females, mating, fertility and gestation indices were calculated for F_1 litters according to the following equations:

$$\text{Female mating index [\%]} = \frac{\text{number of females mated *}}{\text{number of females placed with males}} \times 100$$

* defined as the number of female with vaginal sperm or with implants in utero

$$\text{Female fertility index [\%]} = \frac{\text{number of females pregnant *}}{\text{number of females mated **}} \times 100$$

* defined as number of female with implants in utero
** defined as the number of females with vaginal sperm or with implants in utero

$$\text{Female gestation index [\%]} = \frac{\text{number of females with live pups on the day of birth}}{\text{number of females pregnant *}} \times 100$$

* defined as number of female with implants in utero

The total number of pups delivered and the number of liveborn and stillborn pups were noted, and the live birth index was calculated for F_1 litters:

$$\text{Live birth index [\%]} = \frac{\text{number of liveborn pups at birth}}{\text{total number of pups born}} \times 100$$

The implantations were counted and the postimplantation loss (in %) was calculated. To determine the number of implantation sites, the apparently non-pregnant uteri were stained for about 5 minutes in 1% ammonium sulfide solution according to the method of SALEWSKI.

$$\text{Postimplan tation loss [\%]} = \frac{\text{number of implantati ons} - \text{number of pups delivered}}{\text{number of implantati ons}} \times 100$$

8. Litter data

All F_1 pups were examined as soon as possible on the day of birth to determine the total number of pups and the number of liveborn and stillborn members of each litter. Pups, which died before the first examination on the day of birth, were designated as stillborn pups.
The number of live pups/litter was calculated on the day after birth, and on lactation days 4, 7, 14, and 21. Furthermore, viability and lactation indices were calculated as follows:

$$\text{Viability index [\%]} = \frac{\text{number of live pups on day 4* after birth}}{\text{number of live pups on the day of birth}} \times 100$$

* before standardization of litters (i.e. before culling)

$$\text{Lactation index [\%]} = \frac{\text{number of live pups on day 21 after birth}}{\text{number of live pups on day 4* after birth}} \times 100$$

* after standardization of litters (i.e. after culling)

On the day of birth (PND 0) the sex of F_1 pups was determined by determination of the anogenital distance. Subsequently the sex of the pups was assessed by the external appearance of the anogenital region and/or the mammary line. The sex of the animals was finally confirmed at necropsy. The sex ratio was calculated at PND 0 and PND 21 after birth using the following equation:

$$\text{Sex ratio [\%]} = \frac{\text{number of live male or female pups on day 0/21}}{\text{number of live male and female pups on day 0/21}} \times 100$$

9. Sacrifice and pathology:

All F_0 parental animals were sacrificed by decapitation under isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology with special attention given to the reproductive organs. Animals that were sacrificed in a moribund state were necropsied as soon as possible after death and assessed by gross pathology without determination of organ weights.

On PND 4, as a result of standardization, the surplus pups were sacrificed under isoflurane anesthesia with CO_2. After sacrifice, these pups were examined externally, eviscerated and their organs were assessed macroscopically.
On PND 7, 14 and 21, all F1 generation pups used for blood sampling were sacrificed under isoflurane anesthesia by decapitation. The surplus pups on PND 21 were sacrificed under isoflurane anesthesia with CO_2. After sacrifice, these pups were examined externally, eviscerated and their organs were assessed macroscopically.
All pups without any notable findings or abnormalities were discarded after their macroscopic evaluation. Animals with notable findings or abnormalities were further evaluated on a case-by-case basis, depending on the findings noted.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of test substance in rat diet at room temperature was demonstrated for a period of 42 days before beginning of the study in a comparable batch.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment related clinical observations, which may be attributed to the test substance, were detected in any of the male $\mathbf{F_0}$ parental animals. Clinical observation of $\mathbf{F_0}$ male animals revealed a skin lesion at the throat in one high dose male (#83). This finding is considered to be spontaneous in nature.
There were no test substance-related clinical findings in all $\mathbf{F_0}$ females of all dose groups during premating, gestation and lactation periods for F1 litter. One low-dose $\mathbf{F_0}$ dam (#127) did not nurse its pups properly. Therefore it had no more pups alive on PND 1 (most of its pups were stillborn or died on PND 0). This observation is not considered to be treatment-related.

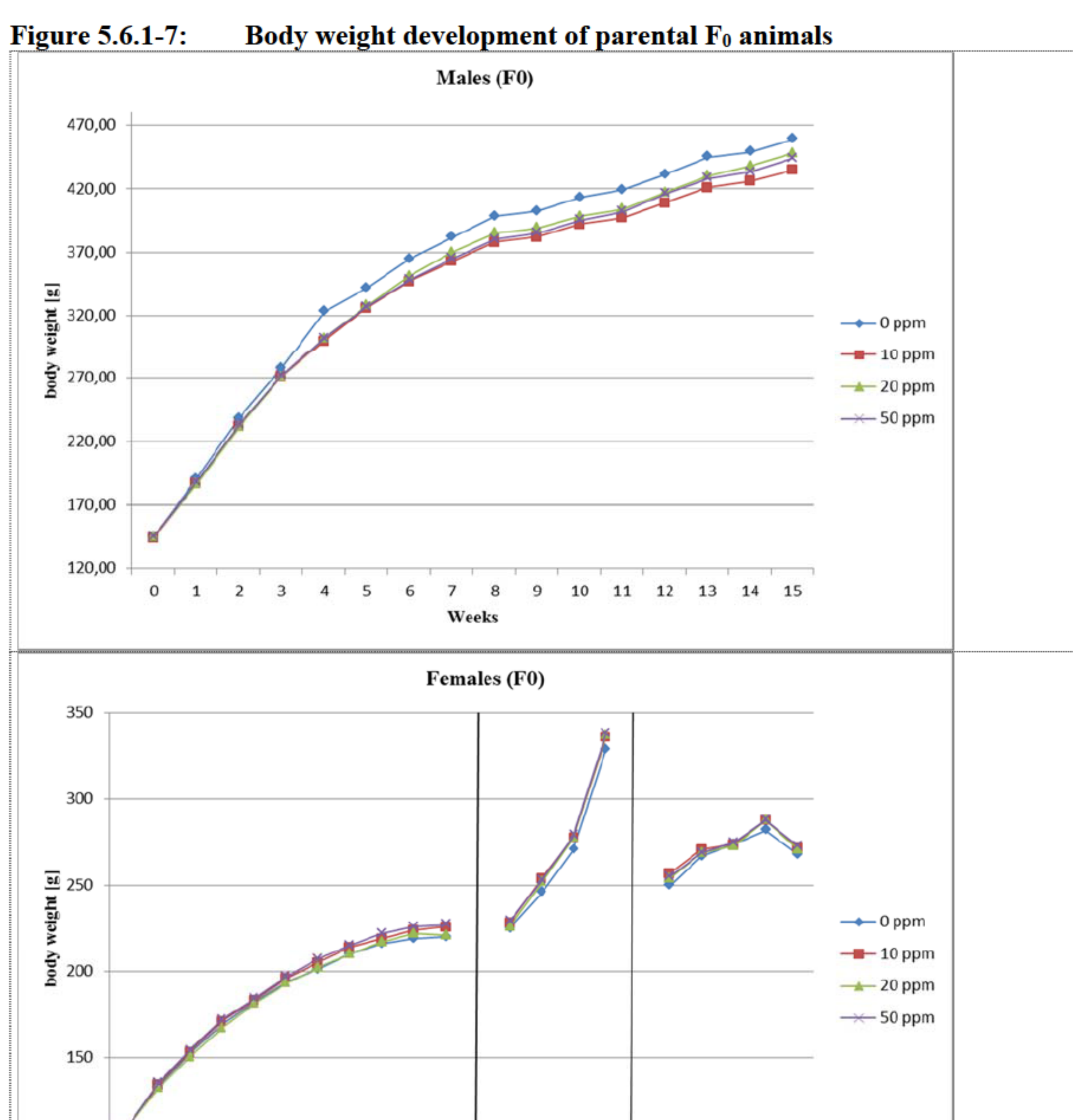
2. Mortality

No treatment-related or spontaneous mortality was observed throughout the study.

C. PARENTAL BODY WEIGHT AND BODY WEIGHT GAIN

Mean body weights and body weight gain of the mid- and high-dose F_0 males (20 and 50 ppm) were comparable to the concurrent control group throughout the study (see Figure 5.6.1-7. However, high-dose body weight was slightly, but statistically significantly, reduced in week 6. Furthermore, F_0 males gained either less body weight during study weeks 0-1 and weeks 3-5 (test group 2 and 3) or more body weight during weeks 13-14 (test group 2).
However, low-dose parental males had statistically significantly lower body weights from study week 5 onwards until the end of the study in week 15. Their body weight gain was statistically significantly decreased during weeks 0-5. During the entire study, the low-dose males gained about 8% less weight than in controls.
All statistical significant differences noted during isolated periods between the substance treated groups and the concurrent control are without any biological relevance and considered not treatment-related (due to the lack of dose-response relationship).
Neither mean body weights nor mean body weight gain of the F_0 parental females in all dose groups were influenced by the test substance during premating, gestation and lactation periods. The statistically significantly increased body weight gain during GD 0-7 (test group 1 and 2) and during PND 7-14 (test group 2) was considered as spontaneous in nature.

Figure 5.6.1-7: Body weight development of parental F_0 animals



D. PARENTAL FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption of the F_0 male and female animals in all test groups (10, 20, and 50 ppm) was generally comparable to the concurrent control group during the entire study, covering premating, gestation and lactation periods (see Figure 5.6.1-8 and Figure 5.6.1-9).

This includes the sporadically significant food consumption values during the different study periods:

Food consumption of the low-dose F_0 males was decreased during premating weeks 4-6. Food consumption of the F_0 females was increased during premating weeks 5-7 and 8-10 in test group 1 (10 ppm) and during week 8-9 in test group 2 (20 ppm). Furthermore, it was increased in test group 1 and 3 calculated for GD 0-7 and in test group 2 for GD 0-14. During lactation the food consumption of the mid-dose F_0 females was increased if calculated for PND 7-14.

Figure 5.6.1-8: Food consumption of parental F_0 animals during premating

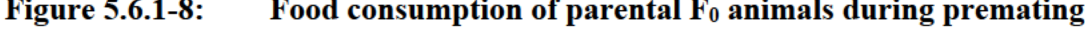
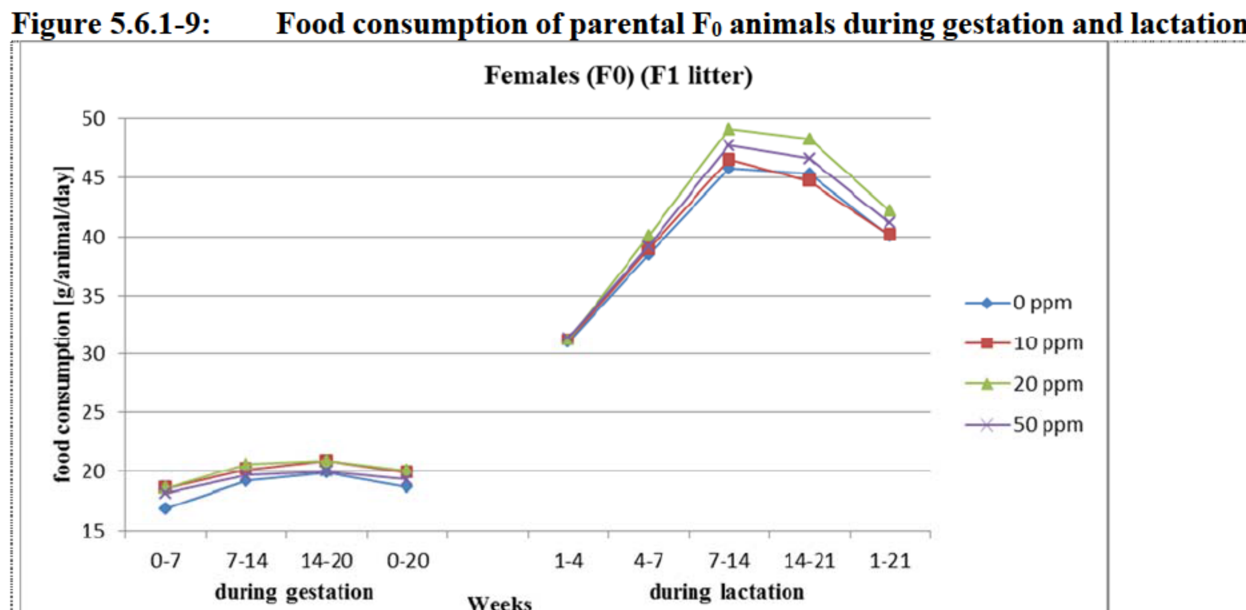


Figure 5.6.1-9: Food consumption of parental F_0 animals during gestation and lactation



For all test groups the intake of dimoxystrobin correlated well with the desired target doses. The actual test substance intake was calculated on the basis of interpolated mean body weights of each test group. With regard to the target dose levels, the mean values and the minimum/maximum deviations of the actual test substance intake per time interval examined are shown in Table 5.6.1-29.

Table 5.6.1-29: Average dimoxystrobin intake (mg/kg bw/d) in parental animals

Dose Group & sex	10 ppm average	min/max	20 ppm average	min/max	50 ppm average	min/max
F_0 males	0.8	0.5 / 1.3	1.6	1.0 / 2.5	4.0	2.6 / 6.5
F_0 females (premating)	0.9	0.7 / 1.3	1.8	1.3 / 2.6	4.5	3.1 / 6.6
F_0 females (F1 litter) - gestation period - lactation period	0.8 0.7	0.8 / 0.8 0.6 / 0.8	1.6 1.5	1.5 / 1.7 1.2 / 1.8	3.8 3.8	3.6 / 4.0 3.1 / 4.3

E. MATING AND GESTATION DATA

1. Male reproductive performance

For all F_0 parental males, which were placed with the females to generate F_1 pups, copulation was confirmed. Thus, the male mating index was 100% in all groups including the controls. Fertility was proven for nearly all F_0 parental males within the scheduled mating interval for F_1 litter. One low-dose male (#39) did not generate F_1 pups.
Thus, the male fertility index was 96% in test group 1 and 100% in all remaining groups including the control. This reflects the normal range of biological variation inherent in the strain of rats used for this study. All respective values are within the range of the historical control data of the test facility.

Parental generation				
Dose [ppm]	**0**	**10/5**	**20/10**	**50/25**
Males placed with females	25	25	25	25
Mated [n]	25	25	25	25
Male mating index [%]	100	100	100	100
did not mate [n]	0	0	0	0
with females pregnant [n]	25	24	25	25
Male fertility index [%]	100	96	100	100
without females pregnant [n]	0	1	0	0
without females pregnant [%]	0	4	0	0

2. Female reproductive performance

The female mating index calculated after the mating period for F_1 litter was 100% in all test groups. The mean duration until sperm was detected (GD 0) varied between 2.4 and 3.0 days without any relation to administered doses. All sperm positive rats delivered pups or had implants in utero with the exception of low-dose female #139 (mated with male #39), that did not become pregnant.
The female fertility index varied between 96% and 100%. All respective values are within the range of historical control data of the test facility and do not show any relation to dosing. The mean duration of gestation was identical in all test groups: 22 days for control, low-, mid- and high-dose groups. The gestation index was 100% in all test groups. Implantation was not affected by the treatment since the mean number of implantation sites was comparable between all test substance-related groups and the control, taking normal biological variation into account (12.9/13.5/13.2 and 13.2 implants/dam in test groups 0-3.
Furthermore, there were no indications for test substance-induced intrauterine embryo-/fetolethality since the postimplantation loss did not show any statistically significant differences between the groups, and the mean number of F_1 pups delivered per dam remained unaffected (12.2/12.5/12.7 and 12.6 pups/dam at 0, 10, 20 and 50 ppm).

The rate of liveborn pups was also not affected by the test substance, as indicated by live birth indices of 100, 98%, 100, and 99% in the control, 10, 20, and 50 ppm dose groups, respectively. Moreover, the number of stillborn pups was comparable between the groups.

Table 5.6.1-30: Summary of female reproduction and delivery data

Parental generation	**F_0**			
Dose [ppm]	**0**	**10/5**	**20/10**	**50/25**
Animals per dose	25	25	25	25
Female fertility				
- placed with males	25	25	25	25
- mated [n]	25	25	25	25
- mating index [%]	100	100	100	100
- pregnant [n]	25	24	25	25
- **Fertility index** [%]	100	96	100	100
Pre coital interval [days]	2.9	2.4	3.0	2.5
Duration of gestation [days]	22.0	22.0	22.0	22.0
Implantation sites, total [n]	323	323	331	331
- dto per dam [n]	12.9	13.5	13.2	13.2
Post implantation loss [n]	17	23	13	16
- dto per dam [n]	0.7	1.0	0.5	0.6
- dto per litter [mean %]	5.0	7.3	4.2	5.3
Females with liveborn	25	24	25	25
- **Gestation index** [%]	100	100	100	100
- with stillborn pups [n]	1	2	0	2
- with all stillborn [n]	0	0	0	0
Pups delivered [n]	306	300	318	315
- per dam [mean n]	12.2	12.5	12.7	12.6
- liveborn [n]	305	294	318	313
- stillborn [n]	1	6	0	2
- **Live birth index** [%]	100	98	100	99

F. PUP DATA

1. Survival

The mean number of delivered F_1 pups per dam and the rates of liveborn and stillborn F_1 pups were evenly distributed about the groups. The respective values reflect the normal range of biological variation inherent in the strain used in this study.

The viability index indicating pup mortality during early lactation (PND 0-4) varied between 97% (control), 98% (group 1), and 99% (groups 2 and 3).
The lactation index indicating pup mortality on PND 4-21 varied between 49% (group 1), 50% (group 3), 54% (control) and 55% (group 2). The lowered lactation index in all test groups is caused by the fact, that selected pups were sacrificed for blood sampling on PND 7, 14 and 21.
Thus, the test substance did not influence pre-weaning pup survival in any of the treated groups.

Table 5.6.1-31: Summary of litter data

Pup generation	F_1			
Dose [ppm]	0	10/5	20/10	50/25
Number of litters	25	24	25	25
- with liveborn pups	25	24	25	25
- with stillborn pups	1	2	0	2
Pups liveborn [n]	305	294	318	313
Pups stillborn [a] [n]	1	6	0	2
Pups died [n]	5	6	3	1
Pups cannibalized [n]	4	1	1	4
Pups culled day 4 [n]	98	105	114	110
Pups day 4 - pre-cull [n]	296	287	314	309
- Viability index [%]	97	98	99	99
Pups day 4 - post cull [n]	198	182	200	199
Pups day 21 [n]	106	90	109	99
- Lactation index [%	54	49	55	50
Sex ratio [% live males]				
- Day 0	48.9	54.8	45.9	48.6
- Day 21	48.1	56.7	45.0	44.4
Male pup weight [g]				
- Day 1 [g]	6.4	6.3	6.5	6.5
- Day 4 - pre cull [g]	9.6	9.8	10.0	10.0
- Day 4 - post cull [g]	9.6	9.8	10.0	10.1
- Day 7 [g]	15.7	16.0	16.3	16.5
- Day 14 [g]	35.1	36.1	36.2	36.7
- Day 21 [g]	58.3	59.5	58.9	60.1
Male body weight gain				
- Day 4 to 21 [g]	48.6	49.8	48.9	50.3
Female pup weight [g]				
- Day 1 [g]	6.1	6.1	6.1	6.2
- Day 4 - pre cull [g]	9.3	9.6	9.5	9.6
- Day 4 - post cull [g]	9.3	9.6	9.6	9.6
- Day 7 [g]	15.2	15.7	15.7	15.7
- Day 14 [g]	34.2	35.4	35.2	35.6
- Day 21 [g]	55.7	56.8	56.3	57.0
Female body weight gain				
- Day 4 to 21 [g]	46.4	47.3	46.7	47.4

2. Sex ratio

The sex ratios at day 0 and 21 were not affected by treatment. All differences were within the historical control range and not indicative of a treatment-related effect [see Table 5.6.1-31]

3. Pup clinical observations /

The F_1 generation pups did not display any clinical signs until weaning.

4. Body weight

No test compound-related influence on F_1 pup body weights and pup body weight changes was noted for all test groups.

5. Pup necropsy findings

A few F_1 pups showed spontaneous findings at necropsy, such as post mortem autolysis, hemorrhagic thymus, dilated renal pelvis, hydroureter, hemorrhagic testis and small testis. These findings occurred without any relation to dosing and/or can be found in the historical control data at comparable or even higher incidences. None of the findings was considered to be related to the treatment.

Table 5.6.1-32: Incidence of gross necropsy observations in F_1 pups

Dose [ppm]	0	10/5	20/10	50/25
	F_1 pups			
Litters evaluated	25	24	25	25
Pups evaluated	302	299	317	311
- Live	301	293	317	309
- Stillborn	1	3	0	2
Post mortem autolysis	0	1	0	1
Hemorrhagic thymus	0	1	0	0
Dilated renal pelvis	6	5	4	13
Hydrouereter	1	0	0	0
Hemorrhagic testis	1	0	0	0
Small testis	1	0	1	0
Total pup necropsy observations - % affected pups/litter	2.7	2.6	1.5	4.5

G. CLINICAL CHEMISTRY AND HEMATOLOGY

F_0 generation (adults)
No treatment-related, adverse changes among hematological parameters as well as transferrin and iron levels were measured.
In males of test group 3 (50 ppm) absolute lymphocyte counts and also total white blood cell counts were higher compared to controls (lymphocytes represent the main fraction of white blood cells). This finding was isolated among the differential blood cell counts. No other hematological parameter was changed in these rats. Therefore, high lymphocyte counts in rats of this test groups were regarded as incidental.

In female rats of test groups 1 and 3 (10 and 50 ppm) absolute neutrophil counts were increased, and female high dose group additionally had high absolute eosinophil counts, without any significant alteration of total white blood cell counts in these animals. The neutrophil counts were not dose-dependently changed and the means were within the historical control range and the absolute eosinophil counts were only marginally above the historical range. Therefore, these alterations were regarded as incidental and not treatment-related.

F_1 pups 7 days pp
No treatment-related changes among hematological parameters as well as transferrin and iron levels were measured.

F_1 pups 14 days pp
No treatment-related changes among hematological parameters as well as transferrin and iron levels were measured.
In female pups of test groups 1 and 3 (10 and 50 ppm) relative lymphocyte counts were decreased and relative neutrophil counts were increased. Additionally, in pups of test group 3 absolute neutrophil counts were higher compared to controls. The mentioned differential blood cell counts were not changed dose-dependently. Total white blood cell (WBC) counts in these pups were not affected. No change in the hematology parameters in pups 7 days pp as well as 21 days pp was measured. Therefore, the described alterations were regarded as incidental and not treatment-related.
At day 14 pp, in all dosed male pups serum transferrin values were higher compared to controls. The values were not dose-dependently altered and they were not changed in pups at day 7 pp and 21 pp. Therefore, this change was regarded as incidental and not treatment-related.

F_1 pups 21 days pp
No treatment-related changes among hematological parameters as well as transferrin and iron levels were measured.

III. CONCLUSIONS

Under the conditions of the present enhanced one-generation reproduction toxicity study the NOAEL for the F_0 parental rats for general, systemic toxicity is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.
The NOEL for fertility and reproductive performance for the F_0 parental rats is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.
The NOEL for developmental toxicity in the F_1 progeny is at least 50 ppm (about 4.3 mg/kg bw/day), the highest dose tested.

Overall conclusion on reproduction toxicity studies with dimoxystrobin and justification that the classification of dimoxystrobin for developmental toxicity is not warranted

Taking into account the results of all three reproduction toxicity studies with dimoxystrobin it is proposed that:

The overall NOAEL for reproductive performance and fertility for the F0 and F1 parental animals is 1200 ppm (136 mg/kg bw/day), the highest dose tested in the 2-generation and the modified 1-generation study.
The overall NOAEL for general parental toxicity is 150 ppm (17 mg/kg bw/day) based on reduced food consumption and body weights at 500 ppm and 1200 ppm in the 2-generation study. This is supported by the slight changes in hematological parameters seen at 150 ppm of the modified one-generation reproduction toxicity study. These slight changes indicate a beginning treatment-related anemia, but were not considered to be adverse.
The overall NOAEL for developmental toxicity is 50 ppm (about 5 mg/kg bw/day in adults; estimated value about 12 mg/kg bw/day for pups) based on reduced pup body weights at 150 ppm in F2 pups (about 17 mg/kg bw/day for adults; about 30-36 mg/kg bw/day corrected for pups). Benchmark dose modelling shows more accurate “NOAELs” for effects on body weights in (F2) pups compared to F1 adult females.
The $BMDL_{05}$ (F1 females, PND 21): 25.5 – 45.3 mg/kg bw (using measured substance intakes in different phases of lactation), the **$BMDL_{05}$ (F2 pups): 39.8 mg/kg bw** (using estimated substance intakes for PND 21).
The changes in pup body weights are not seen at birth, they only develop over time and are most pronounced at lactation day 14-21. There is evidence of slight anemia in pups at PND 21 at ≥ 150 ppm in the modified one-generation study. At ≥ 500 ppm increased heart weights (correlated to cardiomegalies detected at the next higher dose) are observed in pups. At the top dose cardiomegalies and liver discoloration were in PND 21 pups, which are related to offspring anemia, seen at the same doses. The mentioned findings in offspring are clearly not a consequence of in-utero exposure to dimoxystrobin, but are a consequence of a direct dimoxystrobin toxicity. The apparently more severe effects in pups (pup body weight effects in F2 pups seen at 150 ppm; higher effects on hematological parameters in the modified one-generation toxicity study) are related to a considerably higher compound intake of pups at the same dietary dose levels.

Dimoxystrobin has currently a legal classification for developmental toxicity Repro. Cat. 2, H361d. The observed effects on body weights, hearts (cardiomegaly) and blood (anemia) in the offspring of the reproduction toxicity studies constituted the basis for the agreed decision on this classification. Furthermore, a higher susceptibility of the offspring compared to the adults was assumed. A detailed evaluation on the findings in the reproduction toxicity studies with dimoxystrobin has been presented in this chapter and the effects considered for classification are summarized below showing that the classification with Repr. Cat. 2 (H361d) is not justified.

Dimoxystrobin caused decreased body weight developments in pups and dams in the reproductive toxicity studies. There were no effects on pup body weights seen at birth, therefore, an in utero effect can be excluded. The effects only develop over time, being most pronounced at PND 14 and 21 after the start of self-feeding. The calculated dimoxystrobin doses of the pups are considerably higher compared to the dams at the same dietary dose levels. Comparing more accurately calculated doses between pups and dams, effects on pup body weight development occur at higher (F1 litters) or roughly the same doses (F2 litters) than in dams. Benchmark dose calculations show that the no effect levels (= PODs) for body weight effects between dams (25.5-45.3 mg/kg bw; using measured substance intakes during lactation) and pups (about 39.8 mg/kg bw; using estimated pup intakes at PND21) are essentially comparable.
Potential additional exposures via dimoxystrobin contents in the milk would further increase the estimated pup exposures. As the body weight effects only occur during lactation (increasing in severity, when pups start self-feeding) and are not evident at birth, a developmental effect can be excluded.

Iron-deficiency anemia occurred in dams and offspring animals of the reproduction toxicity studies at the same dietary dose levels (≥ 150 ppm). The susceptibility of the pups to develop an anemia is not higher compared to the adults, as the iron deficiency is the first event to occur after dimoxystrobin treatment an identical maternal and offspring NOAEL of 50 ppm was determined in the new enhanced one-generation study. Further a NOAEL of roughly 4 mg/kg bw was determined for changes in serum iron levels in a 7-day mechanistic study in 3-week old animals (see MCA 5.8.2, BASF DocID 2010/1026748). This is the same NOAEL than previously determined and agreed for adult rats (BASF DocID 2002/1014345). No evidence for a higher susceptibility of young animals to develop iron deficiency or anemia after dimoxystrobin exposure compared to adults. A classification for reproduction toxicity is not justified.

Cardiomegaly, which occurs secondary to offspring anemia at the top dose of 1200 ppm, was seen in PND 21 pups (not in PND 4 pups). As no cardiomegaly was observed in F1 adult animals, the effect can be regarded as transient. Therefore, classification for reproduction toxicity based on cardiomegaly is not justified.

The high dose effects on reproductive and developmental indices in the 2-generation study with dimoxystrobin described in the respective chapter were not consistent between the first and the second generation and can conclusively be explained by data compiled from historical control data. Overall, all the effects on reproductive and developmental parameters can be regarded as incidental and not treatment-related.

In conclusion, effects seen in the reproductive toxicity studies with dimoxystrobin were direct effects of dimoxystrobin exposure after the start of self-feeding of the offspring animals (pup body weights, anemia, cardiomegaly, which occurs secondary to offspring anemia). Moreover, no higher susceptibility in young animals could be detected with regard to effects on pup body weights (actual doses, not dietary concentrations, at which maternal and offspring effects occur are comparable) and on serum iron levels and anemia (clear NOAELs detected for young and adult animals).

As a conclusion a comparison with the CLP criteria (Guidance to Regulation (EC) No 1272/2008; 2013) is made to show, that dimoxystrobin is not to be classified with Repr. Cat. 2, H361d. According to the CLP Classification criteria (3.7.2.2) Repr. Cat. 2 is defined as:
“Suspected human reproductive toxicant.
Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.“ (Annex I: 3.7.2.1.1)

The following main adverse effects of dimoxystrobin were identified and considered as critical for the classification decision:

1) Microcytic hypochromic anemia
2) Reduced body weights of the offspring
3) Cardiomegaly in the offspring

Dimoxystrobin reduces the iron uptake and thus leads to the development of an iron deficiency microcytic hypochromic anemia in both parental and offspring animals, with no higher susceptibility of the offspring. This is its general toxic effect and not a specific developmental effect.
It is known that reduced availability of iron leads to depressed body weight development in growing animals. Transient cardiomegaly is a consequence of hypoxia caused by the anemia in pups. Therefore, the effects on body weights and the heart are observed only in the presence of other toxic effects (iron deficiency/anemia). These effects are considered secondary, specific consequences of the anemia. Thus, a classification for developmental toxicity is not warranted. A document providing a comprehensive justification for a proposed re-classification of dimoxystrobin is submitted with this dossier (BASF DocID 2015/1152529).

CA 5.6.2 Developmental toxicity studies

Note: The developmental toxicity studies in rats (BASF DocID 1999/11680) and rabbits (BASF DocID 2000/1016867, 2001/1016351) were already submitted and reviewed in the course of the previous Annex I registration of dimoxystrobin.

BASF DocID 1999/11680: Developmental toxicity study with dimoxystrobin in rats (Study presented in the original Annex I Dossier):

Executive summary:

Dimoxystrobin was administered by oral gavage to groups of 25 presumably pregnant rats at dose levels of 0, 60, 120 and 300 mg/kg bw/day during days 6 to 19 of gestation. Maternal toxicity was observed at dose levels ≥ 120 mg/kg as evident by reduced food consumption, body weights and body weight gain during certain intervals of the treatment period. Additionally, reduced corrected body weight gains were noted.
There were no treatment-related effects on cesarean section parameters. There were no external malformations/variations or visceral malformations. A low number of skeletal malformations was observed in the low and high dose groups. Neither the incidence nor the type or inter-group distribution of malformations indicated a relation to treatment. Examination of fetuses for visceral and skeletal variations revealed no statistically significant differences between treated groups and the control group. Furthermore, the incidences either displayed no dose-response relationship or were within the historical control range.
Based on the findings of this study the maternal NOAEL was determined at 60 mg/kg bw/day. The NOAEL for developmental toxicity was the highest dose tested (300 mg/kg bw/day).

Materials and Methods:
Dimoxystrobin (batch/purity: N 6 Lot 3004: 98.8%) was tested for its prenatal developmental toxicity in Wistar rats. The test substance was administered as an aqueous suspension to 25 mated female rats/group by stomach tube at doses of 0, 60, 120 and 300 mg/kg bw/day on day 6 through day 19 post coitum (day 0 = detection of sperm). A standard dose volume of 10 ml/kg body weight was used for each group. The control group was dosed with the vehicle only (0.5 % Tylose CB 30.000 in doubly distilled water).

Results:

Please note: Only pregnant dams were used for the calculations of mean maternal food consumption, body weight and body weight change. Only pregnant dams with scheduled sacrifice on day 20 p.c. were taken for the calculation of mean gravid uterine weights, mean net maternal body weight change (corrected body weight gain) and summary of reproduction data. For the above reasons the following females were excluded from the above-mentioned calculations:

- Control: none
- Low dose (60 mg/kg): females #33 (not pregnant)
- Mid dose (120 mg/kg): females #54 (not pregnant), and #75 (found dead)
- High dose (300 mg/kg); female #89 (sacrificed moribund, not pregnant)

A. TEST SUBSTANCE ANALYSES

The stability of the aqueous test substance suspensions over a period of 4 hours at room temperature could be demonstrated.

B. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related clinical signs nor any disturbance of the general behavior were observed throughout the study.
Mid dose dam (#75) and high dose dam (#89) showed apathy and lateral position prior to death respectively sacrifice in moribund state.

2. Mortality

No substance-related mortalities were observed in this study

One mid dose dam (#75) was found dead on day 13 p.c. and one high dose dam (#89) was sacrificed moribund on day 16 p.c. The gross pathological examinations of both animals revealed findings indicative for misgavage

C. BODY WEIGHT AND FOOD CONSUMPTION

1. Food consumption

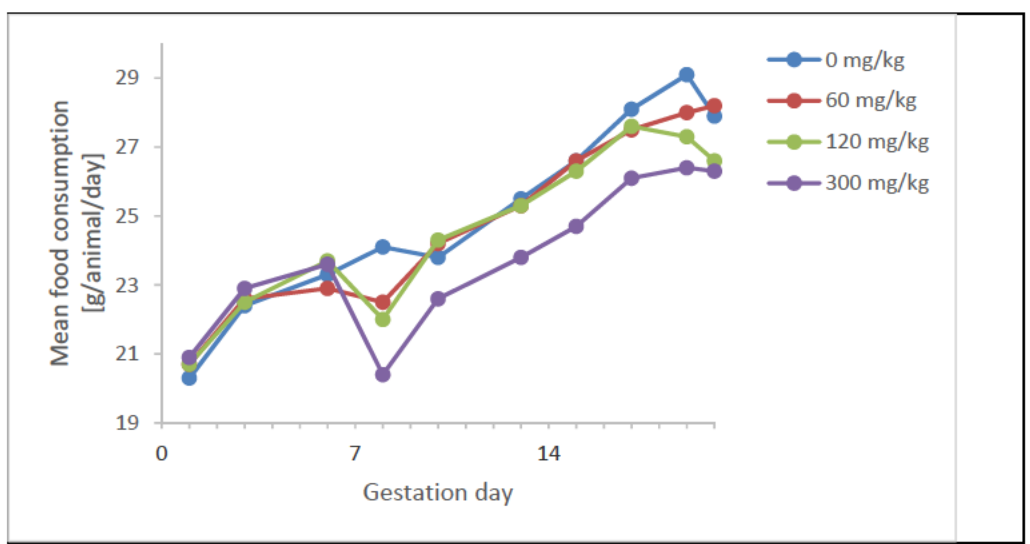
The mean food consumption of the high dose (300 mg/kg) dams was statistically significantly reduced on several days of treatment (Day 6-8 and 10-19 p.c.); if calculated for the entire treatment period (Day 6-19 p.c.) these dams consumed about 8% less food than the concurrent control.
Food consumption in the mid dose group (120 mg/kg) was statistically significantly reduced at initiation of dosing (Day 6-8 p.c.); no relevant deviation if calculated for the entire treatment period.
These changes were considered treatment-related as they were corroborated by a treatment-related impairment of body weight gain [see Table 5.6.2-1 and Table 5.6.2-2, and Figure 5.6.2-1].
Food consumption of low dose dams did not show any difference in food consumption compared to concurrent controls [see Table 5.6.2-1].

2. Body weight and body weight gain

No statistically significant differences of absolute body weights were noted for the substance-treated groups during the administration period [see Table 5.6.2-1]. A statistically significant decrease of body weight gain was observed at the high dose group immediately following commencement of treatment (Day 6-8). If calculated for the entire treatment period body weight gains were about 10% lower compared to concurrent control. A statistically significant decrease of body weight gain of about 12% was observed at the mid dose over the whole treatment period (Day 6-19). These changes in the mid and high dose group were considered to be treatment-related as they were consistent with the effects on food consumption and the lower corrected body weight gain at this dose level.

Body weight gains of the females of the low dose groups were similar to those of the concurrent control.

Figure 5.6.2-1: Food consumption and body weight development in rats administered dimoxystrobin during days 6 to 19 of gestation



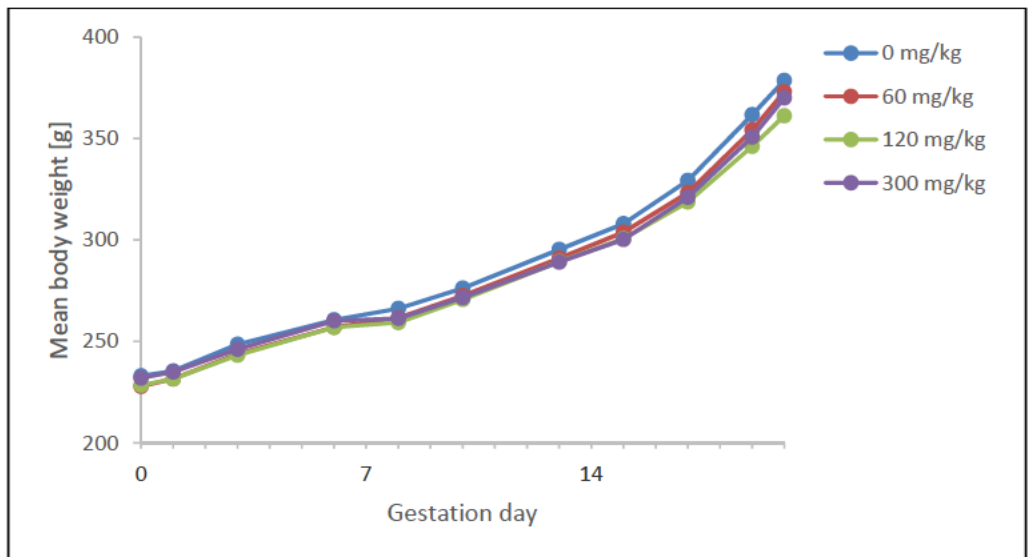


Table 5.6.2-1: Food consumption and body weight development in rat administered dimoxystrobin during days 6 to 19 of gestation

Dose level [mg/kg bw/day]	0	60	120	300
Food consumption [g/animal/day]				
Day 0 to 6 Δ%	22.0 ± 1.6	22.1 ± 1.2 0.5	22.3 ± 1.5 1.4	22.5 ± 1.4 2.3
Day 6 to 19 Δ%	26.2 ± 2.1	25.7 ± 2.1 -1.9	25.5 ± 2.1 -2.7	24.0 ± 2.3 -8.4
Day 0 to 20 Δ%	25.1 ± 2.8	24.9 ± 2.6 -0.8	24.6 ± 2.4 -2.0	23.8 ± 2.1 -5.2
Body weight [g]				
Day 0 Δ%	233.0 ± 8.1	227.8 ± 9.1 -2.2	228.4 ± 7.8 -2.0	231.9 ± 8.1 -0.5
Day 6 Δ%	260.6 ± 10.2	257.0 ± 9.6 -1.4	256.9 ± 12.2 -1.4	260.1 ± 9.7 -0.2
Day 19 Δ%	361.7 ± 20.1	354.2 ± 18.4 -2.1	346.1 ± 27.9 -4.3	350.7 ± 22.1 -3.0
Day 20 Δ%	378.6 ± 22.9	373.0 ± 21.7 -1.5	361.2 ± 32.9 -4.6	370.0 ± 23.1 -2.3
Body weight gain [g]				
Day 0 to 6 Δ%	27.6 ± 6.2	29.2 ± 6.6 5.8	28.5 ± 7.3 3.3	28.2 ± 5.1 2.2
Day 6 to 19 Δ%	101.1 ± 13.5	97.2 ± 12.8 -3.9	**89.2 ± 21.4*** -11.8	90.7 ± 16.2 -10.3
Day 0 to 20 Δ%	145.6 ± 21.4	145.2 ± 18.8 -0.3	132.8 ± 29.6 -8.8	138.1 ± 19.8 -5.2

* p < 0.05, ** p < 0.01 (Dunnett test, two-sided)

D. NECROPSY OBSERVATIONS

1. Gravid uterus weight, carcass weight and corrected (net) body weight gain

Gravid uterus weights were comparable between control and treated groups. The differences between the groups did not reveal dose-dependency. A treatment-related statistically significant decrease of net body weight gain was observed at 300 mg/kg. Furthermore, the corrected body weight gain at 120 mg/kg dose group was slightly decreased without statistical significance. The effects were considered to be treatment-related direct signs of maternal toxicity as they were in line with the decrease in food consumption and body weight gain. The carcass weight of the high dose group was slightly but not statistically significantly reduced [see Table 5.6.2-2].

No treatment related effects were noted at the low dose of 60 mg/kg.

Table 5.6.2-2: Mean gravid uterus weights and net body weight change of pregnant rats administered dimoxystrobin during Days 6 to 19 of gestation

Dose level [mg/kg bw/d]	0	60	120	300
Gravid uterus (g)	81.1 ± 14.5	78.9 ± 12.4	70.0 ± 24.6	83.0 ± 9.8
Carcass (g)	297.4 ± 15.0	294.0 ± 15.7	291.2 ± 17 8	286.9 ± 15.7
Net weight change from Day 6 (g)	36.8 ± 7.8	37.0 ± 9.3	34.3 ± 9.0	**26.9 ± 10.8****

* p < 0.05, ** p < 0.01 (Dunnett test, two-sided)

2. Gross necropsy observations

No treatment-related findings were observed. All findings were observed in single animals only without any relation to treatment. Edema and/or marginal emphysema of the lung were associated with the method of the females sacrifice. Findings like perforation of upper oesophagus and/or acute fibrinous pleuritic, pericarditis and exsudate in the thoracic cavity were typical for misgavaging [see Table 5.6.2-3].

Table 5.6.2-3: Gross necropsy findings in rats administered dimoxystrobin during days 6 to 19 of gestation

Dose group	Animal #	Observation
Control	-	-
60 mg/kg bw/day	-	-
120 mg/kg bw/day	67	Lung: edema/marginal emphysema
	54	Bilateral hydrometra
	75	Findings after gavage error
300 mg/kg bw/day	82	Lung: Edema
	89	Findings after gavage error

E. CESAREN SECTION DATA

25, 24, 24 and 25 females were pregnant at 0, 60, 120 and 300 mg/kg [see Table 5.6.2-4]. None of the pregnant dams aborted, or gave premature birth. One mid dose dam had all fetuses resorbed. This was considered incidental.

There were no treatment-related differences in the number of corpora lutea, implantation sites, pre- and post-implantation losses, resorptions, number of live fetuses between controls and treated groups [see Table 5.6.2-4]. The statistically significant higher post-implantation loss at the mid dose level (120 mg/kg) was due to the higher rate of early resorptions. However, this was mainly caused by one female #72, which had only six early resorptions but no viable fetuses. Due to the isolated occurrence and the lack of dose-dependency this finding was considered incidental. Placental and fetal weights were comparable between all groups and not affected by treatment [see Table 5.6.2-4]

Table 5.6.2-4: Caesarean section data

Dose level [mg/kg bw/d]		0	60	120	300
Pregnancy status					
- mated	[n]	25	25	25	25
- pregnant	[n]	25	24	24	24
conception rate	[%]	100	96	96	96
- aborted	[n]	0	0	0	0
- premature birth	[n]	0	0	0	0
- dams with viable fetuses	[n]	25	24	22	24
- dams with all resorptions	[n]	0	0	1	0
- mortality		0	0	0	0
- pregnant terminal sacrifice	[n]	25	24	23	24
Cesarean section data[a]					
- Corpora lutea	[n]	15.8	15.5	15.0	15.3
total number	[n]	394	372	346	367
- Implantation sites	[n]	14.8	14.7	13.3	14.5
total number	[n]	370	353	307	348
- Pre-implantation loss	[%]	6.4	5.2	12.2	5.0
- Post-implantation loss	[%]	5.2	8.6	14.7*	3.7
- Resorptions	[n]	0.8	1.3	1.5	0.5
total number	[n]	21	30	35	13
- Early resorptions		0.8	1.2	1.3	0.5
total number	[n]	20	28	30	13
- Late resorptions		0.0	0.1	0.2	0.0
total number	[n]	1	2	5	0
- Dead fetuses	[n]	0	0	0	0
- Dams with viable fetuses	[n]	25	24	22	24
- Live fetuses		14.0	13.5	12.4	14.0
total number	[n]	349	323	272	335
- Total live female fetuses	[n]	6.1	6.5	5.9	6.8
total number	[n]	153	156	129	164
Mean	[%]	41.7	43.4	43.3	47.0
- Total live male fetuses	[n]	7.8	7.0	6.5	7.1
total number	[n]	196	167	143	171
Mean	[%]	53.2	48.0	45.9	49.4
- Percent live females		43.8	48.3	47.4	49.0
- Percent live males		56.2	51.7	52.6	51.0
Placental weights	[g]	0.44	0.44	0.45	0.45
- male fetuses	[g]	0.44	0.45	0.45	0.46
- female fetuses	[g]	0.43	0.43	0.45	0.44
Mean fetal weight	[g]	3.9	3.9	3.9	4.0
- males	[g]	3.9	4.0	4.0	4.1
- females	[g]	3.8	3.9	3.8	3.9

[a] Mean ± SD on litter basis; Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett-test, two-sided)

F. EXTERNAL, VISCERAL AND SKELETAL EXAMINATION OF FETUSES

1. External examination

No external malformations or variations were found in in any fetuses of any group [see Table 5.6.2-5]. One unclassified observation, fused placentae with one of the neighbored littermates or resorptions, appeared in one control (#4 of dam #16), one mid dose (#2 of dam #55) and three high dose fetuses (#2 of dam #77, #11 and 13 of dam #86). The scattered occurrence of this one finding dams not suggest any treatment-relationship.

Table 5.6.2-5: Incidence of external malformations and variations

Dose level [mg/kg]	0	60	120	300
Litters Evaluated	25	24	22	24
Fetuses Evaluated	349	323	272	335
Live	349	323	272	335
Dead	0	0	0	0
Total external malformations				
- Fetal incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Litter incidence [a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ±0.0
Total external variations				
- Fetal incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00
Total external unclassified observations				
- Fetal incidence [No. (%)]	1 (0.3)	0 (0.0)	1 (0.4)	3 (0.9)
- Litter incidence [No. (%)]	0 (4.0)	0 (0.0)	1 (4.5)	2 (8.3)
- Affected fetuses/litter (Mean ± SD) [%]	0.2 ±1.18	0.0 ±0.00	0.3 ±1.52	0.9 ±3.15
Individual external unclassified observations				
- fused placenta				
- Fetal incidence [No. (%)]	1 (0.3)	0 (0.0)	1 (0.4)	3 (0.9)
- Litter incidence [No. (%)]	0 (4.0)	0 (0.0)	1 (4.5)	2 (8.3)
- Affected fetuses/litter (Mean ± SD) [%]	0.2 ±1.18	0.0 ±0.00	0.3 ±1.52	0.9 ±3.15

2. Visceral examination

No treatment-related visceral (soft tissue) malformations were noted in this study in any of the fetuses [see Table 5.6.2-6].
Soft tissue variations, namely dilated renal pelvises and dilated ureter, were found in all dose groups including the control. Since these findings were not statistically significant, not dose-dependent and represent very frequent findings in the rat strain a treatment-related origin was excluded. The mean percentage of affected fetuses/litter with total visceral variations (21.0, 17.4, 31.3, 28.9% for the control, 60, 120, and 300 mg/kg bw/day dose groups respectively) was fully within the historical control range (5.0-33.3%) for all dose groups [see Table 5.6.2-6].

Table 5.6.2-6: Incidence of visceral (soft tissue) malformations and variations

Dose level [mg/kg]	0	60	120	300
Litters Evaluated	25	24	22	24
Fetuses Evaluated	166	156	132	160
Live	166	156	132	160
Dead	0	0	0	0
Total visceral malformations				
- Fetal incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Litter incidence [a]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00
Total visceral variations				
- Fetal incidence [No. (%)]	34 (20)	26 (17)	36 (27)	46 (29)
- Litter incidence [No. (%)]	20 (80)	15 (63)	20 (91)	20 (83)
- Affected fetuses/litter (Mean ± SD) [%]	21.0 ±15.35	17.4 ±18.42	31.3 ±24.43	28.9 ±23.75
Individual visceral variations				
- Dilated renal pelvis				
- Fetal incidence [No. (%)]	34 (20)	26 (17)	36 (27)	46 (29)
- Litter incidence [No. (%)]	20 (80)	15 (63)	20 (91)	20 (83)
- Affected fetuses/litter (Mean ± SD) [%]	21.0 ±15.35	17.4 ±18.42	31.3 ±24.43	28.9 ±23.75
- Dilated ureter				
- Fetal incidence [No. (%)]	2 (1.2)	1 (0.6)	4 (3.0)	6 (3.8)
- Litter incidence [No. (%)]	2 (8.0)	1 (4.2)	4 (18)	3 (13)
- Affected fetuses/litter (Mean ± SD) [%]	1.2 ±4.30	0.7 ±3.40	2.6 ±5.73	4.0 ±13.95

No unclassified visceral findings were noted.

3. Skeletal examination

No treatment-related skeletal malformations were noted in this study. Low incidences of skeletal malformations occurred in the low and high dose group [see Table 5.6.2-7].
Absent lumbar vertebra, misshapen scapula, malpositioned and bipartite sternebra, absent and/or fused ribs occurred in 3 low and 3 high dose fetuses. The mean percentage of affected fetuses/litter with skeletal malformations amounted to 0.0, 1.6, 0.0, 1.7% for the control, low, mid and high dose group respectively. All of the skeletal malformations occurred without a dose-relationship, can be found at a comparable frequency in the historical control data range for skeletal malformations (0.0-3.1%), and were therefore assessed as incidental.

Table 5.6.2-7: Incidence of skeletal malformations

Dose level [mg/kg]	**0**	**60**	**120**	**300**
Litters Evaluated	25	24	22	24
Fetuses Evaluated	183	167	140	175
Live	183	167	140	175
Dead	0	0	0	0
Total skeletal malformations				
- Fetal incidence [No. (%)]	0 (0.0)	3 (1.8)	0 (0.0)	3 (1.7)
- Litter incidence [a]	0 (0.0)	3 (13)	0 (0.0)	2 (8.3)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	1.6 ±4.48*	0.0 ±0.00	1.7 ±6.26
Individual skeletal malformations				
- **Absent lumbar vertebra**				
- Fetal incidence [No. (%)]	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
- Litter incidence [No. (%)]	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	0.5 ±2.27	0.0 ±0.00	0.0 ±0.00
- **Misshapen scapula**				
- Fetal incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.5 ±2.55
- **Malpositioned and bipartite sternebra**				
- Fetal incidence [No. (%)]	0 (0.0)	3 (1.8)	0 (0.0)	1 (0.6)
- Litter incidence [No. (%)]	0 (0.0)	3 (13)	0 (0.0)	1 (4.2)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	1.6 ±4.48*	0.0 ±0.00	0.6 ±2.92
- **Fused rib**				
- Fetal incidence [No. (%)]	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
- Litter incidence [No. (%)]	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	0.5 ±2.27	0.0 ±0.00	0.0 ±0.00
- **Absent rib**				
- Fetal incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)
- Litter incidence [No. (%)]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	0.0 ±0.00	0.0 ±0.00	0.6 ±2.92

* p ≤ 0.05, ** p ≤ 0.01 (Fisher's exact test, one-sided; Wilcoxon-test, one-sided)

Table 5.6.2-8: Incidence of skeletal variations

Dose level [mg/kg]	0	60	120	300
Litters Evaluated	25	24	22	24
Fetuses Evaluated	183	167	140	175
Live	183	167	140	175
Dead	0	0	0	0
Total skeletal variations				
- Fetal incidence [No. (%)]	143 (78)	132 (79)	113 (81)	132 (75)
- Litter incidence [No. (%)]	25 (100)	24 (100)	22 (100)	24 (100)
- Affected fetuses/litter (Mean ± SD) [%]	78.0 ±18.92	78.6 ±15.18	79.7 ±18.73	75.6 ±17.25
Selected individual skeletal variations				
- Unossified sternebra – unchanged cartilage				
- Fetal incidence [No. (%)]	8 (4.4)	11 (6.6)	10 (7.1)	15 (8.6)
- Litter incidence [No. (%)]	4 (16)	8 (33)	8 (36)	9 (38)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 4.6-11.6%)*	4.2 ±10.65	6.5 ± 10.38	6.9 ±11.91	8.3 ± 13.22
- Short rib (13th) – cartilage not present				
- Fetal incidence [No. (%)]	19 (10)	26 (16)	29 (21)	35 (20)
- Litter incidence [No. (%)]	12 (48)	14 (58)	12 (55)	15 (63)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 7.7-26.4%)*	9.9 ±13.17	15.2 ± 15.80	19.7 ±23.96	19.3 ± 22.62
- Cervical Rib – cartilage not present				
- Fetal incidence [No. (%)]	2 (1.1)	3 (1.8)	0 (0.0)	5 (2.9)
- Litter incidence [No. (%)]	2 (8.0)	3 (13)	0 (0.0)	3 (13)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0.0-4.1%)*	0.9 ±3.08	1.8 ± 4.81	0.0 ± 0.00	3.0 ± 8.55

Skeletal variations were observed with a high incidence of > 75% of the fetuses and in all litters [see Table 5.6.2-8].
The observed variations were related to skull (bipartite ossification of supraoccipital bone, supraoccipital holes, extra ossification site between parietal and interparietal bones, incomplete ossification of parietal, interpartietal, supraoccupital, and/or the total skull bones), the ventral column (incomplete or missing ossification of thoracic and/or lumbar vertebra), the ribs (short 13th, supernumary 14th or cervical ribs), and the sternum (misshapen, fused or bipartite sternebra, extra sternebral ossification site, incomplete or missing ossification of sternebra).
The mean percentage of affected fetuses/litter with skeletal variations amounted to 78.0, 78.6, 79.7, and 75.6% at 0, 60, 120, or 300 mg/kg be/day.
No statistically significant differences were observed between control and treated groups. The variations were observed without dose response-relationship or at incidences comparable to the historical control range. Therefore, these skeletal variations were not considered to be related to treatment.

Some isolated unclassified observations were observed in all groups including the control in this study. They were related to the vertebral column, the ribs and the sternum. As they were found in similar incidences in all dose groups, and did not show any relation to dosing (mean percentage of affected fetuses/litter 15.6, 10.6, 16.0, 10,9% at 0, 60, 120, 300 mg/kg) they were regarded incidental.

III. CONCLUSION

Based on the effects on maternal food consumption and body weight development the maternal NOAEL was 60 mg/kg bw/day. In absence of any treatment-related developmental effects the developmental NOAEL was 300 mg/kg bw/day.

BASF DocID 2000/1016867: Developmental toxicity study with dimoxystrobin in rabbits (Study presented in the original Annex I Dossier)

Executive summary:

Dimoxystrobin was administered daily to presumably pregnant Himalayan rabbits by stomach tube during gestation days 7-28 post insemination (p.i.) at dose levels of 0, 25, 50 and 100 mg/kg bw/day. A dose related maternal toxicity was noted. One high dose doe was found dead, while another high dose female was sacrificed after abortion. Both effects were considered to be treatment-related. Furthermore, a moderate to excessive reduction of food consumption and impaired body weight gain was seen in high dose females. Diarrhea was present in almost all high dose, 12 mid dose and two low dose does along with no defecation in several high dose animals. Most probably secondary to the massive maternal toxicity immediately after implantation an increased post implantation loss (mainly early resorptions) was noted at 100 mg/kg. This high dose was clearly too toxic to does.
No treatment-related effects on external or visceral (soft tissue) malformations or variations or skeletal variations was observed. There was a statistically significant increase in the skeletal variations fused and misshapen sternebra without a dose-response. The statistical significance in the incidence of fused sternebra was due to the low incidence of this finding in the concurrent control. At the highest dose level - where severe maternal toxicity was evident - the mean value % affected fetuses per litter was substantially influenced by the litter of one doe. The only fetus of this doe displayed fused sternebrae, which resulted in a 100% value of % affected fetuses per litter. Excluding this animal from calculation results in a value of 11.1% well covered within the extended historical control range. Therefore, the increased number of skeletal variations can be considered to be incidental.

Based on the findings in this study the maternal NOAEL was < 25 mg/kg bw/day, whereas the developmental NOAEL was 50 mg/kg bw/day.

Materials and Methods:
Dimoxystrobin (batch/purity: N 15: 98.4%) was tested for its prenatal developmental toxicity in Himalayan rabbits. The test substance was administered as an aqueous suspension to 25 inseminated female Himalayan rabbits/group by stomach tube at doses of 0, 25, 50 and 100 mg/kg bw/day on day 7 through day 28 post insemination (insemination = day 0). A standard dose volume of 10 ml/kg bw was used. The control group was dosed with the vehicle only (0.5% Tylose CB 30.000 in doubly distilled water).

Results:

Please note: Only pregnant does were used for the calculations of mean maternal food consumption, body weight and body weight change. Only pregnant does with scheduled sacrifice on GD 29 were taken for the calculation of mean gravid uterine weights, mean net maternal body weight change (corrected body weight gain) and summary of reproduction data.

For the above reasons the following females were excluded from the above-mentioned calculations:

- Control: none
- Low dose (25 mg/kg bw): none
- Mid dose (50 mg/kg bw) females #52 (not pregnant)
- High dose (100 mg/kg bw): females #80 (not pregnant), #90 (sacrificed after abortion) and #94 (died intercurrently)

A. TEST SUBSTANCE ANALYSES

The stability of the aqueous test substance suspensions over a period of 4 hours at room temperature could be demonstrated.

B. OBSERVATIONS

1. Clinical signs of toxicity

Treatment-related clinical signs in all dose groups consisted of diarrhea (24/25 high dose, 12/25 mid dose, 2/25 low dose), and no defecation (8/25 high dose showing previously diarrhea, 1 mid dose female without diarrhea) [see Table 5.6.2-9].
Blood in bedding was observed in 1 high dose rabbit.

Table 5.6.2-9: Clinical observations in rabbits administered dimoxystrobin during days 6 to 28 of gestation

Dose level [mg/kg]	Animal #	Gestation Day	Observation
Control	-	-	-
25 mg/kg	28	8	diarrhea
	37	8	diarrhea
50 mg/kg	51, 52, 54, 55, 58, 59, 60, 64, 68, 71, 74	7-8	diarrhea
	69	7-8	No defecation
100 mg/kg	All (except 99) 84, 85, 86, 87, 93, 96, 98, 100	7-10 7-8	diarrhea No defecation
	90	25-26 27	Blood in bedding; aborted

2. Mortality

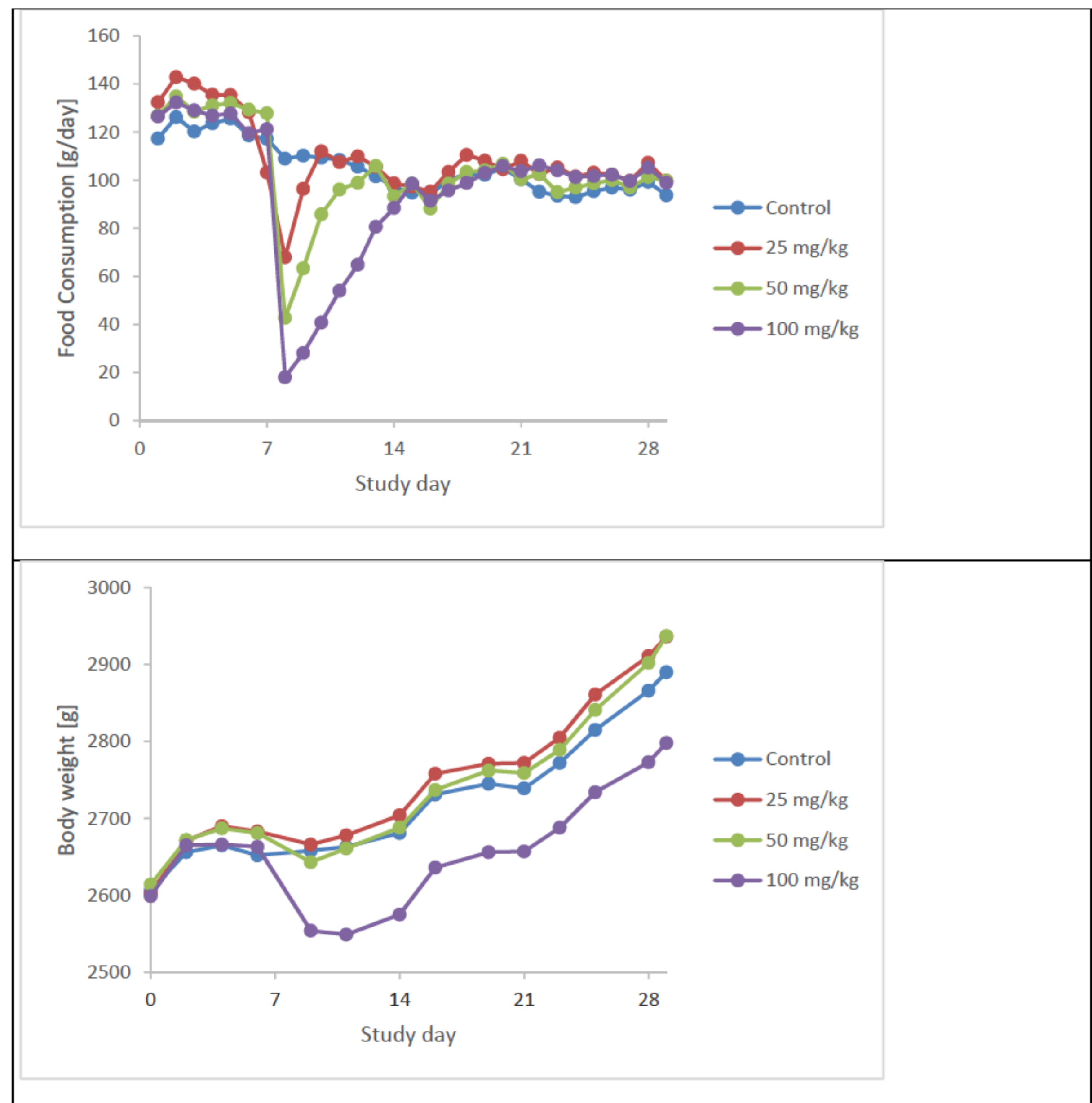
One high dose doe (#94) was found dead on day 8 p.i., while another high dose female (#90) was sacrificed after abortion on day 27 p.i.. Both effects were considered to be treatment-related.
There was no mortality in the control, low and mid dose groups (0, 25, and 50 mg/kg bw)

C. BODY WEIGHT AND FOOD CONSUMPTION

1. Food consumption

Instantly with the start of treatment at GD 7, a marked, statistically significant and treatment related decrease of food consumption was noted in all treated groups [see Table 5.6.2-10]. Mean food consumption fell to 62, 39 and 17% of the control for the day 7 to 8 interval at 25, 50, and 100 mg/kg, respectively. Thereafter and in a dose-dependent matter food consumption improved and reached control levels latest on GD 15. Calculated for the entire period high dose rabbits consumed about 15% less food, mid dose rabbits about 6% less food than concurrent controls.

Figure 5.6.2-2: Food consumption and body weight development in rabbits administered dimoxystrobin during days 7 to 28 of gestation



2. Body weight and body weight gain

Secondary to the temporary decrease of food consumption at initiation of treatment (days 7-9) a dose-dependent body weight loss was noted in all treated groups [Figure 5.6.2-2 and Table 5.6.2-10]. Absolut body weights did not show any statistically significant differences. For the gestation day interval 7-9 a statistically significant decrease of body weight gain was noted in the mid and high dose groups. Mean body weight gain during treatment was significantly decreased at the high dose of 100 mg/kg (about 40%) [see Table 5.6.2-10]. The impaired mean body weight gains were assessed as substance-related.

Table 5.6.2-10: Food consumption and body weight development in rabbit administered dimoxystrobin during days 7 to 28 of gestation

Dose level [mg/kg]	0	25	50	100
Food consumption [g/animal/day]§				
Day 0 to 7 Δ%	121.3	135.0 11.3	130.2 7.3	126.1 4.0
Day 7 to 28 Δ%	100.4	102.2 1.8	94.1 -6.3	85.3 -15.0
Day 0 to 29 Δ%	105.4	110.0 4.4	103.0 -2.3	95.6 -9.3
Body weight gain [g]				
Day 0 to 7 Δ%	46.3	81.3 75.6	67.2 45.1	64.2 38.7
Day 7 to 28 Δ%	213.3	227.6 6.7	221 3.6	**127*** -40.5
Day 0 to 29 Δ%	283.4	334.2 17.9	322.6 13.8	214.1 -24.5

* $p \leq 0.05$, ** $p \leq 0.01$ (Dunnett-test, two sided)

D. NECROPSY OBSERVATIONS

1. Corrected (net) body weight gain, carcass and gravid uterus weights

No treatment-related effects on carcass and corrected (net) body weight gain were observed at any dose level [see Table 5.6.2-11]. The mean gravid uterus weight of the high dose animals was decreased (about 81% of that of the control) without attaining statistical significance. The finding was in line with the increased resorption rate.

Table 5.6.2-11: Mean gravid uterus weights and net body weight change of pregnant rabbits administered dimoxystrobin during Days 7 to 28 of gestation

Dose level [mg/kg bw/d]	0	25	50	100
Gravid uterus (g)	310.5	336.8	353.1	252.6
Carcass (g)	2579.1	2599.1	2583.6	2545
Net weight change from Day 6 (g)	-73.4	-83.9	-97.7	-100.7

2. Gross necropsy observations

There occurred no substance-related necropsy findings in any of the rabbits. A number of gross necropsy findings were noted in all groups including control at Cesarian section [see Table 5.6.2-12]. Findings were either single observations or associated with the method of sacrifice and/or equally distributed over the treatment groups including the control. The most frequent findings were congestions, edema and marginal emphysema of/in lungs and congested livers. Neither the incidence nor the type of observations indicated a relation to treatment.

Table 5.6.2-12: Gross necropsy findings in rabbits administered dimoxystrobin during days 7 to 28 of gestation

Dose group	Animal #	Observation
Control	10	Lungs: edema, marginal emphysema
	14	Lungs: edema
	19	Lungs: edema
	20	Lungs: edema, marginal emphysema Liver: congested
	23	Lungs: edema, marginal emphysema
25 mg/kg bw/day	42	Lungs: edema, marginal emphysema
	44	Lungs: congested
	48	Lungs: edema, marginal emphysema
	49	Lungs: edema
	50	Lungs: edema, marginal emphysema
50 mg/kg bw/day	60	Liver: congested
	61	Lungs: edema, marginal emphysema
	64	Lungs: edema, marginal emphysema
	66	Lungs: edema
	68	Lungs: petechia
	69	Lungs: edema, marginal emphysema
	72	Lungs: edema, marginal emphysema
	74	Lungs: marginal emphysema
	75	Lungs: edema, marginal emphysema
100 mg/kg bw/day	76	Liver: necrosis
	82	Lungs: edema, marginal emphysema
	88	Lungs: edema
	90	Particular findings on implants in females which aborted
	92	Liver: congested
	94	Particular findings on implants in females died
	97	Liver: congested Lungs: congested, edema

E. CESAREN SECTION DATA

25, 25, 23 and 24 does were pregnant at 0, 25, 50 and 100 mg/kg [see Table 5.6.2-13]. As already indicated one high dose female died and one was sacrificed after abortion.

The number of corpora lutea, implantations and the pre-implantation loss was comparable between all groups.
Three high dose females (#84, 92, 97) had total resorptions. In addition, a non-statistically significant increase of the post-implantation loss was noted at the high dose. This was due to an increased number of early resorptions. The increase of early resorptions is considered to be secondary to the marked maternal toxicity observed at the high dose.

Table 5.6.2-13: Pregnancy status and caesarean section data of does treated with dimoxystrobin during days 7 to 28 of pregnancy

Dose level [mg/kg bw/d]	0	25	50	100
Pregnancy status				
Females				
- mated [n]	25	25	25	25
- pregnant [n]	25	25	23	24
conception rate [%]	100	100	92	96
- aborted [n]	0	0	0	1
- premature birth [n]	0	0	0	0
- females with viable fetuses [n]	25	25	23	19
- females with all resorptions [n]	0	0	0	3
- mortality	0	0	0	2
- pregnant terminal sacrifice [n]	25	25	23	22
Cesarean section data[a]				
- Corpora lutea [n]	8.0	7.9	8.5	8.0
total number [n]	200	198	196	177
- Implantation sites [n]	7.0	6.9	7.5	7.0
total number [n]	175	173	172	153
- Pre-implantation loss [%]	13.1	14.1	13.1	14.3
- Post-implantation loss [%]	14.2	5.0	6.6	27.5
- Resorptions [%]	14.2	5.0	6.6	27.5
number [n]	1.0	0.4	0.5	**2.2***
total number [n]	24	10	11	48
- Early resorptions [%]	11.6	2.7	5.2	25.7
number [n]	0.8	0.2	0.3	**2.0***
total number [n]	19	5	8	45
- Late resorptions [%]	2.6	2.3	1.5	1.8
number [n]	0.2	0.2	0.1	0.1
total number [n]	5	5	3	3
- Dead fetuses [n]	0	0	0	0
- Females with viable fetuses [n]	25	25	23	19
- Live fetuses	6.0	6.5	7.0	5.5
total number [n]	151	163	161	105
Mean [%]	85.8	95.0	93.4	84.0
- Total live female fetuses [n]	3.0	3.3	3.8	2.8
total number [n]	74	83	87	53
Mean [%]	40.7	47.7	50.8	41.6
- Total live male fetuses [n]	3.1	3.2	3.2	2.7
total number [n]	77	80	74	52
Mean [%]	45.1	47.3	42.6	42.4
- Percent live females	49.0	50.9	54.0	50.5
- Percent live males	51.0	49.1	46.0	49.5
Placental weights [g]	4.3	4.2	4.2	4.4
- male fetuses [g]	4.4	4.2	4.3	4.4
- female fetuses [g]	4.3	4.2	4.0	4.2

Table 5.6.2-13: Pregnancy status and caesarean section data of does treated with dimoxystrobin during days 7 to 28 of pregnancy

Dose level [mg/kg bw/d]	0	25	50	100
Pregnancy status				
Females				
- mated [n]	25	25	25	25
- pregnant [n]	25	25	23	24
conception rate [%]	100	100	92	96
- aborted [n]	0	0	0	1
- premature birth [n]	0	0	0	0
- females with viable fetuses [n]	25	25	23	19
- females with all resorptions [n]	0	0	0	3
- mortality	0	0	0	2
- pregnant terminal sacrifice [n]	25	25	23	22
Cesarean section data[a]				
Mean fetal weight [g]	38.0	38.5	37.8	38.1
- males [g]	38.2	38.6	38.1	38.4
- females [g]	37.9	38.3	37.2	37.6

[a] Mean ± SD on litter basis; Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett-test, two-sided)

The sex ratio as well as placental and fetal weights were not affected by treatment. No substance-related differences occurred between control, low and mid dose group animals.

F. EXTERNAL, VISCERAL AND SKELETAL EXAMINATION OF FETUSES

1. External examination

External malformations were recorded in each one low, mid and high dose fetus [see Table 5.6.2-14]. Low dose fetus 1 of female #33 displayed microphthalmia. Mid dose fetus 4 of female #67 had meningoencephalocele. High dose fetus 3 of female #83 had a short tail. Findings were regarded as incidental and not treatment-related.
External variations observed consisted of paw hyperflexion in single control, mid and high dose fetuses. Neither the incidence nor the type of external variations indicated a relation to treatment.
There appeared two unclassified external observations. Placenta necrobiotic combined with blood coagulum around placenta was observed in one control fetus 6 of female #4.

Table 5.6.2-14: Incidence of external malformations and variations

Dose level [mg/kg bw/day]	0	25	50	100
Litters Evaluated	25	25	23	19
Fetuses Evaluated	151	163	161	105
Live	151	163	161	105
Dead	0	0	0	0
Total external malformations				
- Fetal incidence [N (%)]	0 (0.0)	1 (0.6)	1 (0.6)	1 (1.0)
- Litter incidence [a]	0 (0.0)	1 (4.0)	1 (4.3)	1 (5.3)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	2.0 ±10.00	0.7 ±3.48	1.8 ±7.65
Total external variations				
- Fetal incidence [N (%)]	1 (0.7)	0 (0.0)	1 (0.6)	3 (2.9)
- Litter incidence [N (%)]	1 (4.0)	0 (0.0)	1 (4.3)	2 (11)
- Affected fetuses/litter (Mean ± SD) [%]	0.6 ±2.86	0.0 ±0.00	0.6 ±2.98	3.9 ±11.61
Individual external variations				
- Paw hyperflexion				
- Fetal incidence [N (%)]	1 (0.7)	0 (0.0)	1 (0.6)	3 (2.9)
- Litter incidence [N (%)]	1 (4.0)	0 (0.0)	1 (4.3)	2 (11)
- Affected fetuses/litter (Mean ± SD) [%]	0.6 ±2.86	0.0 ±0.00	0.6 ±2.98	3.9 ±11.61

( ) Values in brackets give % fetal respectively litter incidence

2. Visceral examination

A variety of visceral (soft tissue) malformations were observed in all groups including control [see Table 5.6.2-15]. The malformations occurred either singly, without dose response-relationship or at incidences comparable to the historical control range. The only statistically significant difference was observed for muscular ventricular septal defects in low dose fetuses. The incidence of this malformation was within the historical control range. All other findings were observed in single cases only. None of the findings was considered to be treatment-related.

Table 5.6.2-15: Incidence of visceral (soft tissue) malformations

Dose level [mg/kg]	**0**	**25**	**50**	**100**
Litters Evaluated	25	25	23	19
Fetuses Evaluated	151	163	161	105
Live	151	163	161	105
Dead	0	0	0	0
Total visceral malformations				
- Fetal incidence [No. (%)]	3 (1.3)	3 (1.8)	3 (1.9)	2 (1.9)
- Litter incidence	2 (8.0)	3 (12)	3 (13)	2 (11)
- Affected fetuses/litter (Mean ± SD) [%]	1.4 ±4.82	1.6 ±4.55	2.3± 6.15	2.8 ±8.70
Selected Individual visceral malformations				
- Muscular ventricular septum defect				
- Fetal incidence [N (%)] *(HCD 0-2.6%)*	0 (0.0)	3 (1.8)	2 (1.2)	0 (0.0)
- Litter incidence [N (%)] *(HCD 0-17.6%)*	0 (0.0)	3 (12)	2 (8.7)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0-2.6%)*	0.0 ±0.00	**1.6*** ±4.55	1.4 ±4.80	0.0 ±0.00

( ) Values in brackets give % fetal respectively litter incidence
* p ≤ 0.05, ** p ≤ 0.01 (Fisher's exact test, one-sided; Wilcoxon-test, one-sided)

Two different visceral variations was observed in all treated groups [see Table 5.6.2-16]. Malpositioned carotid branch was the most common finding with the highest incidence in mid dose fetuses, without attaining statistically significance. The other finding (dilated renal pelvis) was observed in a single case only. None of the findings was considered to be treatment-related.

Table 5.6.2-16: Incidence of visceral (soft tissue) variations

Dose level [mg/kg]	**0**	**25**	**50**	**100**
Litters Evaluated	25	25	23	19
Fetuses Evaluated	151	163	161	105
Live	151	163	161	105
Dead	0	0	0	0
Total visceral variations				
- Fetal incidence [N (%)]	11 (7.3)	11 (6.7)	17 (11)	11 (10)
- Litter incidence [N (%)]	10 (40)	9 (36)	11 (48)	8 (42)
- Affected fetuses/litter (Mean ± SD) [%]	8.1 ±13.09	6.7 ±10.42	9.2 ±11.09	10.2 ±15.12
Individual visceral variations				
- Malpositioned carotid branch				
- Fetal incidence [N (%)]	11 (7.3)	11 (6.7)	16 (9.9)	11 (10)
- Litter incidence [N (%)]	10 (40)	9 (36)	11 (48)	8 (42)
- Affected fetuses/litter (Mean ± SD) [%]	8.1 ±13.09	6.7 ±10.42	8.7 ±10.74	10.2 ±15.12
- Dilated renal pelvis				
- Fetal incidence [N (%)]	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
- Litter incidence [N (%)]	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	0.0 ±0.00	0.5 ±2.32	0.0 ±0.00

( ) Values in brackets give % fetal respectively litter incidence

3. Skeletal examination

A number of skeletal malformations were observed in all groups including controls [see Table 5.6.2-17]. The malformations occurred either singly, without dose response-relationship or at incidences comparable to the historical control range. There were no statistically significant differences for the total or individual skeletal malformations. The highest incidence of severely fused sternebra (bony plate) occurred with 3 affected fetuses in the control group.

Table 5.6.2-17: Incidence of skeletal malformations and variations

Dose level [mg/kg]	0	25	50	100
Litters Evaluated	25	25	23	19
Fetuses Evaluated	151	163	161	105
Live	151	163	161	105
Dead	0	0	0	0
Total skeletal malformations				
- Fetal incidence [N (%)]	4 (2.6)	2 (1.2)	3 (1.9)	5 (4.8)
- Litter incidence	3 (12)	2 (8.0)	3 (13)	4 (21)
- Affected fetuses/litter (Mean ± SD) [%]	3.4 ±9.87	2.5 ±10.21	2.4 ±7.46	6.9 ±16.66
Individual skeletal malformations				
- Sternebra severely fused (Bony plate)				
- Fetal incidence [N (%)]	3 (2.0)	0 (0.0)	0 (0.0)	1 (1.0)
- Litter incidence [N (%)]	3 (12)	0 (0.0)	0 (0.0)	1 (5.3)
- Affected fetuses/litter (Mean ± SD) [%]	2.6 ±7.23	0.0 ±0.00	0.0 ±0.00	1.8 ±7.65
- Absent lumbar vertebra				
- Fetal incidence [N (%)]	2 (1.3)	0 (0.0)	1 (0.6)	1 (1.0)
- Litter incidence [N (%)]	1 (4.0)	0 (0.0)	1 (4.3)	1 (5.3)
- Affected fetuses/litter (Mean ± SD) [%]	1.6 ±8.00	0.0 ±0.00	0.5 ±2.32	0.8 ±3.28
Total skeletal variations				
- Fetal incidence [N (%)]	111 (74)	114 (70)	100 (62)	77 (73)
- Litter incidence [N (%)]	25 (100)	25 (100)	23 (100)	19 (100)
- Affected fetuses/litter (Mean ± SD) [%]	73.7 ±18.73	73.0 ±22.87	62.3 ±22.53	76.7 ±18.06
Individual skeletal variations				
- Fused sternebra				
- Fetal incidence [N (%)] *(HCD 0-10.7%)*[1)]	2 (1.3)	9 (5.5)	10 (6.2)	11 (10)
- Litter incidence [N (%)] *(HCD 0-50.0%)*[1)]	2 (8.0)	9* (36)	8 (35)	9** (47)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0-13.5%)*[1)]	0.9 ±3.08	5.1* ±6.97	6.5** ±10.07	15.2** ±24.50 11.1[2)] (excluding female #87)
- Misshapen sternebra				
- Fetal incidence [N (%)] *(HCD 0-11.6%)*[1)]	5 (3.3)	16 (9.8)	9 (5.6)	7 (6.7)
- Litter incidence [N (%)] *(HCD 0-55.0%)*[1)]	4 (16)	11* (44)	8 (35)	6 (32)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0-15.8%)*[1)]	2.6 ±6.37	10.5* ±14.47	5.5 ±8.85	7.6 ±12.39
- Supernumary Rib (13th)				
- Fetal incidence [N (%)] *(HCD 2.5-11.7%)*[1)]	7 (4.6)	7 (4.3)	7 (4.3)	12 (11)
- Litter incidence [N (%) *(HCD 16.0-52.5%)*[1)]	6 (24)	4 (16)	7 (30)	7 (37)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 2.1-11.1%)*[1)]	5.6 ±11.06	4.3 ±13.76	4.8 ±8.59	10.0 ±15.62

* $p \leq 0.05$, ** $p \leq 0.01$ (Fisher's exact test, one-sided; Wilcoxon-test, one-sided)

[1)] extended historical control data covering a time span of roughly ± 5 years around the experimental date

[2)] when excluding dam #87 (which had 100% affected fetuses (=1 affected pup)/litter) from the evaluation the incidence decreases to 11.1%

Skeletal variations were observed in about 62 to 74% of the fetuses in all litters [see Table 5.6.2-17]. No statistically significant differences (exception: fused or misshapen sternebra) were observed between control and treated groups. The variations were observed without dose response-relationship or at incidences comparable to the historical control range. This includes the statistically significantly increased incidence of fetuses with misshapen sternebra in the low dose group only. Furthermore, the incidences are covered within an extended historical control data covering a time span of roughly ± 5 years around the experimental date (The historical control data considered during the last evaluation only comprised studies conducted before the respective study summarized.). Therefore, these skeletal variations were not considered to be related to treatment.

There was an apparent increase in the fetal, litter and affected fetuses/litter incidence of fused sternebrae at all dose levels without a dose-response relationship. The statistical significance was due to the low incidence of this finding in the concurrent control. At the highest dose level - where severe maternal toxicity was evident - the % affected fetuses per litter was substantially influenced by the reduced litter size. Especially, the litter of female #87 influenced the group mean value substantially. The only fetus of this female displayed fused sternebrae, which resulted in a 100% value of % affected fetuses per litter. Excluding this female from calculation results in a group mean of 11.1% instead of 15.2%. The recalculated value of 11.1% is within the extended historical control range described above.

The incidence of supernumerary rib (13th) was not statistically significantly increased in the high dose group. No dose-relationship could be observed and the incidence is well covered by the extended historical control data range.

No skeletal unclassified observations were observed in this study.

III. CONCLUSION

Severe maternal toxicity was seen at 100 mg/kg bw, indicated by maternal deaths (2 females died). At ≥ 50 mg/kg bw increased incidences of no defecation was observed and the animals showed diarrhea at all dose levels. Marked to excessive, but transient drop of food consumption and body weight loss was observed at all dose levels.
At 100 mg/kg bw/day this resulted in increased resorptions (mainly early resorptions) and post implantation loss. As a consequence gravid uterus weights were lower at this dose level without attaining statistical significance. No treatment-related malformations or variations were recorded.

Based on these effects the maternal NOAEL was < 25 mg/kg and developmental NOAEL was 50 mg/kg bw/day.

BASF DocID 2001/1016351: (Second) Developmental toxicity study with dimoxystrobin in rabbits (Study presented in the original Annex I Dossier)

Executive summary:

Dimoxystrobin was administered daily to presumably pregnant Himalayan rabbits by stomach tube during gestation days 7-28 post insemination (p.i.) at dose levels of 0, 5, 20 and 75 mg/kg bw/day. Two high dose does were found dead. This effect was considered to be treatment-related. Moreover, dose-related maternal toxicity was noted as indicated by a moderate to excessive reduction of food consumption and impaired body weight gain. Diarrhea was seen in 16 high dose, and 6 mid dose females and no defecation in 10 high dose does, most of them showing previously diarrhea and was assessed as treatment-related. A slightly increase in early resorptions and post implantation loss was noted secondary to the massive maternal toxicity at the high dose of 75 mg/kg. This high dose was clearly too toxic to does.
No treatment-related effects on external or visceral (soft tissue) malformations or variations or skeletal variations was observed. There was an increase in the skeletal variation fused sternebra without attaining statistically significance, which was well covered within the extended historical control range. Further statistically significant increases in skeletal variations did not show any dose-relationship and were not found in the first rabbit prenatal developmental toxicity study. Therefore, the increased number of skeletal variations can be considered to be incidental.

Based on the findings in this study the maternal NOAEL was 5 mg/kg bw/day. Developmental effects were seen at the high dose of 75 mg/kg bw/day only.

Materials and Methods:

Dimoxystrobin (batch/purity: N 15: 98.4%) was administered as an aqueous suspension to 25 inseminated female Himalayan rabbits/group by stomach tube at doses of 0, 5, 20 and 75 mg/kg bw on day 7 through day 28 post insemination (insemination = day 0). A standard dose volume of 10 ml/kg bw was used. The control group was dosed with the vehicle only (0.5% Tylose CB 30.000 in doubly distilled water).

Results:

Please note: Only pregnant does were used for the calculations of mean maternal food consumption, body weight and body weight change. Only pregnant does with scheduled sacrifice on GD 29 were taken for the calculation of mean gravid uterine weights, mean net maternal body weight change (corrected body weight gain) and summary of reproduction data.

For the above reasons the following females were excluded from the above-mentioned calculations:

- Control: female #17 (sacrificed after abortion)
- Low dose (5 mg/kg bw): female #30 (accidental death)
- Mid dose (20 mg/kg bw): none
- High dose (75 mg/kg bw): females #77 and 78 (died intercurrently)

A. TEST SUBSTANCE ANALYSES

The stability of the aqueous test substance suspensions over a period of 4 hours at room temperature could be demonstrated.

B. OBSERVATIONS

1. Clinical signs of toxicity

Treatment-related clinical signs in the mid and high dose groups consisted of diarrhea (16/25 high dose, 6/25 mid dose), and no defecation (10/25 high dose, most of them showing previously diarrhea) [see Table 5.6.2-18]. Blood in bedding was observed in 1 high dose rabbit. This rabbit delivered no viable fetuses but had only early resorptions. These effects were considered treatment-related. One low dose doe showed ne defecation towards the end of treatment, which was assessed as incidental due to the late occurrence.

Table 5.6.2-18: Clinical observations in rabbits administered dimoxystrobin during days 6 to 28 of gestation

Dose level [mg/kg]	Animal #	Gestation Day	Observation
Control	17	29	Abortion, sacrificed
5 mg/kg	33	27-29	No defecation
20 mg/kg	51, 54, 63, 64, 70, 74	8	Diarrhea
75 mg/kg	76, 79, 81, 87, 89, 91-100	7-8	Diarrhea
	81, 84, 85, 87, 93, 96-100	8-14	No defecation
	98	19-24	Blood in bedding

2. Mortality

Two high dose does (#77 and 78) were found dead on day 8 p.i.. This effect was considered to be treatment-related.

One low dose doe (#30) was found dead on day 16 p.i.. This was an incidental death since necropsy showed findings indicative for misgavage. One control doe (#17) was sacrifice after abortion on day 29 p.i..

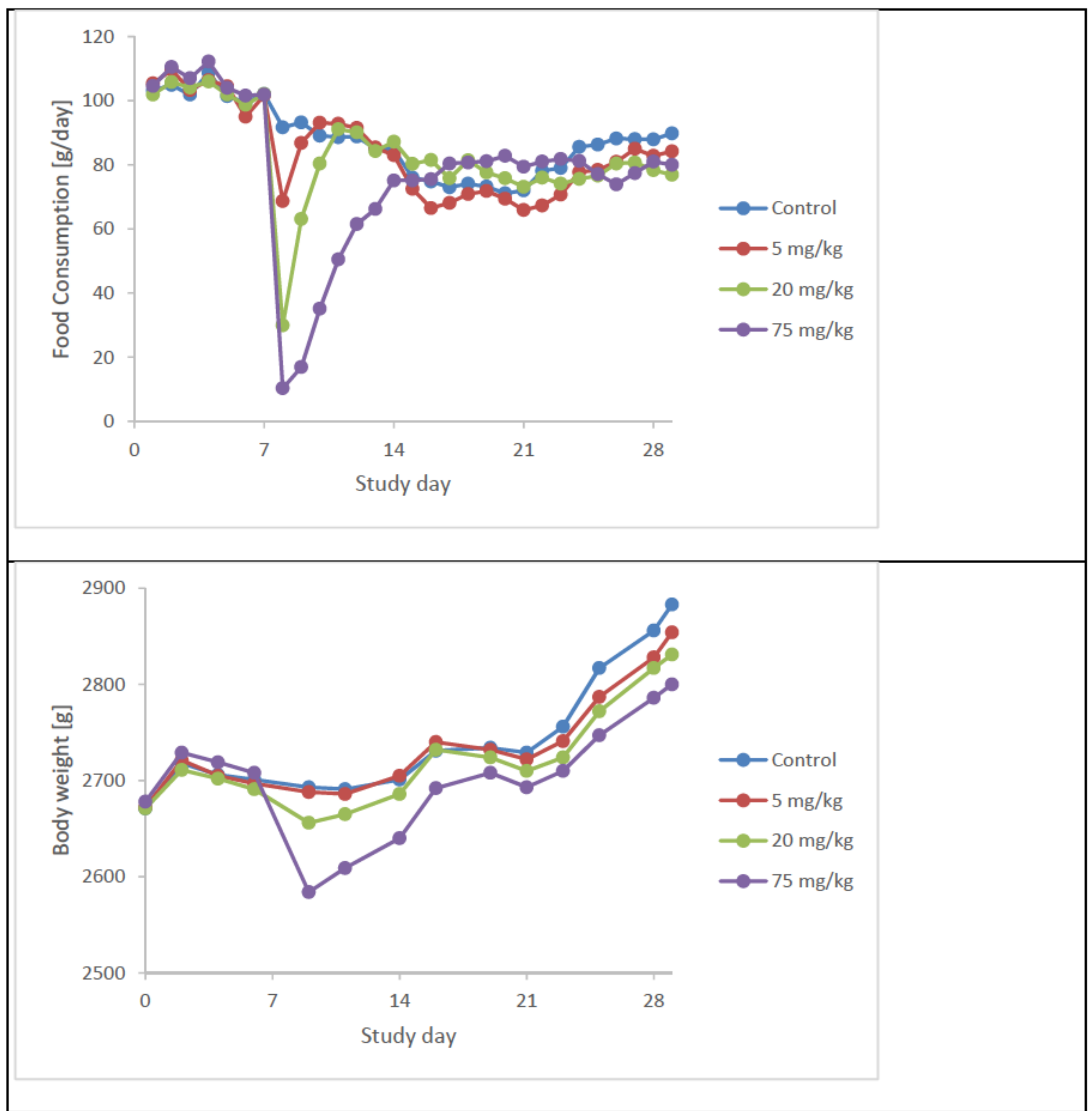
There were no further substance-related or spontaneous mortalities in any of the groups.

C. BODY WEIGHT AND FOOD CONSUMPTION

1. Food consumption

Instantly with the start of treatment at GD 7, a marked, statistically significant and treatment related decrease of food consumption was noted in all treated groups [see Figure 5.6.2-3]. Mean food consumption fell to 75, 33 and 11% of the control for the day 7 to 8 interval at 5, 20, and 75 mg/kg, respectively. Thereafter and in a dose-dependent matter food consumption improved and reached control levels latest on GD 15. Calculated for the entire period high dose rabbits consumed about 18% less food than concurrent controls.

Figure 5.6.2-3: Food consumption and body weight development in rabbits administered dimoxystrobin during days 7 to 28 of gestation



2. Body weight and body weight gain

Secondary to the temporary decrease of food consumption at initiation of treatment (days 7-9) a dose-dependent body weight loss was noted in mid and high dose groups [Figure 5.6.2-3 and Table 5.6.2-19]. Absolut body weights did not show any statistically significant differences. For the gestation day interval 7-9 a statistically significant decrease of body weight gain was noted in the mid and high dose groups. Mean body weight gain during treatment was significantly decreased at the high dose of 75 mg/kg (about 55%) and non-statistically significantly at the mid dose of 20 mg/kg (about 19%) [see Table 5.6.2-19]. The impaired mean body weight gains in the mid and high dose group were assessed as substance-related.

Table 5.6.2-19: Food consumption and body weight development in rabbit administered dimoxystrobin during days 7 to 28 of gestation

Dose level [mg/kg]	0	5	20	75
Food consumption [g/animal/day]§				
Day 0 to 7 Δ%	103.1	103.7 0.6	102.9 -0.2	106.0 2.8
Day 7 to 28 Δ%	82.3	77.6 -5.7	76.8 -6.7	67.8 -17.6
Day 0 to 29 Δ%	87.6	84.1 -4.0	83.1 -5.1	77.4 -11.6
Body weight gain [g]				
Day 0 to 7 Δ%	29.7	25.2 -15.2	20.5 -31.0	29.2 -1.7
Day 7 to 28 Δ%	155.2	136.4 -12.1	125.3 -19.3	**69.2*** -55.4
Day 0 to 29 Δ%	212.1	188.4 -11.2	160.2 -24.5	**107.6*** -49.3

** $p \leq 0.01$ (Dunnett-test, two sided)

D. NECROPSY OBSERVATIONS

1. Corrected (net) body weight gain, carcass and gravid uterus weights

No treatment-related effects on carcass and corrected (net) body weight gain were observed at any dose level [see Table 5.6.2-20]. The mean gravid uterus weight of the high dose animals was decreased (about 85% of that of the control) without attaining statistically significance. The finding was in line with the slightly increased resorption rate.

Table 5.6.2-20: Mean gravid uterus weights and net body weight change of pregnant rabbits administered dimoxystrobin during Days 7 to 28 of gestation

Dose level [mg/kg bw/d]	**0**	**5**	**20**	**75**
Gravid uterus (g)	328.9	323.6	292.1	279.4
Carcass (g)	2561.1	2530.5	2539.1	2521.1
Net weight change from Day 6 (g)	-131.1	-161.1	-152.4	-195.5

2. Gross necropsy observations

There occurred no substance-related necropsy findings in any of the rabbits. A number of gross necropsy findings were noted in all groups including control at Cesarian section [see Table 5.6.2-21]. Findings were either single observations or associated with the method of sacrifice and/or equally distributed over the treatment groups including the control. The most frequent findings were congestions, edema of/in lungs and congested livers. Neither the incidence nor the type of observations indicated a relation to treatment.

Table 5.6.2-21: Gross necropsy findings in rabbits administered dimoxystrobin during days 7 to 28 of gestation

Dose group	Animal #	Observation
Control	11	Lungs: congested
	17	Large intestine: tympanic distension Thoracic cavity: filled with serous fluid Lungs: congested Stomach: filled with fluid bloody Particular findings on implants in females which aborted
	19	Lungs: congested
	20	Lungs: congested
	25	Lungs: edema Thoracic cavity: filled with serous fluid
5 mg/kg bw/day	28	Lungs: congested
	30	Thoracic cavity: filled with bloody fluid Lungs: congested Particular findings on implants in females died intercurrently
	41	Lungs: congested
20 mg/kg bw/day	56	absence of uterine horns
	57	Lungs: congested Liver: congested
	65	Lungs: edema Liver: pale Thoracic cavity: filled with serous fluid
	66	Lungs: congested
	75	Lungs: edema Liver: pale
75 mg/kg bw/day	77	Stomach: tympanic distension Watery feces Particular findings on implants in females died intercurrently
	78	Stomach: ulceration(s) Watery feces Particular findings on implants in females died intercurrently
	87	Lungs: congested
	91	Blind ending uterine horn(s)
	98	Lungs: congested Liver: congested
	100	Blind ending uterine horn(s)

E. CESAREN SECTION DATA

25 does were pregnant at 0, 5, 20 and 75 mg/kg each [see Table 5.6.2-22]. As already indicated two high dose and one low dose female died.

The number of corpora lutea, implantations and the pre-implantation loss was comparable between all groups.
The total resorption rate was slightly but not statistically significantly increased in the high dose group due to an increase in early resorptions. This was mainly caused by one doe (#98), which had no viable fetuses but inly early resorptions. Consequently, the post-implantation loss value was slightly increased without attaining statistical significance. The increase of early resorptions is considered to be secondary to the marked maternal toxicity observed at the high dose as evident by maternal deaths, the marked reduction of food consumption, body weight loss and diarrhea during the early phase of treatment.

Table 5.6.2-22: Pregnancy status and caesarean section data of females treated with dimoxystrobin during days 7 to 28 of pregnancy

Dose level [mg/kg bw/d]		0	5	20	75
Pregnancy status					
Females					
- mated	[n]	25	25	25	25
- pregnant	[n]	25	25	25	25
conception rate	[%]	100	100	100	100
- aborted	[n]	1	0	0	0
- premature birth	[n]	0	0	0	0
- females with viable fetuses	[n]	24	24	25	22
- females with all resorptions	[n]	0	0	0	1
- mortality		1	1	0	2
- pregnant terminal sacrifice	[n]	24	24	25	23
Cesarean section data[a]					
- Corpora lutea	[n]	8.6	8.9	8.5	8.5
total number	[n]	206	214	213	195
- Implantation sites	[n]	7.6	7.5	6.8	6.7
total number	[n]	183	181	213	155
- Pre-implantation loss	[%]	12.4	14.9	19.7	21.7
- Post-implantation loss	[%]	10.1	8.8	12.5	16.0
- Resorptions	[%]	10.1	8.8	12.5	16.0
number	[n]	0.8	0.6	0.8	0.9
total number	[n]	19	15	21	20
- Early resorptions	[%]	8.4	8.4	9.4	15.5
number	[n]	0.7	0.6	0.6	0.8
total number	[n]	16	14	16	19
- Late resorptions	[%]	1.6	0.4	3.1	0.5
number	[n]	0.1	0.0	0.2	0.0
total number	[n]	3	1	5	1
- Dead fetuses	[n]	0	0	0	0
- Females with viable fetuses	[n]	24	24	25	22
- Live fetuses		6.8	6.9	6.0	6.1
total number	[n]	164	166	150	135
Mean	[%]	89.9	91.2	87.5	87.8
- Total live female fetuses	[n]	3.6	3.1	3.1	2.8
total number	[n]	86	75	78	62
Mean	[%]	48.1	41.9	43.8	37.6
- Total live male fetuses	[n]	3.3	3.8	2.9	3.3
total number	[n]	78	91	72	73
Mean	[%]	41.8	49.3	43.7	50.3
- Percent live females		52.4	45.2	52.0	45.9
- Percent live males		47.6	54.8	48.0	54.1

Table 5.6.2-22: Pregnancy status and caesarean section data of females treated with dimoxystrobin during days 7 to 28 of pregnancy

Dose level [mg/kg bw/d]	0	5	20	75
Pregnancy status				
Females				
- mated [n]	25	25	25	25
- pregnant [n]	25	25	25	25
conception rate [%]	100	100	100	100
- aborted [n]	1	0	0	0
- premature birth [n]	0	0	0	0
- females with viable fetuses [n]	24	24	25	22
- females with all resorptions [n]	0	0	0	1
- mortality	1	1	0	2
- pregnant terminal sacrifice [n]	24	24	25	23
Cesarean section data[a]				
Placental weights [g]	4.3	4.1	4.4	4.3
- male fetuses [g]	4.3	4.2	4.6	4.3
- female fetuses [g]	4.3	4.0	4.3	4.2
Mean fetal weight [g]	36.1	34.7	36.0	35.3
- males [g]	36.2	34.8	36.1	35.3
- females [g]	35.8	34.2	35.9	34.4

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett-test, two-sided)

The sex ratio as well as placental and fetal weights were not affected by treatment. No substance-related differences occurred between control, low and mid dose group animals.

F. EXTERNAL, VISCERAL AND SKELETAL EXAMINATION OF FETUSES

1. External examination

No treatment related external malformations were recorded in treated groups. The malformations occurred either singly, without dose response-relationship or at incidences comparable to the historical control range [see Table 5.6.2-23].
Microglossia was observed in two low, one mid and two high dose fetuses. There was no dose-response relationship and the mean percentage of affected fetuses/litter did not attain statistical significance. The same was true for the finding malroted limb.
External variations observed consisted of paw hyperflexion in all treated dose groups including control to a comparable number. Neither the incidence nor the type of external variations indicated a relation to treatment.
Moreover, none of the observed external malformations or variations were found in the preceding prenatal developmental toxicity study up to and including the high dose level of 100 mg/kg bw/day.

No unclassified external observations were found in neither the control nor the treated dose groups.

Table 5.6.2-23: Incidence of external malformations and variations

Dose level [mg/kg bw/day]	0	5	20	75
Litters Evaluated	24	24	25	22
Fetuses Evaluated	164	166	150	135
Live	164	166	150	135
Dead	0	0	0	0
Total external malformations				
- Fetal incidence [N (%)]	0 (0.0)	8 (1.8)	1 (0.7)	3 (2.2)
- Litter incidence [a]	0 (0.0)	2 (8.3)	1 (4.0)	1 (4.5)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	1.7 ±6.26	0.6 ±2.86	1.9 ±9.14
Individual external malformations				
- Microglossia				
- Fetal incidence [N (%)]	0 (0.0)	2 (1.2)	1 (0.7)	2 (1.5)
- Litter incidence [N (%)]	0 (0.0)	1 (4.2)	1 (4.0)	1 (4.5)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	1.2 ±5.83	0.6 ±2.86	1.3 ±6.09
- Malrotated limb				
- Fetal incidence [N (%)]	0 (0.0)	2 (1.2)	1 (0.7)	2 (1.5)
- Litter incidence [N (%)]	0 (0.0)	1 (4.2)	1 (4.0)	1 (4.5)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	1.2 ±5.83	0.6 ±2.86	1.3 ±6.09
Total external variations				
- Fetal incidence [N (%)]	4 (2.4)	7 (4.2)	5 (3.3)	5 (3.7)
- Litter incidence [N (%)]	4 (17)	5 (21)	4 (16)	4 (18)
- Affected fetuses/litter (Mean ± SD) [%]	1.8 ±4.24	5.3 ±11.26	2.6 ±6.42	2.8 ±6.97
Individual external variations				
- Paw hyperflexion				
- Fetal incidence [N (%)]	4 (2.4)	7 (4.2)	5 (3.3)	5 (3.7)
- Litter incidence [N (%)]	4 (17)	5 (21)	4 (16)	4 (18)
- Affected fetuses/litter (Mean ± SD) [%]	1.8 ±4.24	5.3 ±11.26	2.6 ±6.42	2.8 ±6.97

( ) Values in brackets give % fetal respectively litter incidence

2. Visceral examination

A variety of visceral (soft tissue) malformations were observed in all groups including control [see Table 5.6.2-24]. The malformations occurred either singly, without dose response-relationship or at incidences comparable to the historical control range. There were no statistically significant differences in treated compared to control fetuses. A slight increase in membranous ventricular septum defect was seen in the control, mid and high dose with 1, 1, and 3 affected fetuses without attaining statistical significance. The incidence of this malformation was within the historical control range. Furthermore, ventricular septum defects were seen only in the low and mid dose of the first prenatal developmental toxicity study in rabbits, but not in the highest dose tested (100 mg/kg bw), showing the variability of this effect. It was therefore considered to be incidental. All other findings were observed in single cases only. None of the findings was considered to be treatment-related.

Table 5.6.2-24: Incidence of visceral (soft tissue) malformations

Dose level [mg/kg]	**0**	**5**	**20**	**75**
Litters Evaluated	24	24	25	22
Fetuses Evaluated	164	166	150	135
Live	164	166	150	135
Dead	0	0	0	0
Total visceral malformations				
- Fetal incidence [No. (%)]	1 (0.6)	3 (1.8)	2 (1.2)	3 (2.2)
- Litter incidence	1 (4.2)	3 (13)	2 (8.0)	3 (14)
- Affected fetuses/litter (Mean ± SD) [%]	0.5 ±2.55	1.7 ±4.64	1.8± 7.02	2.4 ±6.56
Selected Individual visceral malformations				
- **Membranous ventricular septum defect**				
- Fetal incidence [N (%)] *(HCD 0-2.6%)*	1 (0.6)	0 (0.0)	1 (0.7)	3 (2.2)
- Litter incidence [N (%)] *(HCD 0-17.6%)*	1 (4.2)	0 (0.0)	1 (4.0)	3 (14)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0-2.6%)*	0.5 ±2.55	0.0 ±0.00	0.5 ±2.50	2.4 ±6.56

( ) Values in brackets give % fetal respectively litter incidence

Visceral variations were observed in all treated groups [see Table 5.6.2-25]. Malpositioned carotid branch was the most common finding with the highest incidence in mid dose fetuses, without attaining statistically significance. The other finding (dilated cerebral ventricle) was observed in a single case only. None of the findings was considered to be treatment-related.

Table 5.6.2-25: Incidence of visceral (soft tissue) variations

Dose level [mg/kg]	**0**	**5**	**20**	**75**
Litters Evaluated	24	24	25	22
Fetuses Evaluated	164	166	150	135
Live	164	166	150	135
Dead	0	0	0	0
Total visceral variations				
- Fetal incidence [N (%)]	31 (19)	29 (17)	34 (23)	27 (20)
- Litter incidence [N (%)]	18 (75)	11 (71)	18 (72)	14 (64)
- Affected fetuses/litter (Mean ± SD) [%]	17.8 ±15.69	17.4 ±14.85	24.6 ±20.86	20.5 ±18.61
Individual visceral variations				
- Malpositioned carotid branch				
- Fetal incidence [N (%)]	30 (18)	25 (15)	34 (23)	24 (18)
- Litter incidence [N (%)]	17 (71)	16 (67)	18 (72)	12 (55)
- Affected fetuses/litter (Mean ± SD) [%]	17.2 ±16.09	15.2 ±13.23	24.6 ±20.86	18.0 ±18.99
- Dilated cerebral vertricle				
- Fetal incidence [N (%)]	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
- Litter incidence [N (%)]	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	0.6 ±2.92	0.0 ±0.00	0.0 ±0.00

( ) Values in brackets give % fetal respectively litter incidence

Low incidences infarct of liver (2 fetuses of the mid and 1 fetus of the high dose) and fluid-filled abdomen (1 fetus of the mid dose only) were the only unclassified findings. The incidence was not indicative for a relation to treatment.

3. Skeletal examination

A number of skeletal malformations were observed in all groups including controls [see Table 5.6.2-26]. The malformations occurred either singly, without dose response-relationship or at incidences comparable to the historical control range. The total skeletal malformations were statistically significantly increased in the high dose group on a litter and affected fetuses/litter basis without a dose-relationship. Individual skeletal malformations did not shown significant changes. Severely fused sternebra were slightly but statistically significantly increased in the high dose group (fetal incidence of 3). The incidence was observed to the same incidence in the control of the preceding prenatal developmental study in rabbits performed with dimoxystrobin and was therefore regarded as being incidental. No other individual skeletal malformation was significantly increased.

Table 5.6.2-26: Incidence of skeletal malformations and variations

Dose level [mg/kg]	**0**	**5**	**20**	**75**
Litters Evaluated	24	24	25	22
Fetuses Evaluated	164	166	150	135
Live	164	166	150	135
Dead	0	0	0	0
Total skeletal malformations				
- Fetal incidence [N (%)]	3 (1.8)	6 (3.6)	3 (2.0)	11 (8.1)
- Litter incidence	3 (13)	6 (25)	2 (8.0)	9* (41)
- Affected fetuses/litter (Mean ± SD) [%]	2.2 ±6.16	3.4 ±6.07	1.6 ±5.65	9.5* ±14.07
Individual skeletal malformations				
- Sternebra severely fused (Bony plate)				
- Fetal incidence [N (%)]	0 (0.0)	0 (0.0)	1 (0.7)	3 (2.2)
- Litter incidence [N (%)]	0 (0.0)	0 (0.0)	1 (4.0)	3 (14)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	0.0 ±0.00	0.5 ±2.50	2.2* ±6.16
Total skeletal variations				
- Fetal incidence [N (%)]	100 (61)	107 (64)	92 (61)	94 (70)
- Litter incidence [N (%)]	24 (100)	24 (100)	25 (100)	22 (100)
- Affected fetuses/litter (Mean ± SD) [%]	62.7 ±24.39	61.7 ±20.88	59.4 ±25.72	71.5 ±17.00
Individual skeletal variations				
- Fused sternebra; unchanged cartilage				
- Fetal incidence [N (%)] *(HCD 0-10.7%)[1]*	5 (3.0)	8 (4.8)	3 (2.0)	16 (12)
- Litter incidence [N (%)] *(HCD 0-47.1%)[1]*	4 (17)	5 (21)	2 (8.0)	8 (36)
- Affected fetuses/litter (Mean ± SD) [%] *(HCD 0-13.5%)[1]*	2.7 ±6.29	4.6 ±9.92	2.5 ±10.21	11.2 ±18.17
- Incomplete Ossification of cervical centrum; unchanged cartilage				
- Fetal incidence [N (%)]	16 (9.8)	17 (10)	28 (19)	22 (16)
- Litter incidence [N (%)]	8 (33)	9 (38)	**17*** (68)	12 (55)
- Affected fetuses/litter (Mean ± SD) [%]	9.5 ±16.62	9.3 ±15.01	**18.3*** ±17.68	14.7 ±16.84
- Hemicentric cervical centrum; unchanged cartilage				
- Fetal incidence [N (%)]	0 (0.0)	4 (2.4)	1 (0.7)	0 (0.0)
- Litter incidence [N (%)]	0 (0.0)	3 (13)	1 (4.0)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	**2.1*** ±5.88	0.6 ±2.86	0.0 ±0.00
- incomplete ossification of thoracic centrum; unchanged cartilage				
- Fetal incidence [N (%)]	0 (0.0)	1 (0.6)	3 (2.0)	0 (0.0)
- Litter incidence [N (%)]	0 (0.0)	1 (4.2)	3 (12)	0 (0.0)
- Affected fetuses/litter (Mean ± SD) [%]	0.0 ±0.00	0.5 ±2.55	**1.4*** ±3.85	0.0 ±0.00

* $p \leq 0.05$, ** $p \leq 0.01$ (Fisher's exact test, one-sided; Wilcoxon-test, one-sided)

[1)] extended historical control data covering a time span of roughly ± 5 years around the experimental date

Skeletal variations were observed in about 61 to 70% of the fetuses in all litters [see Table 5.6.2-26].
The variations were observed either singly, without dose response-relationship or at incidences comparable to the historical control range.
This includes the statistically significantly increased incidence of incomplete ossification of cervical centrum, which was statistically significantly increased only in the mid dose group. Furthermore, incomplete ossification of cervical centrum was not increased in the first prenatal developmental rabbit study up to the high dose of 100 mg/kg bw.
Incomplete ossification of thoracic centrum was statistically significantly increased in the mid dose only, but did not occur in the high dose group. Therefore, the effect is not considered treatment-related. Again, this finding was not evident in the first prenatal developmental rabbit study up to the high dose of 100 mg/kg bw, but was actually highest in the control animals with fetal incidence 6, litter incidence 5 and affected fetuses/litter 3.2.
Slight incidence of hemicentric cervical centrum was statistically significantly increased in the low dose only and did not show any dose-relationship.

An increased incidence in fused sternebrae was observed in the high dose group (11.2% affected fetuses/litter), which was not statistically significantly different from control. The incidence is well covered by the extended historical control range described above.

III. CONCLUSION

Severe maternal toxicity was seen at 75 mg/kg bw, indicated by maternal deaths (2 females died) and no defecation. At ≥ 25 mg/kg bw the incidence of diarrhea was increased. Marked to excessive, but transient drop of food consumption and lower mean body weights / body weight gains were seen at ≥ 25 mg/kg bw,
At 75 mg/kg bw/day this resulted in increased non-statistically significant resorptions (mainly early resorptions) and post implantation loss. As a consequence gravid uterus weights were lower at this dose level without attaining statistical significance. No treatment-related malformations or variations were recorded.

Based on these effects the maternal NOAEL was 5 mg/kg. Developmental toxicity was observed at 75 mg/kg bw/day.

Based on the results of both prenatal toxicity studies in rabbits the overall maternal NOAEL was 5 mg/kg. The developmental NOAEL was 50 mg/kg bw/day (from the first rabbit study).

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CA 5.7 Neurotoxicity Studies

Studies evaluated in the draft assessment report (DAR, July 2003):
Based on the structure of dimoxystrobin (it is not an organophosphate), the active substance would not be expected to produce delayed neuropathy. Also none of the impurities in the proposed technical specification for which approval is sought are organophosphates**.** Therefore no delayed polyneuropathy studies, however, acute and subchronic neurotoxicity studies have been conducted with dimoxystrobin.

These studies have been evaluated and peer reviewed during the previous Annex I inclusion process and were considered to be acceptable. For the convenience of the reviewer, these are summarized below as extracted from the DAR (2003).

Table 5.7-1: Summary of neurotoxicity studies with dimoxystrobin

Study	NOAEL (mg/kg bw/d)	Critical effects	Reference
Acute neurotoxicity study Wistar rats 0, 500, 1000, 2000 mg/kg bw (single administration, gavage)	neurotoxicity: 2000 mg/kg bw/day systemic toxicity: <500 mg/kg bw/day	No neurotoxic effects were observed Mucoid feces	BASF DocID 1999/11250
Subchronic neurotoxicity study Wistar rats 0, 300, 1500, 4500 (males), 9000 (females) ppm (diet)	neurotoxicity: males: 4500 ppm (305 mg/kg bw/day) females: 9000 ppm (677 mg/kg bw/day) systemic toxicity: males: < 300 ppm (<21 mg/kg bw/d) females: 300 ppm (24 mg/kg bw)	No neurotoxic effects were observed Reduced bw gain	BASF DocID 2000/1013110

Conclusion
Dimoxystrobin was tested for potential neurotoxicity in an acute and a subchronic study in rats. These studies included extensive functional observation batteries as well as specific neurohistopathological investigations. In these studies, as well as in other studies with dimoxystrobin, no indications for a neurotoxic potential were observed.

Based on the data provided for the previous Annex I inclusion process, the list of EU agreed endpoints specified:

Neurotoxicity/Delayed neurotoxicity	Not neurotoxic in acute and repeated dose neurotoxicity studies in rats

Studies submitted in this supplementary dossier (not yet peer-reviewed):
No new neurotoxicity studies have been performed.

There are no new studies available with dimoxystrobin that could affect the overall evaluation for neurotoxicity and delayed neurotoxicity. Thus, the conclusion for relevant endpoints for the current renewal remains as follows:

Acute neurotoxicity	Acute, rat: No neurotoxic effects were observed NOAEL 2000 mg/kg bw/day **No classification required**
Repeated neurotoxicity	90-day, rat: No neurotoxic effects were observed NOAEL males: 4500 ppm (305 mg/kg bw/day) females: 9000 ppm (677 mg/kg bw/day) **No classification required**
Additional studies (e.g. delayed neurotoxicity, developmental neurotoxicity)	No data - not required

For convenience of the reviewer brief summaries of the respective studies as extracted from the DAR on Dimoxystrobin of the RMS United Kingdom (July 2003) are provided under the respective chapters.

CA 5.7.1 Neurotoxicity studies in rodents

Acute neurotoxicity study with dimoxystrobin in rats (BASF DocID 1999/11250)

Guidelines: In accordance with OECD 424 (adopted 1997) and complying with EPA requirements.
Deviations: No major deviations. An extensive neurohistopathological examination was conducted but there were some slight deviations from the examination recommended by OECD. Historical positive control data for the laboratory were provided.
GLP: Yes
Acceptance: The study was considered acceptable in the previous EU evaluation process.

Dimoxystrobin (Batch/purity: N 15: 98.4%) was administered to groups of 10 male and 10 female Wistar rats as a single oral administration by gavage at dose levels of 0, 500, 1000 and 2000 mg/kg bw. The vehicle was a 0.5% aqueous solution of carboxymethyl-cellulose (Tylose CB 30000), and the administration volume was 20 mL/kg bw.
No substance-related findings were noted in a preliminary peak-finding study following dosing with up to 2000 mg/kg bw (measurements at 15 and 30 minutes, every hour from 1-6 hours, and at 1, 2 and 3 days). Hence in the main study first measurements of FOB and motor activity were made within 2-6h of dosing.

There were no deaths, no clinical signs of toxicity and no effects on body weight. At 2000 mg/kg bw, urine staining of the anogenital region (1 rat day 7), soft or mucoid faeces (2 rats day 0) and diarrhoea (2 rats day 0) were observed. At 1000 mg/kg bw urine staining of the anogenital region (1 rat day 7) and mucoid faeces (9 rats day 0) were detected and only mucoid faeces (2 rats day 0) occurred at 500 mg/kg bw.
As no other signs indicative of an effect on the autonomic nervous system (e.g. salivation) were observed, the soft faeces were assessed as being a (local) effect on the gastrointestinal tract, but not an effect on the central nervous system.

All clinical effects were reversible within the 14-day observation period. There were no other substance-related effects detected by the FOB and there was no statistically significant effect on overall motor activity. No lesions were noted on gross pathological examination. Light-microscopic investigation of the nervous system and gastrocnemius muscle did not reveal any lesions.

Conclusion
The effects observed in this study were assessed as being due to acute systemic toxicity and/or local effects on the digestive tract, but not to neurotoxicity.
The NOAEL for neurotoxicity under the conditions of this study was therefore 2000 mg/kg bw in both sexes.

Subchronic oral neurotoxicity study with dimoxystrobin in rats (BASF DocID 2000/1013110)

Guidelines: In accordance with OECD 424 (adopted 1997) and complying with EPA requirements.
Deviations: First FOB and motor activity measurements during the exposure period were made a little later than recommended by OECD. An extensive neurohistopathological examination was conducted but there were some slight deviations from the examination recommended by OECD. Historical positive control data for the laboratory were provided.
GLP: Yes
Acceptance: The study was considered acceptable in the previous EU evaluation process.

Dimoxystrobin (Batch/purity: N 15: 98.4%) was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0, 300, 1500, 4500 (males) and 9000 ppm (females) for 3 months. Dose levels were selected based on the findings of the 3-month oral toxicity study in rats. Test substance intake was 21, 102, and 305 mg/kg bw/day for males of the 300, 1500, and 4500 ppm dose groups. For females a substance intake of 24, 116, and 677 mg/kg bw/day was observed for the 300, 1500, and 9000 ppm dose groups.

There were no deaths. At the top dose, dark discoloration of faeces was observed in females. A dose-related reduction in body weight gain was seen in males at all dose levels (by 10% at the lowest dose). Statistically significant reductions in food consumption by males were seen at all dose levels (only very occasionally at the lowest dose level). A dose-related reduction in body weight gain was seen in females at the top two dose levels (by up to 13% at 1500 ppm, although no statistically significant differences were seen at this dose level). Occasional statistically significant reductions in food consumption were noted in females at the top 2 dose levels. There were no clear effects on food efficiency.
FOB observations revealed no substance-related effects. Soft faeces were seen in a few rats at 1500 and 9000 ppm on days 22 and 50, with one rat at 1500 ppm on day 85.
There was no statistically significant effect on overall motor activity.
No gross lesions were noted. There was no effect on absolute brain weight, and no substance-related lesions of the nervous system and gastrocnemius muscle were observed on histological examination.

Conclusion
No signs of neurotoxicity were detected. The NOAEL for neurotoxicity was therefore 4500 ppm (305 mg/kg bw) in males and 9000 ppm (677 mg/kg bw) in females. There was evidence of systemic toxicity (notably reduced body weight gain) at all dose levels in males and at the top two dose levels in females. Hence 300 ppm (males 21 mg/kg bw/day, females 24 mg/kg bw/day) was a LOAEL for systemic toxicity in males and a NOAEL for systemic toxicity in females.

CA 5.7.2 Delayed polyneuropathy studies

As there was no indication for neurotoxicity and/or neuropathy from any of the studies conducted and as dimoxystrobin does not belong to a chemical class suspected to induce delayed neuropathies, no study is considered to be necessary. Thus, no further study was conducted.

CA 5.8 Other Toxicological Studies

CA 5.8.1 Toxicity studies of metabolites

Studies evaluated in the draft assessment report (DAR, July 2003):
Studies submitted for Annex I listing consisted of acute oral toxicity, bacterial mutagenicity, mammalian point mutation, and chromosome aberration studies with metabolite **505M01**. The genotoxicity tests were negative and no hazard was identified in the acute oral toxicity study.

Category of test	Dose range	Results	Reference (BASF DocID)
Acute oral toxicity Wistar rats 505M01	2000, 4000 mg/kg bw	LD_{50} >2000 mg/kg bw 2000 mg/kg bw: no mortality 4000 mg/kg bw: 1 animal died	2001/1001875
Ames mutagenicity test TA 1535, 100, 1537, 98 E. coli WP2 uvrA ±S9 mix 505M01	22 – 5500 µg/plate	Negative	2000/1018822
V79/HPRT mutagenicity test ±S9 mix 505M01	156.3, 312.5, 625, 1250, 2500 µg/mL	Negative	2001/1001616
In vitro cytogenetics : Chromosome aberration in Chinese hamster V79 cells ±S9 mix 505M01	625, 1250, 2500 µg/mL	Negative (not clastogenic and no effect on polyploidy)	2001/1009006

Metabolite 505M01 is a photolysis metabolite of dimoxystrobin in environmental studies and a metabolite in rotational crops and hens. It was formed in measurable amounts in rats following cleavage of the ether bridge (0.675% in urine, 0.385% in faeces). The acute oral LD_{50} of 505M01in Wistar rats was >2000 mg/kg bw. It can be concluded that the metabolite is of low acute toxicity. In all mutagenicity tests no mutagenic potential was detected. Metabolite 505M01 appears to contain no structural alerts for potential DNA reactivity according to the model of Ashby and Tennant (1991; BASF DocID 1991/11664).

Based on the available data the following assessment was drawn in the Annex I listing of Dimoxystrobin:

Other toxicological studies	Acute oral toxicity: LD_{50} >2000 mg/kg bw
	Ames - negative
	HPRT (V79) – negative
	Chromosome aberration (V79) – negative (not clastogenic and no effect on polyploidy)

Within the past few years, metabolites in food of plant and animal origin were getting more into focus within the regulatory evaluation process. In 2012, EFSA published a Scientific Opinion on approaches to evaluate the toxicological relevance of metabolites and degradates of pesticides (see: EFSA Journal 2012;10(07): 2799. [187 pp.] doi:10.2903/j.efsa.2012.2799). The opinion identifies the threshold of toxicological concern (TTC) concept as an appropriate screening tool and has therefore been used for the metabolite assessment of dimoxystrobin.

Dimoxystrobin is extensively metabolised in all matrices (mammal, plant and soil/water) resulting in numerous metabolites identified. Meanwhile further insights into behavior of dimoxystrobin in plants, animals and in the environment has been obtained and thus several metabolites were identified which require further consideration (see section M-CA 6.9).

There are mainly four key transformation steps:

- Cleavage between the ring systems
- Modification/hydroxylation of the side chain
- Hydroxylation of the aromatic ring system or the methyl groups at the aromatic ring system
- Carboxylation of the methyl group at the aromatic ring system

A grouping of plant and livestock metabolites into 5 groups was performed according to structure and metabolic pathway. Details of this grouping can be found in M-CA 6.7 and M-CA 6.9. One metabolite of each group was chosen as representative metabolite, representing the toxicological properties of the group. An overview of this grouping is given below:

- Group 1: Cleavage Products
- Group 2: Hydroxylation products
- Group 3: Carboxylation products
- Group 4: Products with modifications at the side chain
- Group 5: Hydroxylation and carboxylation products

To decide for which group the toxicological endpoints (ADI, ARfD) of the parent molecule can be applied, the plant and livestock metabolites were compared with the rat metabolites:
If a representative metabolite is present in a rat metabolism study with more than 10% of the applied dose for these metabolites and their respective groups the toxicological endpoints of the parent molecule can be applied. Depending on the detected metabolite quantities (expressed as mean % dose) the weight of evidence approach was selected or additional investigations were initiated.

Group 1 Cleavage Products (see Table 5.8.1-1):

The cleavage of the ether bond between the two ring systems is a relevant metabolic degradation step in rats resulting in more than 15 identified metabolites. Of the plant and livestock cleavage metabolites included in the TTC grouping only 505M01 was identified in the rat. As the assigned dose for 505M01 in the rat metabolism study is < 1%, additional toxicity testing was performed with the representative metabolite to facilitate the use of the TTC concept. The metabolites of group 1 cannot be considered as fully covered by the investigations performed with the parent compound.

Table 5.8.1-1: Grouping of metabolites: Group 1 Cleavage products

Metabolite	Occurrence	Metabolite Structure	Occurrence in rats [mean, % of dose]
505M01[#]	Goat (only detected after enzyme treatment), confined rotational crop	HO, H, N, H_3C, O, N, O, CH_3	Urine < 1% Faeces < 0.5%
505M80	Confined rotational crop	HO, O, O, HO, HO, OH, H, N, H_3C, O, N, O, CH_3	Not identified in rat metabolism

[#]representative metabolite

Group 2 Hydroxylation products (see Table 5.8.1-2):

The hydroxylation of the aromatic ring system is an important metabolic degradation step in rat, livestock and plant metabolism. The majority of the metabolites included in the TTC grouping concept belong to this group. The representative metabolite 505M04 was not identified in the rat. Therefore, additional toxicological tests have been performed.

Table 5.8.1-2: Grouping of metabolites: Group 2 Hydroxylated metabolites

Metabolite	Occurrence	Metabolite Structure	Occurrence in rats
505M02	Goat, confined rotational crop (feed items)		Faeces 40%**
505M04[#]	Goat (milk only)		Not identified in rat metabolism
505M06	Confined rotational crop (lettuce leaf)		Not identified in rat metabolism
505M49	Goat (liver, kidney)		Not identified in rat metabolism
505M50	Goat (liver)		Bile 23%**
505M51	Goat (liver, kidney)		Not identified in rat metabolism
505M63*	Confined rotational crop (feed items)		Faeces 40%**

Metabolite	Occurrence	Metabolite Structure	Occurrence in rats
505M78	Goat (liver, kidney)		Not identified in rat metabolism
505M79	Goat (liver, kidney)		Not identified in rat metabolism
505M84	Goat (liver, kidney)		Not identified in rat metabolism
505M86	Goat (milk only)		Not identified in rat metabolism
505M91	Confined rotational crop (lettuce leaf)		Not identified in rat metabolism
505M93	Confined rotational crop (lettuce leaf)		Not identified in rat metabolism
505M95	Confined rotational crop (lettuce leaf)		Not identified in rat metabolism
505M107*	Rape (feed items)		Not identified in rat metabolism

*feed item metabolite, **sum of several, co-eluting metabolites, #representative metabolite

Group 3 Carboxylation products (Table 5.8.1-3):

The oxidation of the methyl groups at the aromatic ring system is an important metabolic degradation step in rats resulting in 15 identified metabolites. In the studied plant and livestock metabolisms this route is less pronounced. Because of their potential occurrence in groundwater the toxicological properties of 505M08 and 505M09 were investigated in more detail. Toxicity data based on 505M08 and 505M09 is representative for metabolites of group 3. The obtained data support the use of the TTC concept for the evaluation of group 3. The metabolites of group 3 cannot be considered as fully covered by the investigations performed with the parent compound.

Table 5.8.1-3: Grouping of metabolites: Group 3 Carboxylated metabolites

Metabolite	Occurrence	Metabolite Structure	Occurrence in rats
505M08#	Water/soil, traces in rape	COOH, O, H, N, H_3C, N, O, CH_3, CH_3, O	Urine 10%** Feces 40%**
505M09#	Goat (liver, kidney, milk), traces in rape, confined rotational crop	CH_3, O, H, N, H_3C, N, O, CH_3, COOH, O	Urine 10%** Feces 40%** Bile 6% Plasma <0.1% Liver < 1% Kidney < 0.5%
505M81	Goat (liver, kidney)	CH_3, O, H, N, H_3C, N, O, CH_3, O, HOOC, O, O, O, HO, HO, OH	Bile 8%

**sum of several, co-eluting metabolites, #either metabolite could be chosen as representative metabolite

Metabolites in **Groups 1, 2 and 3** will be addressed in this section. An estimation of the potential and actual exposure through diet is provided in M-CA 6.9.

Group 4 Products with modifications at the side chain (see Table 5.8.1-4):

Group 4 contains metabolites with modified sidechains, a significant metabolic pathway in rats (> 15 metabolites). In plant and livestock studies metabolites with modified sidechains did only occur in feed items and the calculated consumer exposure values assuming dietary intake via products of animal origin are < 0.0000025 mg/kg. Therefore, no further toxicological assessment is to be performed. The metabolites of group 4 are just listed for reasons of completeness.

Table 5.8.1-4: Grouping of metabolites: Group 4 Metabolites with modified sidechains

Metabolite	Occurrence	Metabolite Structure	Occurrence in rats
505M88*	Confined rotational crop (feed items)		Not identified in rat metabolism
505M89*	Confined rotational crop (feed items)		Not identified in rat metabolism
505M94*	Confined rotational crop (feed items)		Not identified in rat metabolism

*feed item metabolite

Group 5 Hydroxylation and carboxylation products (Table 5.8.1-5):

The hydroxylation of the aromatic ring combined with the oxidation of the methyl groups is an important metabolic degradation step in rats and in livestock and plant metabolism. The representative metabolite 505M33 was a significant rat metabolite identified in the rat metabolism study with dimoxystrobin with an assigned dose of > 10% TAR. For this reason the toxicological endpoints of dimoxystrobin are also applying to the metabolites of Group 5. No further toxicological assessment is to be performed.

Table 5.8.1-5: Grouping of metabolites: Group 5 Hydroxylated and carboxylated metabolites

Metabolite	Occurrence	Metabolite Structure	Occurrence in rats
505M33[#]	Confined rotational crop (radish root)		Urine <1%** Bile 23%**
505M76	Goat (liver)		Not identified in rat metabolism
505M105	Goat (liver)		Not identified in rat metabolism

**sum of several, co-eluting metabolites, [#]representative metabolite, significant rat metabolite > 10% in rat bile

The overall conclusion for relevant endpoints for the current re-registration are drawn as follows:

Other toxicological studies (SANCO/11802 data point 5.8)

Toxicity studies of metabolites as referred to in the introduction

Group 1) Cleavage products:

505M01 (Representative Metabolite)

- not genotoxic in vitro based on experimental data
- not acutely toxic (oral route)
- below the TTC-threshold for non-genotoxic compound.

Conclusion: **not toxicologically relevant** based on metabolite specific information

505M80

- by weight of evidence not genotoxic in vitro
- by weight of evidence not acutely toxic
- below the TTC-threshold for non-genotoxic compound.

Conclusion: **not toxicologically relevant** based on grouping approach

Group 2) Hydroxylation products:

505M04 (Representative Metabolite)

- not genotoxic in vitro based on experimental data
- below the TTC-threshold for non-genotoxic compound.

Conclusion: **not toxicologically relevant** based on available information

505M02; 505M06; 505M50; 505M078; 505M84; 505M95 (hydroxylation at the phenyl ring)

- by weight of evidence not genotoxic in vitro
- below the TTC-threshold for non-genotoxic compound.

Conclusion: **not toxicologically relevant** based on grouping approach

505M49; 505M51; 505M63; 505M079; 505M91; 505M93 (hydroxylation of the methyl group)

- by weight of evidence not genotoxic in vitro
- below the TTC-threshold for non-genotoxic compound.

Conclusion: **not toxicologically relevant** based on grouping approach

505M107 (hydroxylation of the methyl group or at the phenyl ring)
- by weight of evidence not genotoxic in vitro
- below the TTC-threshold for non-genotoxic compound.

Conclusion: **not toxicologically relevant** based on grouping approach

505M86 (two hydroxylations in the molecule)
- by weight of evidence not genotoxic in vitro
- below the TTC-threshold for non-genotoxic compound.

Conclusion: **not toxicologically relevant** based on grouping approach

Group 3) Carboxylation products:

505M08
- not genotoxic based on experimental data
- not acutely toxic (oral route)

Conclusion: **not toxicologically relevant** based on available information

505M09
- not genotoxic based on experimental data
- not acutely toxic (oral route)
- below the TTC-threshold for non-genotoxic compound.

Conclusion: **not toxicologically relevant** based on available information

505M81
- by weight of evidence not genotoxic in vitro
- below the TTC-threshold for non-genotoxic compound.

Conclusion: **not toxicologically relevant** based on grouping approach

Group 4) Modifications at side chain:

505M88, 505M89, 505M94
- metabolites only found in feed items
- calculated consumer exposure values assuming dietary intake via products of animal origin are < 0.0000025 mg/kg.

Conclusion: **not toxicologically relevant** based on weight of evidence

Group 5) Hydroxylation and carboxylation products:

505M33, 505M76, 505M105
- metabolite 505M33 is a significant rat metabolite, identified in rat metabolism with an assigned dose of > 10% in the bile

Conclusion: covered by studies performed with parent dimoxystrobin

The toxicological assessment was performed based on experimental studies and/or QSAR analysis.

Presence of Structural alerts – QSAR evaluation of metabolites
For all metabolites irrespective of the presence of toxicological data, the presence for potential structural alerts was evaluated with different QSAR models. Models used were the OASIS TIMES and VEGA (Caesar, SarPy and Toxtree).

OASIS TIMES
OASIS TIMES is a hybrid statistical and knowledge-based model for toxicity prediction. The Tissue Metabolism Simulator (TIMES), developed by LMC (Bourgas University, Bulgaria; http://oasis-lmc.org/) integrates on the same platform a metabolic simulator and QSAR models for predicting toxicity of selected metabolites. The metabolic simulator generates plausible metabolic maps from a comprehensive library of biotransformations and abiotic reactions. It allows prioritization of chemicals according to toxicity of their metabolites. Of OASIS TIMES the prediction models for Ames test and in vitro chromosome aberration were considered and therefore predictivity is limited to these test systems only.

The reports for the evaluations made are available under BASF DocID 2014/1323732 and 2015/1106118 for prediction of Ames mutagenicity and chromosomal aberration in vitro.

The reactivity model describing interactions of chemicals with DNA is based on an alerting group approach. Only those toxicophores extracted from the training set having clear interpretation for the molecular mechanism causing the ultimate effect included in the model. The mechanistic interrelation between alerts and related parametric ranges generalizing the effect of the rest of the molecules on the alert is also considered. The structural component of the model is based on the structural similarity between chemicals in the training set which were correctly predicted by the model. The structural neighborhood of atom-centered fragments is used to determine this similarity. The training set consists of 1514 chemicals for Ames and 808 chemicals for chromosomal aberration.
The derived model is combined with metabolic simulator TIMES used for predicting metabolic activation of chemicals with the S9 mix. The metabolic simulator is trained to reproduce documented maps for mammalian liver metabolism for 261 chemicals. Parent chemicals and each of the generated metabolites are submitted to a battery of models to screen for a general effect and mutagenicity mechanisms. Thus, chemicals are predicted to be mutagenic as parents only, parents and metabolites, and metabolites only. Mutagenicity could be due to the parent chemical only or as a result of its metabolic activation (i.e., the parent is inactive but it is transformed to a mutagenic metabolite), or both parent structure and metabolites could be mutagenic.

This OASIS QSAR system is also included in the OECD Toolbox (but not in combination with TIMES), in order to make use of (Q)SAR approaches also in the assessment of chemicals under REACH. The BASF-internal full version has the advantage that it is capable to consider metabolic transformation.

VEGA
Using the VEGA platform, access to a series of QSAR (quantitative structure-activity relationship) models for regulatory purposes was obtained. Of the models offered by VEGA [http://www.vega-qsar.eu/] the three independent statistical/rules based prediction models for mutagenicity (Ames) were selected.

The data obtained for dimoxystrobin and its impurities can be found under BASF DocID 2015/1106120.

The first one is an implementation of **CAESAR**, which makes predictions based on the comparison of the structure of interest to the CAESAR database of mutagenicity data of substances in the structure database. A score is provided for the match of the structures, and the mutagenicity data of the closest related substances compared to the structure of interest. Consequently, if a structure is not adequately presented in the database, the prediction is only of very limited validity.

The second algorithm **SarPy** searches for isolated structural alerts of substructures in the molecule. Again this is based on the mutagenicity data provided in the structure database. Only predictivity for mutagenicity based on the Ames test was generated.

The third algorithm **Toxtree** is based on Benigni-Bossa Mutagenicity rules of structural alerts for mutagenicity. It works as a decision tree for estimating carcinogenicity, based on a list of structural alerts (SAs). The SAs for mutagenicity are molecular functional groups or substructures known to be linked to the mutagenic activity of chemicals. As one or more SAs embedded in a molecular structure are recognised, the system flags the potential mutagen of the chemical. The model goes through a first step in which a set of 12 SAs related to mutagenicty is checked The SAs are the following (SA numbers refer to the original Benigni/Bossa study):
- SA 1: Acyl halides
- SA 6: Propiolactones or propiosultones
- SA 11: Simple aldehyde
- SA 12: Quinones
- SA 13: Hydrazine
- SA 14: Aliphatic azo and azoxy
- SA 16: alkyl carbamate and thiocarbamate
- SA 18: Polycyclic Aromatic Hydrocarbons
- SA 21: alkyl and aryl N-nitroso groups
- SA 22: Azide and triazene groups
- SA 25: Aromatic nitroso group
- SA 28bis: Aromatic mono- and dialkylamine
- SA 29: Aromatic diazo

CAVEAT on reliability of QSAR modules implied

With regard to the QSAR evaluations as implied in OECD TIMES and in VEGA it should be noted that for nearly all analysis the algorithm reported an out of structural domain error. Each of this QSAR models is built on a set of chemicals that forms its chemical domain, space or applicability domain. That means that the prediction is best if a structure of interest is represented in the original baseline dataset. Substances outside of the dataset are evaluated in comparison to the chemical space, and only in case that the chemical space adequately covers all structural elements or the queried structure, the prediction is considered to be adequately covered by experimental data. Predictions outside of the applicability domain have far lower predictability. In addition all mentioned QSAR models check for structural alerts, like those identified by the Benigni-Bossa rules that have been implicated in mutagenic actions.

As a consequence, the predictivity is solely based on the proposed DNA-interaction via the structural alert, not (VEGA) or not appropriately (OASIS TIMES) taking into account possible functional group interaction and stereochemical hindrance. It is well established that structure elements have to be evaluated within the context of a structure.

In general, the predictivity of various QSAR models for genotoxicity equivalent to the Ames test has been considered to be reasonable accurate. Predictivity rates expressed as accuracy and specificity are usually >80%. This is in particular true, if information from more than one QSAR model is combined.

QSAR models for chromosome aberration however are far less well established. One of the underlying limitations is that typical in vitro assays for chromosome aberration, like chromosome aberration in V79 cells or the in vitro micronucleus assay have false discovery rates of approximately 30%. This means that three out of ten molecules are falsely categorized. In addition a high number of in vitro positive substances are negative in adequate in vivo assays. The latter is often a function of the underlying mode of action and the kinetic behavior of a substance. Both are not adequately covered by QSAR predictions.

Predictions for chromosome damage were performed with OASIS Times. The prediction model used in the OECD toolbox is similar to that of OASIS Times, with the major difference being the different underlying database of reference compounds and the combination with the metabolism generator in OASIS Times.

The VEGA system - with the CAESAR SarPy and ToxTree modules used - only predicts bacterial mutagenicity.

Threshold of toxicological concern concept

A further approach to assess whether chemical structures are of concern for which no or only limited information on the toxicological profile is available, is to consider whether the predicted exposure is above or below a threshold of toxicological concern. The threshold of toxicological concern (TTC) (Cramer et al., 1978; BASF DocID 1978/1001324; Kroes et al., 2004; BASF DocID 2004/1036074; Munro et al., 1996; BASF DocID 1996/1005180) has meanwhile been considered in the EU e.g. for the evaluation of chemicals under the REACH regulation [*ECHA (2012) Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health*] and has been employed or considered for the evaluation of food flavourings [*EFSA, 2010e. Guidance on the data required for the risk assessment of flavourings to be used in or on foods. European Food Safety Authority.*
The EFSA Journal 8(6): 1623. Available at: http://www.efsa.europa.eu/en/efsajournal/doc/1623.pdf] or pesticidal degradation products by EFSA [*EFSA, 2012b. Scientific Opinion: Exploring options for providing preliminary advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC). European Food Safety Authority (EFSA) Scientific Committee, Parma, Italy; EFSA Journal, 10(7), 2750 and EFSA, 2012a. Scientific opinion on evaluation of the toxicological relevance of pesticide metabolites for dietary risk assessment. EFSA Panel on Plant Protection Products and their Residues (PPR), European Food Safety Authority (EFSA), Parma, Italy; EFSA Journal, 10(07), 2799*].

The proposed threshold levels are 0.0025 µg/kg bw/day for potentially genotoxic compounds and 1.5 µg/kg bw/day for non-genotoxic Cramer Class III compounds.

The available toxicological database for metabolites will be discussed in the following paragraphs. This starts with an assessment of the QSAR predictions of the parent dimoxystrobin and is followed by the metabolites indicated above. For a better overview a tabulation of the QSAR results of dimoxystrobin and metabolites is provided below.

Table 5.8.1-6: Overview on QSAR evaluation of Dimoxystrobin and metabolites (Ames mutagenicity)

	QSAR prediction of mutagenicity (Ames)				
	OASIS Times (v2.27.13), Ames, with S9		VEGA		
			VEGA, Caesar 2.1.12	VEGA, SarPy 1.0.6-DEV	VEGA, TOXTREE 1.0.0-DEV
	Prediction (compound evaluated)	Overall prediction*	Prediction (compound evaluated)	Prediction (compound evaluated)	Prediction (compound evaluated)
Dimoxystrobin	Negative Out of domain	Negative Out of domain	Negative Could be out of domain	Negative Could be out of domain	Negative Out of domain
505M01 Reg.No. 358104	Negative Out of domain	Negative Out of domain	Negative Out of domain	Negative Out of domain	Negative Out of domain
505M04 Reg.No. 4035807	Negative Out of domain	Negative Out of domain	Negative In domain	Negative In domain	Negative Out of domain
505M02	Negative Out of domain	Negative Out of domain	Negative In domain	Negative In domain	Negative Out of domain
505M84	Negative Out of domain	Negative Out of domain	Negative In domain	Negative In domain	Negative Out of domain
505M08 Reg.No. 354562	Negative Out of domain	Negative Out of domain	Negative In domain	Negative In domain	Negative Out of domain
505M09 Reg.No. 354563	Negative Out of domain	Negative Out of domain	Negative In domain	Negative In domain	Negative Out of domain

*: Overall prediction of compound evaluated including presumed in silico metabolites.

Table 5.8.1-7: Overview on QSAR evaluation of Dimoxystrobin and metabolites (Chromosome aberration)

	QSAR prediction of chromosomal aberrations	
	OASIS Times (v2.27.13), CA, with S9	
	Prediction (compound evaluated)	Overall prediction*
Dimoxystrobin	Negative Out of domain	Positive Relevant metabolites in domain Alert: Phenols
505M01 Reg.No. 358104	Negative Out of domain	Negative Out of domain
505M04 Reg.No. 4035807	Negative Out of domain	Negative Out of domain
505M02	Positive Out of domain Alert: Substituted phenols	Positive Out of domain Alert: Substituted phenols
505M84	Positive Out of domain Alert: Substituted phenols	Positive Out of domain Alert: Substituted phenols, hydroxylated phenols
505M08 Reg.No. 354562	Negative Out of domain	Negative Out of domain
505M09 Reg.No. 354563	Negative Out of domain	Negative Out of domain

Dimoxystrobin (BAS 505 F):

a. QSAR predictions on dimoxystrobin:

OASIS TIMES (V.2.27.15.146; Mutagenicity S-9 activated v09.09) [see molecule 1 of reports BASF DocID 2015/1106126 and 2015/1106127]
There were **no** Ames mutagenicity alerts for dimoxystrobin or in-silico generated metabolites and no structural alerts were received. The parent structure was within the applicability domain, whereas the metabolite structures were not within the applicability domain.

Regarding in vitro chromosome aberration the prediction was negative for the parent substance. The structure was out of the applicability domain. For 9 out of 24 in-silico generated metabolites the structural alert "Substituted Phenols" was received and the prediction was positive for the metabolites with S9. The total predicted in vitro chromosome aberration was positive.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) [see molecule 1 of report BASF DocID 2015/1106128]
Dimoxystrobin could be out of the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. Two of the 6 most similar molecules were predicted and actual mutagens. The accuracy index (1.0), ACF matching index (1.0), and the descriptors range check were reliable. The similarity index (0.814) and concordance index (0.668) were moderately reliable.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) [see molecule 1 of report BASF DocID 2015/1106129]
Dimoxystrobin could be out of the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. Two of the 6 most similar molecules were actual mutagens. The similarity index (0.814) and ACF matching index (1.0) were reliable. The accuracy index (0.668) and concordance index (0.668) were moderately reliable.

VEGA: Mutagenicity model (Toxtree; version 1.0.0-DEV) [see molecule 1 of report BASF DocID 2015/1106134]
Dimoxystrobin is out of the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. One of the 6 most similar molecules was a predicted and actual mutagen. The accuracy index (1.0) and concordance index (1.0) were reliable. The similarity index (0.78) was moderately reliable and the ACF matching index (0.6) was not reliable.

b. Genotoxicity studies with dimoxystrobin:

The following genotoxicity studies are available with dimoxystrobin:

Test System	Test Object	Purity	Results	Reference BASF DocID
In vitro Mutagenicity in bacterial cells (Ames test) (with and without Metabolic activation)	*S. typhimurium* TA-98-100-1535-1537 *E. coli WP2 uvrA* Batch : N6	98.8%	Negative	1998/10411 1999/10454
In vitro Mutagenicity in mammalian cells (with and without metabolic activation)	CHO/HPRT Batch: N15	98.4%	Negative	1999/10539
In vitro cytogenicity Chromosome aberration assay (with and without activation)	Chinese hamster V79 cells Batch: N15	98.4%	Negative (Neither clastogenic effect on chromosome number)	1999/11417
In vivo cytogenicity micronucleus assay	Mouse bone marrow cells Batch: N15	98.4%	Negative	1998/10412 1998/10628

c. Conclusion on dimoxystrobin:

No conclusive structural alert was identified for dimoxystrobin regarding mutagenicity. The prediction was "non-mutagen" in all models used and is in concordance with available in vitro data (see M-CA 5.4).

Regarding in vitro chromosome aberration the structural alert "Substituted Phenols" was received for some hydroxylated metabolites. The structural alert is not reflected by experimental data (see M-CA 5.4). In vitro chromosome aberration (BASF DocID 1999/11417) and in vivo chromosome aberration (MNT, BASF DocID 1998/10412 and 1998/10628) were negative. This alert is not predictive and is therefore rejected.

It is concluded that dimoxystrobin has no genotoxic properties based on the results from several in vitro and in vivo genotoxicity studies.

A. Cleavage products of dimoxystrobin and their conjugates

I. Definition of group 1: Cleavage products of dimoxystrobin and their conjugates

For the group of metabolites containing cleavage products and their conjugates, the following molecules were taken into consideration:

II. Evaluation of group 1 members: Cleavage products of dimoxystrobin and their conjugates

1. 505M01 (Reg.No. 358104)

a. QSAR predictions on 505M01

OASIS TIMES (V.2.27.13 TB; Mutagenicity S-9 activated v08.08) (see molecule 2 of reports BASF DocID 2014/1323732 and 2015/1106118)
There were **no** Ames mutagenicity alerts for 505M01 or in-silico generated metabolites structural alerts were detected. In all cases the structures were not within the applicability domain.
For in-vitro chromosome aberration the prediction for 505M01 was **negative** (out of domain) with no specific structural alert. All in-silico generated metabolites were also predicted negative (out of domain) with no specific structural alert.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) (see molecule 2 of report BASF DocID 2015/1106120)
505M01 was out of model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. One of the six most similar molecules had positive predicted and experimental data. The other ones had the prediction non-mutagen, which was experimentally confirmed. The concordance of the total underlying database was low (0.334) and thus is not very robust. The molecules had a moderately similar structure to 505M01 (similarity 0.811 to 0.832).

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) (see molecule 2 of report BASF DocID 2015/1106120)
505M01 was out of model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. Three of the six most similar molecules had negative predicted and experimental data. Two of them were predicted negative but yielded positive experimental values. One molecule was predicted positive yielding negative experimental values. The concordance of the total underlying database was low (0.334) and thus is not very robust. The molecules had a moderately similar structure to 505M01 (similarity 0.811 to 0.832).

VEGA: Mutagenicity model (TOXTREE; version 1.0.0-DEV) (see molecule 2 of report BASF DocID 2015/1106120)
505M01 is out of the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. The concordance of the total underlying database was low (0.0) and thus is not very robust. The molecules had a moderately similar structure to 505M01 (similarity 0.783 to 0.831). Furthermore, the model is not reliable regarding Accuracy index and ACF matching index.

Conclusion on QSAR evaluations of 505M01
The QSAR evaluation of 505M01 is of low to moderate reliability and by weight of evidence there was no conclusive alert for genotoxicity.

b. Toxicological evaluation of 505M01

No conclusive alerts for genotoxicity were identified by the QSAR evaluation conducted. Furthermore, toxicity data are available for 505M01, which have been already evaluated. In summary, metabolite 505M01 is of low acute toxicity, the acute oral LD50 was > 2000 mg/kg bw for male and female rats (BASF DocID 2001/1001875). It was clearly not mutagenic in the bacterial reverse mutation assay (Ames assay) with and without metabolic activation (BASF DocID 2001/1018822). The HPRT assay provided no evidence for metabolite 505M01 causing gene mutation in mammalian cells (BASF DocID 2001/1001646). Furthermore, 505M01 was not clastogenic and did not increase the incidence of polyploidy cells in an in vitro chromosome aberration assay (BASF DocID 2001/1009006).

505M01 is considered to be not genotoxic based on the available information and no further testing is required. Therefore, 505M01 is considered to be **not toxicologically relevant**.

With regard to consumer exposure the TTC concept for a non-genotoxic compound was applied. Thus an ADI of 0.0015 mg/kg bw/day as well as an ARfD of 0.005 mg/kg bw/day was established. The estimated exposure to 505M01 is low (0.1% of TTC of 0.0015 mg/kg bw/day).

505M01
505M80 (conjugate of M01)

As the sugar conjugates (especially O-glycosyls) are likely to be cleaved in the intestinal tract, (see Bergmann et al., 2010: "Scientific report by Ages: Impact of metabolic and degradation process on toxicological properties of residues of pesticides in food commodities") the respective parent molecule should be considered relevant. Therefore, metabolite 505M01 was chosen as the representative metabolite of group 1. For 505M80 the same conclusion was drawn as for the **representative metabolite 505M01**.

B. Hydroxylation products of dimoxystrobin and their conjugates

I. Definition of group 2: Hydroxylation products of dimoxystrobin and their conjugates

Group 2 contains all hydroxylation products and their conjugates. The positions of hydroxylation are described below.

- M02, M06, M78, M84, M95 (hydroxylation at the phenyl ring)
- **M04**, M49, M50, M63, M79, M91, M93 (hydroxylation of the methyl group)
- M107 (hydroxylation of the methyl group or at the phenyl ring)
- M86 (two hydroxylations in the molecule)

As the representative metabolite for this group **505M04** with a hydroxylation of the methyl group was chosen.

II. Evaluation of group 2 members: Hydroxylation products of dimoxystrobin and their conjugates

1. 505M04 (Reg.No. 4035807)

a. QSAR predictions on 505M04

OASIS TIMES (V.2.27.13 TB; Mutagenicity S-9 activated v08.08) (see molecule 6 of report BASF DocID 2015/1106120)
There were **no** Ames mutagenicity alerts for 505M04 or in-silico generated metabolites structural alerts were detected. In all cases the structures were not within the applicability domain.
For in-vitro chromosome aberration the prediction for 505M04 was **negative** (out of domain) with no specific structural alert. All in-silico generated metabolites were also predicted negative (out of domain) with no specific structural alert.

VEGA: Mutagenicity model (CAESAR, version 2.1.12) (see molecule 6 of report BASF DocID 2015/1106120)
505M04 was in the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. One of the six most similar molecules had positive predicted and experimental data. The other ones had the prediction non-mutagen, which was experimentally confirmed. The concordance of the total underlying database was high (1.0) and thus is robust. The molecules had a moderately similar structure to 505M04 (similarity 0.800 to 0.823).

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) (see molecule 6 of report BASF DocID 2015/1106120)
505M04 was in the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. One of the six most similar molecules had negative predicted but positive experimental data. The other five molecules were predicted negative, which was confirmed by experimental data. The concordance of the total underlying database was high (1.0) and thus is robust. The molecules had a moderately similar structure to 505M04 (similarity 0.800 to 0.823).

VEGA: Mutagenicity model (TOXTREE; version 1.0.0-DEV) (see molecule 6 of report BASF DocID 2015/1106120)
505M04 is out of the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. The concordance of the total underlying database was moderate (0.501) and thus is not very robust. The molecules had a moderately similar structure to 505M01 (similarity 0.762 to 0.780). Furthermore, the model is not reliable regarding ACF matching index (0.6), whereby the accuracy index was high (1.0).

Conclusion on QSAR evaluations of 505M04
The QSAR evaluation of 505M04 is of moderate reliability and by weight of evidence there was no conclusive alert for genotoxicity.

b. Toxicological evaluation of 505M04

No conclusive alerts for genotoxicity were identified by the QSAR evaluation conducted. Generally, hydroxylation of a molecule within the metabolizing process is considered as a detoxification step. Also in the EFSA Scientific Opinion it is stated, that hydroxylation of the ring system without any cleavage of the ring, or hydroxylation of another ring position than the parent was identified as probably not causing higher toxicity of metabolites. Furthermore, additional genotoxicity data are available for 505M04. The metabolite 505M04 was clearly not mutagenic in the bacterial reverse mutation assay (Ames assay, BASF DocID 2015/1028370) with and without metabolic activation. Furthermore, 505M04 was not clastogenic or aneugenic in an in vivo micronucleus assay (BASF DocID 2015/1040946).

Table 5.8.1-8: Overview on available toxicity data of 505M04 (Metabolite of dimoxystrobin)

Category of test	Dose range	Results	Reference (BASF DocID)
Ames mutagenicity test TA 1535, 100, 1537, 98 E. coli WP2 uvrA ±S9 mix 505M04	33 – 5100 µg/plate	Negative	2015/1028370
Micronucleus test in Human Lymphocytes ±S9 mix 4 and 20 h exposure 505M04	3.9 – 600 µg/mL (-S9) 13.3 – 2045 µg/mL (+S9)	Negative	2015/1040946

505M04 is considered to be not genotoxic based on the available information and no further testing is required. Therefore, 505M04 is considered to be **not toxicologically relevant**.

With regard to consumer exposure the TTC concept for a non-genotoxic compound was applied. Thus an ADI of 0.0015 mg/kg bw/day as well as an ARfD of 0.005 mg/kg bw/day was established. The estimated exposure to 505M04 is low (26.4% of TTC of 0.0015 mg/kg bw/day).

Studies on metabolite 505M04

Report: CA 5.8.1/1
Woitkowiak C., 2015a
Reg.No. 4035807 (metabolite of BAS 505 F) - Salmonella typhimurium / Escherichia coli - Reverse mutation assay
2015/1028370

Guidelines: OECD 471, (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/B.14 No. L 142, EPA 870.5100

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

Executive Summary

S. typhimurium and E. coli were exposed to Reg.No. 4035807 (Metabolite of dimoxystrobin; Batch: 01658-157, Purity: 97.8%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in a standard plate and preincubation test. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. In the standard plate and preincubation test Reg.No. 4035807 was tested at concentrations of 33 to 5100 µg/plate. In the plate incorporation assay a weak bacteriotoxic effect was observed in the tester strain TA 98 in the presence of S9 mix at 5100 µg/plate. In the preincubation assay a weak bacteriotoxic effect was observed occasionally depending on the strain and test conditions at concentrations of 1000 µg/plate onward. Precipitation of the test substance did not occur up to the highest tested concentration.
A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in the presence or absence of metabolic activation in any of the experiments. The number of revertant colonies in the negative controls was within the range of the historical negative control data for each tester strain in the presence or absence of S9 mix. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.
According to the results of the study, test substance Reg.No. 4035807 was not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2015/1028370)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 4035807 (Metabolite of dimoxystrobin)

Description: Solid, beige

Lot/Batch #: 01658-157

Purity: 97.8% (tolerance ±1.0%)

Stability of test compound: The test substance was stable over the study period under the storage conditions and guaranteed until July 2024. The stability in DMSO was verified analytically.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Solvent/final concentration: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

To demonstrate the efficacy of the S9 mix in this assay, the S9 batch was further characterized with benoz(a)pyrene in the strains TA 100 and TA 98 as required in OECD 471.

3. Activation:

S9 was produced from the livers of induced male Wistar rats. The rats received phenobarbital (80 mg/kg bw; i.p.) and β-naphthoflavone (80 mg/kg bw; orally) each on three consecutive days. 24 h after the last administration the animals were sacrificed and the livers were prepared. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
Salmonella typhimurium:
The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay: In the first experiment triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2550, 5100 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without S9) for all tester strains indicated above. A second experiment was performed as the number of revertant colonies in the negative control was within the range of the historical control data, but the values were very low, therefore a new tester strain was used for the further experiments (new TA 100).

Pre-incubation assay: In the third experiment triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2550, 5100 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains.

B. TEST PERFORMANCE:

1. Dates of experimental work: 14-Jan-2015 to 30-Jan-2015

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates). In the experiments with E. coli the warm soft agar contains 0.5 mM tryptophan instead of histidine + biotin. After incubation for 48-72 h at 37°C, his^+ or trp^+ revertants were counted.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.
After incubation in the dark for 48 to 72 hours at 37°C the bacterial colonies were counted.

4. Statistics:

No special statistical tests were performed.

5. Evaluation criteria:

Solubility:

Precipitation of the test material was recorded and indicated in the tables. As long as precipitation did not interfere with the colony scoring, 5 mg/plate was generally selected and analyzed (in cases of nontoxic compounds) as the maximum dose at least in the 1st Experiment even in the case of relatively insoluble test compounds to detect possible mutagenic impurities. Furthermore, doses > 5 mg/plate might also be tested in repeat experiments for further clarification/substantiation.

Toxicity:

Toxicity detected by a

- Decrease in the number of revertants (factor ≤ 0.6)
- Clearing or diminution of the background lawn (reduced his^- or trp^- background growth)

was recorded for all test groups both with and without S9 mix in all experiments and indicated in the tables. Single values with a factor ≤ 0.6 were not detected as toxicity in low dose groups.

Mutagenicity:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling (bacteria strains with high spontaneous mutation rate, like TA 98, TA 100 and E.coli WP2 uvrA) or tripling (bacteria strains with low spontaneous mutation rate, like TA 1535 and TA 1537) of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions. The stability of the test substance at room temperature in the vehicle DMSO was verified analytically.

B. TOXICITY AND SOLUBILITY

A weak bacteriotoxic effect (slight decrease in the number of his^+ revertants) was observed in the standard plate test only in the tester strain TA 98 with S9 mix at 5100 µg/plate.
In the preincubation assay bacteriotoxicity (slight decrease in the number of his^+ revertants) was occasionally observed depending on the strain and test conditions from about 1000 µg/plate onward.
No test substance precipitation was found with and without S9 mix.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiment with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested [see **Table 5.8.1-14**]. The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Table 5.8.1-9: Bacterial gene mutation assay with Reg.No. 4035807 - Mean number of revertants

Experiment 1/(2): Plate incorporation assay*										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	28 ± 6	22 ± 2	40 ± 4 (108 ± 9)	24 ± 2 (93 ± 4)	10 ± 2	10 ± 1	11 ± 1	8 ± 3	56 ± 9	55 ± 4
Test substance										
33 µg/plate	25 ± 6	25 ± 11	22 ± 7 (107 ± 12)	21 ± 6 (91 ± 14)	13 ± 7	12 ± 4	10 ± 6	8 ± 3	54 ± 14	71 ± 5
100 µg/plate	23 ± 3	20 ± 3	25 ± 3 (116 ± 7)	22 ± 1 (88 ± 12)	12 ± 3	12 ± 2	14 ± 6	8 ± 3	62 ± 11	57 ± 11
333 µg/plate	21 ± 3	18 ± 3	23 ± 4 (102 ± 13)	20 ± 4 (101 ± 19)	9 ± 2	14 ± 2	10 ± 2	14 ± 4	63 ± 3	67 ± 2
1000 µg/plate	21 ± 5	14 ± 3	25 ± 1 (101 ± 5)	21 ± 3 (90 ± 3)	12 ± 4	13 ± 5	7 ± 2	11 ± 3	56 ± 11	59 ± 14
2550 µg/plate	23 ± 1	19 ± 2	23 ± 4 (100 ± 9)	20 ± 4 (101 ± 17)	13 ± 7	13 ± 1	10 ± 1	8 ± 1	62 ± 11	48 ± 4
5100 µg/plate	15 ± 5	20 ± 6	23 ± 7 (97 ± 2)	21 ± 4 (90 ± 8)	11 ± 4	12 ± 5	8 ± 3	6 ± 3	60 ± 10	50 ± 7
Pos. control§	1505 ± 198	426 ± 25	492 ± 160 (1961 ± 294)	1962 ± 417 (3957 ± 207)	248 ± 39	4574 ± 184	130 ± 22	925 ± 240	137 ± 26	932 ± 51
Experiment 3: Preincubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	26 ± 3	13 ± 3	91 ± 19	87 ± 6	9 ± 2	10 ± 2	8 ± 3	13 ± 4	66 ± 12	60 ± 14
Test substance										
33 µg/plate	22 ± 4	16 ± 1	95 ± 17	96 ± 4	11 ± 7	10 ± 5	11 ± 4	8 ± 2	69 ± 11	66 ± 10
100 µg/plate	23 ± 4	17 ± 2	102 ± 12	83 ± 7	11 ± 2	12 ± 4	6 ± 1	9 ± 2	71 ± 8	68 ± 12
333 µg/plate	22 ± 2	19 ± 5	97 ± 15	96 ± 17	13 ± 2	13 ± 2	9 ± 3	8 ± 6	65 ± 6	58 ± 8
1000 µg/plate	25 ± 6	11 ± 2	93 ± 8	96 ± 7	10 ± 4	10 ± 1	11 ± 3	5 ± 3	74 ± 6	61 ± 7
2550 µg/plate	21 ± 3	16 ± 3	92 ± 11	95 ± 6	14 ± 4	8 ± 1	7 ± 2	6 ± 3	76 ± 6	57 ± 5
5100 µg/plate	23 ± 4	11 ± 2	90 ± 16	80 ± 10	13 ± 5	8 ± 2	5 ± 1	5 ± 1	65 ± 4	57 ± 5
Pos. control§	1293 ± 186	414 ± 95	1859 ± 208	1958 ± 45	219 ± 27	2912 ± 173	150 ± 16	520 ± 46	139 ± 21	393 ± 63

*: Numbers may differ from original data due to rounding

§ = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSION

According to the results of the present study, the test substance Reg.No. 4035807 (Metabolite of dimoxystrobin) is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report: CA 5.8.1/2
Bohnenberger S., 2015a
Reg.No. 4035807 (metabolite of BAS 505 F): Micronucleus test in human lymphocytes in vitro
2015/1040946

Guidelines: OECD 487 (2014)

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

Reg.No. 4035807 (Metabolite of dimoxystrobin) (Batch: 01658-157; Purity: 97.8%) was tested for its potential to induce micronuclei in human lymphocytes *in vitro* in the absence and presence of metabolic activation by S9 mix. Two independent experiments were performed where the cells were incubated for 4 (±S9 mix) or 20 hours (-S9 mix) with the test substance at concentrations in the range of 3.9 to 2045 µg/mL. The vehicle DMSO served as negative control, mitomycin C (4 h) and demecolcin (20 h) as positive controls in the absence of metabolic activation and cyclophosphamide as positive control in the presence of metabolic activation. Treatments started after a 48 hour stimulation period with phytohemeagglutinine. Thereafter cytochalasin B was added and the cultures were fixed and stained finally after another 20 hours. Cytokinesis-block proliferation index and cytostasis were determined in 500 binucleated cells/culture as cytotoxicity parameters and number of micronucleated cells were determined in 1000 binucleated cells/culture for evaluation of mutagenicity.

In Experiment I precipitation of the test item in the culture medium was observed at 2045 µg/mL in the presence of S9 mix at the end of treatment observed with the unaided eye. In all other experimental parts, no precipitation was observed. No relevant influence on osmolarity or pH value was observed. In Experiment I and II in the absence and presence of S9 mix, clear cytotoxicity was observed at the highest evaluated concentration. In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying micronuclei was observed. The micronucleus rates of the cells after treatment with the test item (0.25 – 1.15 % micronucleated cells) were close to the range of the solvent control values (0.25 – 1.00 % micronucleated cells) and within the range of the laboratory historical control data. The positive control chemicals led to the expected increase in cells containing micronuclei, thus demonstrating the sensitivity of the test system.

In conclusion, Reg.No. 4035807 (Metabolite of dimoxystrobin) is considered to be non-mutagenic in this *in vitro* micronucleus test when tested up to cytotoxic concentrations.

(BASF DocID 2015/1040946)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg. No. 4035807 (Metabolite of dimoxystrobin)

Description: solid / beige

Density: NA

Lot/Batch #: 01658-157

Purity/content: 97.8%

Stability of test compound: The stability of the test item under storage conditions over the study period was guaranteed by the sponsor (expiry date July, 2024).

Vehicle used: DMSO (0.5% final concentration)

2. Control Materials:

Negative: No negative control was employed in this study.

Vehicle control: Culture medium with 0.5% DMSO

Positive control: Without metabolic activation:
Mitomycin C (MMC, 3 µg/mL; pulse treatment) dissolved in deionized water;
Demecolcin (50 ng/mL; continuous treatment) dissolved in deionized water
With metabolic activation:
Cyclophosphamide (CCP, 12.5 µg/mL) dissolved in saline (0.9% NaCl)

3. Activation: S9 was produced from the livers of rats pretreated with β-naphthoflavone/phenobarbital. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature mixed with an appropriate volume of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM

50 µL S9 mix per mL culture medium were added yielding a final protein concentration of 0.75 mg/mL in the cultures.

4. Test organism: Human peripheral blood lymphocytes

5. Culture media:

Culture medium: Dulbecco´s Modified Eagles medium/Ham´s F12 (1:1) with GlutaMAX^{TM} (200 mM) supplemented with 10% (v/v) fetal bovine serum (FBS), Pen/Strep (100 U/mL/100 µg/mL), HEPES (10 mM), heparin (125 U.S.P.-U/mL), phytohemeagglutinine (PHA, 3 µg/mL).

6. Test concentrations:

Micronucleus assay

Experiment I
(4-h exposure, ±S9): 13.3, 23.2, 40.7, 71.2, 124.6, 218, 381.6, 667.8, 1168.6, 2045 µg/mL

Experiment II
(4-h exposure, +S9): 25, 50, 100, 200, 250, 300, 350, 400, 500, 600 µg/mL
(20-h exposure, -S9): 3.9, 6.8, 11.9, 20.9, 36.6, 64.0, 112.0, 195.9, 342.9, 600 µg/mL

B. TEST PERFORMANCE

1. Dates of experimental work: 26-Nov-2014 to 20-Jan-2015

2. Dose selection:

Dose selection was performed according to the current OECD Guideline for the *in vitro* micronucleus test. The highest test item concentration should be 2000 µg/mL, 2 µL/mL or 10 mM, whichever is the lowest. At least three test item concentrations should be evaluated for cytogenetic damage.

A preliminary cytotoxicity test was performed to determine the concentrations to be used in the main experiment. Cytotoxicity is characterized by the percentages of reduction in the CBPI in comparison with the controls (% cytostasis) by counting 500 cells per culture in duplicate. The experimental conditions in this pre-experimental phase were identical to those required and described below for the mutagenicity assay. The pre-test was performed with 10 concentrations of the test item separated by no more than a factor of $\sqrt{10}$ and a solvent and positive control. All cell cultures were set up in duplicate. Exposure time was 4 hrs (with and without S9 mix). The preparation interval was 40 hrs after start of the exposure.

With regard to the purity (97.8 %) of the test item, 2045.0 µg/mL of Reg.No. 4035807 (Metabolite of dimoxystrobin) were applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations ranging from 13.3 to 2045 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. In the pre-test for toxicity, precipitation of the test item was observed at the end of treatment at 2045 µg/mL in the presence of S9 mix. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.

Using a reduced Cytokinesis-block proliferation index (CBPI) as an indicator for toxicity clear toxic effects were observed after 4 hours treatment with 218.0 µg/mL and above in the absence of S9 mix and with 381.6 µg/mL and above in the presence of S9 mix. Therefore, 600.0 µg/mL (with and without S9 mix) were chosen as top concentration in Experiment II.

3. Micronucleus test:

Pulse exposure:

About 48 h after seeding 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL culture medium was added. After 4 h the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were resuspended in and washed with "saline G" (pH 7.2, containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4). The washing procedure was repeated once as described. The cells were resuspended in complete culture medium with 10 % FBS (v/v) and cultured for a 16-hour recovery period. After this period Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Continuous exposure

About 48 h after seeding 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with complete medium (with 10 % FBS) containing the test item. After 20 hours the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in and washed with "saline G". The washing procedure was repeated once as described. After washing the cells were re-suspended in complete culture medium containing 10 % FBS (v/v). Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Preparation of cells

The cultures were harvested by centrifugation 40 h after beginning of treatment. The cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were re-suspended in approximately 5 mL saline G and spun down once again by centrifugation for 5 minutes. Then the cells were resuspended in 5 mL KCl solution (0.0375 M) and incubated at 37°C for 20 minutes. 1 mL of ice-cold fixative mixture of methanol and glacial acetic acid (19 parts plus 1 part, respectively) was added to the hypotonic solution and the cells were resuspended carefully. After removal of the solution by centrifugation the cells were resuspended for 2 x 20 minutes in fixative and kept cold. The slides were prepared by dropping the cell suspension in fresh fixative onto a clean microscope slide. The cells were stained with Giemsa.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Chi square test.

5. Cytotoxicity evaluation:

Evaluation of cytotoxicity and cytogenetic damage

Evaluation of the slides was performed using NIKON microscopes with 40 x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The micronuclei have to be stained in the same way as the main nucleus. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 binucleate cells per culture were evaluated for cytogenetic damage on coded slides, except for the positive control in Experiment I in the absence of S9 mix, where only 500 binucleated cells were evaluated. The frequency of micronucleated cells was reported as % micronucleated cells. To describe a cytotoxic effect the CBPI was determined in 500 cells per culture and cytotoxicity is expressed as % cytostasis. A CBPI of 1 (all cells are mononucleate) is equivalent to 100 % cytostasis.

$$CBPI = \frac{(MONC \times 1) + (BINC \times 2) + (MUNC \times 3)}{n}$$

CBPI	Cytokinesis-block proliferation index
n	Total number of cells
MONC	Mononucleate cells
BINC	Binucleate cells
MUNC	Multinucleate cells

$$\text{Cytostasis } \% = 100 - 100\,[(CBPI_T - 1) / (CBPI_C - 1)]$$

T	Test item
C	Solvent control

6. Evaluation criteria:

Acceptability criteria:

- The rate of micronuclei in the solvent controls falls within the historical laboratory control data range.
- The rate of micronuclei in the positive controls is statistically significant increased.
- The quality of the slides must allow the evaluation of a sufficient number of analyzable cells.

Evaluation criteria:

A test item can be classified as non-clastogenic and non-aneugenic if:

- the number of micronucleated cells in all evaluated dose groups is in the range of the historical laboratory control data and
- no statistically significant or concentration-related increase of the number of micronucleated cells is observed in comparison to the respective solvent control.

A test item can be classified as clastogenic and aneugenic if:

- the number of micronucleated cells is not in the range of the historical laboratory control data and
- either a concentration-related increase in three test groups or a statistically significant increase in the number of micronucleated cells is observed.

If the above mentioned criteria for the test item are not clearly met, the test item will be classified as equivocal or a confirmatory experiment may be performed. However, results may remain questionable regardless of the number of times the experiment is repeated.

An increase in the number of micronucleated mononucleate cells may indicate that the test item has aneugenic potential.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

All formulations were prepared freshly before treatment and used within two hours of preparation. The formulation was assumed to be stable for this period unless specified otherwise by the Sponsor. Therefore, no analytical determinations were performed.

B. CYTOTOXICITY

In each experimental group two parallel cultures were analysed. 1000 binucleated cells per culture were evaluated for cytogenetic damage on coded slides, except for the positive control in Experiment I in the absence of S9 mix, where only 500 binucleated cells were evaluated due to strong clastogenic effects. To determine a cytotoxic effect the CBPI was determined in 500 cells per culture and cytotoxicity is described as % cytostasis.

The highest treatment concentration in this study, 2045 µg/mL was chosen with regard to the purity (97.8 %) of the test item and with respect to the OECD Guideline 487 for the *in vitro* mammalian cell micronucleus test.

In Experiment I precipitation of the test item in the culture medium was observed at 2045 µg/mL in the presence of S9 mix at the end of treatment observed with the unaided eye. In all other experimental parts, no precipitation was observed. No relevant influence on osmolarity or pH value was observed.

In Experiment I and II in the absence and presence of S9 mix, clear cytotoxicity was observed at the highest evaluated concentration.

B. MICRONUCLEUS ASSAY

In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying micronuclei was observed. The micronucleus rates of the cells after treatment with the test item (0.25 – 1.15 % micronucleated cells) were close to the range of the solvent control values (0.25 – 1.00 % micronucleated cells) and within the range of the laboratory historical control data.

In both experiments, either Demecolcin (50.0 ng/mL), MMC (3.0 µg/mL) or CPA (12.5 µg/mL) were used as positive controls and showed distinct increases in cells with micronuclei.

Table 5.8.1-10: Summary of results of the in vitro micronucleus test in human lymphocytes with Reg.No. 4035807 (Metabolite of dimoxystrobin)

Exp.	Preparation interval	Test item concentration in µg/mL	Proliferation index CBPI	Cytostasis in %[a]	Micronucleated cells in %[b]
Exposure period 4 hrs without S9 mix					
I	40 hrs	Solvent control[1]	1.96		0.30
		Positive control[2#]	1.30	69.0	**22.50***
		71.2	1.85	11.5	0.25
		124.6	1.71	26.6	0.25
		218.0	1.46	52.2	0.30
Exposure period 20 hrs without S9 mix					
II	40 hrs	Solvent control[1]	1.77		0.45
		Positive control[3]	1.86	n.c.	**2.85***
		11.9	1.67	13.4	0.45
		20.9	1.52	33.1	0.45
		36.6	1.42	45.9	0.75
		64.0	1.34	55.6	0.65
Exposure period 4 hrs with S9 mix					
I	40 hrs	Solvent control[1]	1.98		0.25
		Positive control[4]	1.63	36.2	**2.40***
		71.2	1.93	5.5	0.45
		124.6	1.73	26.0	0.45
		218.0	1.60	39.0	0.40
		381.6	1.31	68.4	0.35
II	40 hrs	Solvent control[1]	1.80		1.00
		Positive control[4]	1.72	9.8	**2.30***
		50.0	1.72	10.5	0.75
		100.0	1.62	22.4	1.05
		200.0	1.37	53.3	1.15

a: For the positive control groups and the test item treatment groups the values are related to the solvent controls
b: The number of micronucleated cells was determined in a sample of 2000 binucleated cells
#: The number of micronucleated cells was determined in a sample of 1000 binucleated cells
*: The number of micronucleated cells is statistically significantly higher than corresponding control values ($p \leq 0.05$)
1 DMSO 0.5 % (v/v)
2 MMC 3.0 µg/mL
3 Demecolcin 50.0 ng/mL
4 CPA 12.5 µg/mL

III. CONCLUSION

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce micronuclei as determined by the *in vitro* micronucleus test in human lymphocytes. Therefore, Reg.No. 4035807 (Metabolite of dimoxystrobin) is considered to be non-mutagenic in this *in vitro* micronucleus test when tested up to cytotoxic concentrations.

2. 505M02

a. QSAR predictions on 505M02

OASIS TIMES (V.2.27.13 TB; Mutagenicity S-9 activated v08.08) (see molecule 3 of reports BASF DocID 2014/1323732 and 2015/1106118)

There were **no** Ames mutagenicity alerts for 505M02 or in-silico generated metabolites structural alerts were detected. In all cases the structures were not within the applicability domain.

For in-vitro chromosome aberration the prediction for 505M02 was **positive** (out of domain) referring to the structural alert info “Substituted Phenols”. Three out of 12 in-silico metabolites were predicted positive (out of domain) with the alert info ‘Substituted Phenols’. The remaining in-silico metabolites were negative (domain info: N/A or no domain).

VEGA: Mutagenicity model (CAESAR, version 2.1.12) (see molecule 3 of report BASF DocID 2015/1106120)

505M02 was in the model applicability domain. The prediction is **‘non-mutagen’** with no specific structural alerts. The similarity index of the total underlying database was moderate (0.812) and thus is reliable with restriction. The concordance index, accuracy index, ACF matching index, and the model descriptors range check revealed that the model was reliable regarding these aspects.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) (see molecule 2 of report BASF DocID 2015/1106120)

505M02 was in the model applicability domain. The prediction is **‘non-mutagen’** with no specific structural alerts. The similarity index, concordance index, accuracy index, and ACF matching index revealed that the model was reliable regarding these aspects.

VEGA: Mutagenicity model (TOXTREE; version 1.0.0-DEV) (see molecule 2 of report BASF DocID 2015/1106120)

505M02 is out of the model applicability domain. The prediction is **‘non-mutagen’** with no specific structural alerts. The concordance of the total underlying database was moderate (0.500) and thus is not very robust. The molecules had a moderately similar structure to 505M02 (similarity 0.763 to 0.786). Furthermore, the model is not reliable regarding ACF matching index (0.6), whereby the accuracy index was high (1.0).

Conclusion on QSAR evaluations of 505M02

The QSAR evaluation of 505M02 is of moderate to high reliability and by weight of evidence there was no conclusive alert for genotoxicity. All models predicted the metabolite as **‘non-mutagen’.**

b. Toxicological evaluation of 505M02

No conclusive alerts for genotoxicity were identified by the QSAR evaluation conducted. In general, hydroxylation of the ring system without any cleavage of the ring, or hydroxylation of another ring position than the parent was identified as probably not causing higher toxicity of metabolites. Therefore, hydroxylated metabolites are considered to be less toxic than the parent compound, which has shown to be not genotoxic in several experiments.
No additional genotoxicity data are available for 505M02 itself but for the analogue substance 505M04, which was chosen as the representative metabolite of group 2 (Hydroxylation products). Negative mutagenic and clastogenic results were observed with 505M04 in an Ames test and a Micronucleus test in vivo. By weight of evidence, the negative QSAR prediction together with the negative experimental results of the parent substance and the analogue substance 505M04 indicate that 505M02 possesses no genotoxic potential.

505M02 is considered to be not genotoxic based on the available information and no further testing is required. Therefore, 505M02 is considered to be **not toxicologically relevant**.

With regard to consumer exposure the TTC concept for a non-genotoxic compound was applied. Thus an ADI of 0.0015 mg/kg bw/day as well as an ARfD of 0.005 mg/kg bw/day was established. Metabolite 505M02 is only found in feed items, therefore calculated consumer exposure values assuming dietary intake via products of animal origin are < 0.0015 mg/kg bw/day.

3. 505M84

a. QSAR predictions on 505M84

OASIS TIMES (V.2.27.13 TB; Mutagenicity S-9 activated v08.08) (see molecule 4 of reports BASF DocID 2014/1323732 and 2015/1106118)
There were **no** Ames mutagenicity alerts for 505M84 or in-silico generated metabolites structural alerts were detected. In all cases the structures were not within the applicability domain. For one metabolite (No 23) a structural alert ‘Quinones’ was received not influencing the negative prediction. For in-vitro chromosome aberration the prediction for 505M02 was **positive** (out of domain) referring to the structural alert info “Substituted Phenols”. Eighteen out of 25 in-silico metabolites were predicted positive (domain info: N/A) with the alert info ‘Substituted Phenols’ or ‘Hydroxylated Phenols’. The remaining in-silico metabolites were negative (domain info: N/A).

VEGA: Mutagenicity model (CAESAR, version 2.1.12) (see molecule 4 of report BASF DocID 2015/1106120)
505M84 was in the model applicability domain. The prediction is **‘non-mutagen’** with no specific structural alerts. The similarity index of the total underlying database was moderate (0.812) and thus is reliable with restriction. The concordance index, accuracy index, and ACF matching index revealed that the model was reliable regarding these aspects.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) (see molecule 4 of report BASF DocID 2015/1106120)
505M84 was in the model applicability domain. The prediction is **‘non-mutagen’** with no specific structural alerts. The similarity index, concordance index, accuracy index, and ACF matching index revealed that the model was reliable regarding these aspects.

VEGA: Mutagenicity model (TOXTREE; version 1.0.0-DEV) (see molecule 4 of report BASF DocID 2015/1106120)
505M84 is out of the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. The concordance of the total underlying database was low (0.500) and thus is not very robust. The molecules had a moderately similar structure to 505M84 (similarity 0.767 to 0.783). Furthermore, the model is not reliable regarding ACF matching index (0.6), whereby the accuracy index was high (1.0).

Conclusion on QSAR evaluations of 505M84
The QSAR evaluation of 505M84 is of moderate to high reliability and by weight of evidence there was no conclusive alert for genotoxicity. All models predicted the metabolite as **'non-mutagen'.**

b. Toxicological evaluation of 505M84

No conclusive alerts for genotoxicity were identified by the QSAR evaluation conducted. In general, hydroxylation of the ring system without any cleavage of the ring, or hydroxylation of another ring position than the parent was identified as probably not causing higher toxicity of metabolites. Therefore, hydroxylated metabolites are considered to be less toxic than the parent compound, which has shown to be not genotoxic in several experiments.
No additional genotoxicity data are available for 505M84 itself but for the analogue substance 505M04, which was chosen as the representative metabolite of group 2 (Hydroxylation products). Negative mutagenic and clastogenic results were observed with 505M04 in an Ames test and a Micronucleus test in vivo. By weight of evidence, the negative QSAR prediction together with the negative experimental results of the parent substance and the analogue substance 505M04 indicate that 505M84 possesses no genotoxic potential.

505M84 is considered to be not genotoxic based on the available information and no further testing is required. Therefore, 505M84 is considered to be **not toxicologically relevant**.

With regard to consumer exposure the TTC concept for non-genotoxic compounds was applied. Thus an ADI of 0.0015 mg/kg bw/day as well as an ARfD of 0.005 mg/kg bw/day was established. The estimated exposure (up to 9.4% of TTC of 0.005 mg/kg bw/day for acute and 0.7% of TTC of 0.0015 mg/kg bw/day for chronic exposure) is low.

C. Carboxylation products of dimoxystrobin and their conjugates

I. Definition of group 3: Carboxylation products of dimoxystrobin and their conjugates

Group 3 contains all carboxylated metabolites and their conjugates M08, M09. As metabolites **M08** and **M09** are also relevant groundwater metabolites, either could be chosen as the representative metabolites for group 3 based on the available study data. Genotoxicity data, as well as studies on acute oral toxicity are already available for these two metabolites as summarized below.

II. Evaluation of group 3 members: Carboxylation products of dimoxystrobin and their conjugates

1. 505M08 (Reg.No. 354562)

a. QSAR predictions on 505M08

OASIS TIMES (V.2.27.13 TB; Mutagenicity S-9 activated v08.08) (see molecule 9 of reports BASF DocID 2014/1323732 and 2015/1106118)
There were **no** Ames mutagenicity alerts for 505M08 or in-silico generated metabolites structural alerts were detected. In all cases the structures were not within the applicability domain.
For in-vitro chromosome aberration the prediction for 505M08 was **negative** (out of domain) with no specific structural alert. All of the 19 in-silico metabolites were predicted negative (domain info: N/A or no domain).

VEGA: Mutagenicity model (CAESAR, version 2.1.12) (see molecule 9 of report BASF DocID 2015/1106120)
505M08 was in the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. The similarity index of the total underlying database was moderate (0.830) and thus is reliable with restriction. The concordance index, accuracy index, and ACF matching index revealed that the model was reliable regarding these aspects.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) (see molecule 9 of report BASF DocID 2015/1106120)
505M08 was in the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. The similarity index, concordance index, accuracy index, and ACF matching index revealed that the model was reliable regarding these aspects.

VEGA: Mutagenicity model (TOXTREE; version 1.0.0-DEV) (see molecule 9 of report BASF DocID 2015/1106120)
505M08 is out of the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. The concordance of the total underlying database was moderate (0.509) and thus is not very robust. The molecules had a moderately similar structure to 505M08 (similarity 0.766 to 0.801). Furthermore, the model is not reliable regarding ACF matching index (0.6) and moderately reliable regarding the accuracy index (0.509).

Conclusion on QSAR evaluations of 505M08
The QSAR evaluation of 505M08 is of moderate to high reliability and by weight of evidence there was no conclusive alert for genotoxicity.

b. Toxicological evaluation of 505M08

No conclusive alerts for genotoxicity were identified by the QSAR evaluation conducted. Furthermore, additional general toxicity and genotoxicity data are available for 505M08. Metabolite 505M08 is of low acute toxicity, the acute oral LD50 was > 2000 mg/kg for rats. It was not mutagenic in a bacterial reverse mutation assay (Ames assay) with and without metabolic activation. Based on the results of a HPRT assay it was concluded, that the metabolite did not induce forward mutations in mammalian cells in vitro. In an in vitro CA assay metabolite 505M08 was a weak chromosome-damaging (clastogenic) agent at the high dose exceeding solubility. Therefore, secondary effects resulting from extreme culture conditions are most likely the cause for this finding. An in vivo micronucleus test clearly showed that metabolite 505M08 did not induce the formation of micronuclei in mouse polychromatic erythrocytes, and did therefore not show any clastogenic activity in vivo. Thus, it can be concluded that metabolite 505M08 is not genotoxic.

Table 5.8.1-11: Overview on available toxicity data of 505M08 (Metabolite of dimoystrobin)

Category of test	Dose range	Results	Reference (BASF DocID)
Acute oral toxicity Wistar rats 505M08	2000 mg/kg bw	LD_{50} >2000 mg/kg bw 2000 mg/kg bw: no mortality	2004/1021192
Ames mutagenicity test TA 1535, 100, 1537, 98 E. coli WP2 uvrA ±S9 mix 505M08	20 – 5000 µg/plate	Negative	2004/1027668
CHO/HPRT mutagenicity test ±S9 mix 505M08	225 – 3600 µg/mL	Negative	2005/1006707
In vitro cytogenetics : Chromosome aberration in Chinese hamster V79 cells ±S9 mix 505M08	400 - 4500 µg/mL (4 h exposure) 225 – 3600 µg/mL (18 h exposure)	Weak clastogenic (at extremely high concentrations only (> 10 mM) and in the presence of precipitation)	2005/1004853
Micronucleus test in bone marrow of NMRI mice single oral application 505M08	500, 1000, 2000 mg/kg bw (24 h post exposure) 2000 mg/kg bw (48 h post exposure)	Negative	2005/1006738
Serum iron and duodenal weight determination Wistar rats oral application (7 days) 505M08	41.6 mg/kg bw (500 ppm)	NOAEL = 41.6 mg/kg bw No effects on serum iron concentration or duodenal weights	2005/1006725

505M08 is considered to be not genotoxic based on the available information and no further testing is required. Therefore, 505M08 is considered to be **not toxicologically relevant**.

Metabolite 505M08 is an intermediate metabolite in rat metabolism which is rapidly converted to its downstream metabolites and a groundwater metabolite (see Doc N4 of this dossier). As a result of the toxicological assessment, any risk for consumers via drinking water can be excluded and no further assessment is necessary.

2. 505M09 (Reg.No. 354563)

a. QSAR predictions on 505M09

OASIS TIMES (V.2.27.13 TB; Mutagenicity S-9 activated v08.08) (see molecule 10 of reports BASF DocID 2014/1323732 and 2015/1106118)
There were **no** Ames mutagenicity alerts for 505M09 or in-silico generated metabolites structural alerts were detected. In all cases the structures were not within the applicability domain.
For in-vitro chromosome aberration the prediction for 505M09 was **negative** (out of domain) with no specific structural alert. All of the 19 in-silico metabolites were predicted negative (domain info: N/A or no domain).

VEGA: Mutagenicity model (CAESAR, version 2.1.12) (see molecule 10 of report BASF DocID 2015/1106120)
505M09 was in the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. The similarity index of the total underlying database was moderate (0.820) and thus is reliable with restriction. The concordance index, accuracy index, and ACF matching index revealed that the model was reliable regarding these aspects.

VEGA: Mutagenicity model (SarPy; version 1.0.6-DEV) (see molecule 10 of report BASF DocID 2015/1106120)
505M09 was in the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. The similarity index, concordance index, accuracy index, and ACF matching index revealed that the model was reliable regarding these aspects.

VEGA: Mutagenicity model (TOXTREE; version 1.0.0-DEV) (see molecule 10 of report BASF DocID 2015/1106120)
505M09 is out of the model applicability domain. The prediction is **'non-mutagen'** with no specific structural alerts. The concordance of the total underlying database was moderate (0.509) and thus is not very robust. The molecules had a moderately similar structure to 505M09 (similarity 0.764 to 0.797). Furthermore, the model is not reliable regarding ACF matching index (0.6) and moderately reliable regarding the accuracy index (0.509).

Conclusion on QSAR evaluations of 505M09
The QSAR evaluation of 505M09 is of moderate to high reliability and by weight of evidence there was no conclusive alert for genotoxicity.

b. Toxicological evaluation of 505M09

No conclusive alerts for genotoxicity were identified by the QSAR evaluation conducted. Furthermore, additional general toxicity and genotoxicity data are available for 505M09. Metabolite 505M09 is of low acute toxicity, the acute oral LC50 was > 2000 mg/kg for rats. It was not mutagenic in a bacterial reverse mutation assay (Ames assay) with and without metabolic activation. Based on the results of a HPRT assay it was concluded, that the metabolite did not induce forward mutations in mammalian cells in vitro. In an in vitro CA assay it was clearly shown, that metabolite 505M09 is neither a clastogenic (chromosome-damaging) nor an aneugenic agent under in vitro conditions. The in vivo micronucleus test clearly showed that metabolite 505M09 did not induce the formation of micronuclei in mouse polychromatic erythrocytes, and did therefore not show any clastogenic activity in vivo. Based on close structural similarity to metabolite 505M08, this supports the conclusion for 505M08, that clastogenicity was evident as a result of cytotoxicity rather than a compound specific effect. It can be concluded that metabolite 505M09 is not genotoxic.

Table 5.8.1-12: Overview on available toxicity data of 505M09 (Metabolite of dimoxystrobin)

Category of test	**Dose range**	**Results**	**Reference (BASF DocID)**
Acute oral toxicity Wistar rats 505M09	2000 mg/kg bw	LD_{50} >2000 mg/kg bw 2000 mg/kg bw: no mortality	2004/1021193
Ames mutagenicity test TA 1535, 100, 1537, 98 E. coli WP2 uvrA ±S9 mix 505M09	20 – 5000 µg/plate (SPT) 4 – 2500 µg/plate (PIT)	Negative	2004/1027669
CHO/HPRT mutagenicity test ±S9 mix 505M09	100 – 3600 µg/mL	Negative	2005/1006706
In vitro cytogenetics: Chromosome aberration in Chinese hamster V79 cells ±S9 mix 505M09	400 - 3600 µg/mL (4 h exposure) 250 – 2000 µg/mL (18 h exposure)	Negative	2005/1004854
Micronucleus test in bone marrow of NMRI mice single oral application 505M09	500, 1000, 2000 mg/kg bw (24 h post exposure) 2000 mg/kg bw (48 h post exposure)	Negative	2005/1006737
Serum iron and duodenal weight determination Wistar rats oral application (7 days) 505M09	41.2 mg/kg bw (500 ppm)	NOAEL = 41.2 mg/kg bw No effects on serum iron concentration or duodenal weights	2005/1006725

505M09 is considered to be not genotoxic based on the available information and no further testing is required. Therefore, 505M09 is considered to be **not toxicologically relevant**.

Metabolite 505M09 is an intermediate metabolite in rat metabolism which is rapidly converted to its downstream metabolites and a groundwater metabolite (see Doc N4 of this dossier). Furthermore, 505M09 was found in confined rotational crop (feed items), hen, goat, and in traces in rape.

With regard to consumer exposure the TTC concept for non-genotoxic compounds was applied. Thus an ADI of 0.0015 mg/kg bw/day as well as an ARfD of 0.005 mg/kg bw/day was established. The estimated exposure (up to 25% of TTC of 0.005 mg/kg bw/day for acute and 22% of TTC of 0.0015 mg/kg bw/day for chronic exposure) is low.

Studies on metabolite 505M08

Report:	CA 5.8.1/3 [redacted] 2004a Reg.No. 354 562 (metabolite of BAS 505 F) - Acute oral toxicity study in rats 2004/1021192
Guidelines:	OECD 423, EEC 2004/73 B.1 tris, EPA 870.1100
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an acute oral toxicity study, single doses of 2000 mg/kg body weight of Reg. No. 354562 (batch: 01196-241, purity: 97.8%) in CMC-solution (0.5%) were given to 2 administration groups of each 3 fasted female animals by gavage in a sequential manner. Animals were observed for 14 days.
No mortality occurred after dosing with 2000 mg/kg bw. Accordingly, the oral LD50 was found to be greater than 2000 mg/kg bw.

Rat, oral: > 2000 mg/kg bw

The mean body weight increased within the normal range throughout the study period. No clinical observations were observed during clinical examination. No macroscopic pathologic abnormalities were noted in the animals examined at termination of the study. The available data on acute oral toxicity of the test substance do not meet the criteria for classification according to EC Directive on dangerous preparations 1999/45/EC (DPD) and Regulation (EC) No 1272/2008 (CLP). Classification for acute oral is therefore not warranted.

(BASF DocID 2004/1021192)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg. No. 354562 (Test-substance No. 03/0215-1)
Description:	solid (powder) / white
Density:	NA
Lot/Batch #:	01196-241
Purity/content:	97.8%
Stability of test compound:	The stability of the test item under storage conditions over the study period was guaranteed by the sponsor, and the sponsor holds this responsibility. The stability of the test item in the vehicle was determined indirectly by concentration control analysis.
2. Vehicle:	CMC (clean sodium carboxymethylcellulose, 0.5%) in doubly distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar / HanBrl:WIST (SPF)
Sex:	female
Age:	approximately 8-12 weeks
Weight at dosing:	174 - 187 g
Source:	RCC Ltd. Laboratory Animal Services, Wölferstrasse 4, CH-4414 Füllinsdorf, Switzerland
Acclimation period:	At least 5 days
Diet:	Kliba-Labordiät (Maus/Ratte Haltung "GLP"), Provimi Kliba SA, Kaiseraugst, Basel, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages, tape DK-III (Becker & Co., Castrop-Rauxel, Germany)
Environmental conditions:	
Temperature:	22 ± 2°C
Humidity:	30 - 70 %
Air changes:	NA
Photo period:	Alternating 12-hour light and dark cycles, artificial light from 6 am to 6 pm

B. STUDY DESIGN AND METHODS

1. Dates of work: 23-Aug-2004 - 14-Sep-2004

2. Animal assignment and treatment:

Single doses of 2000 mg test substance/kg bw in 0.5% CMC were given to 2 administration groups of each 3 fasted female animals by gavage in a sequential manner. Clinical signs and symptoms were recorded several times on the day of administration and afterwards at least once each workday for the individual animals up to 14 days post-administration. A check for any dead or moribund animal was made twice each workday and at least once on Saturdays, Sundays and on public holidays. Individual body weights were determined shortly before administration, weekly thereafter and on the last day of observation. The animals were sacrificed by CO_2-inhalation and subjected to necropsy including gross pathological examination on the last day of the observation period.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality was observed (see Table 5.8.1-13).

Table 5.8.1-13: Mortality of rats administered Reg. 5079618 by the oral route

Step No.	Sex	Dose (mg/kg bw)	Mortality / animals treated
1	Females	2000	0/3
2	Females	2000	0/3

B. CLINICAL OBSERVATIONS

No clinical observations were observed during clinical examination.

C. BODY WEIGHT

The mean body weight increased within the normal range throughout the study period.

D. NECROPSY

No abnormalities were observed at gross necropsy.

III. CONCLUSION

Under the conditions of this study, the oral LD_{50} in rats for Reg. No. 354562 was determined to be greater than 2000 mg/kg bw. According to DPD and CLP classification criteria, a classification is not warranted based on the results of this study.

Report: CA 5.8.1/4
Engelhardt G.,Leibold E., 2004a
Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and preincubation test) with Reg.No. 354 562 (metabolite of BAS 505 F)
2004/1027668
Guidelines: OECD 471, EEC 2000/32 B.13/B.14, EPA 870.5100
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

S. typhimurium and E. coli were exposed to Reg.No. 354562 (Metabolite of dimoxystrobin; Batch: 01196-241, Purity: 97.8%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. In the plate incorporation assay and the preincubation test Reg.No. 354563 was tested in concentrations of 20 to 5000 µg/plate. In both, the plate incorporation assay and the preincubation assay occasionally a weak bacteriotoxic effect was observed depending on the strain and test conditions at concentrations from 2500 µg/plate onward. Precipitation of the test substance did not occur up to the highest tested concentration.
A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.
According to the results of the study the test substance Reg.No. 354562 was not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2004/1027668)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg.No. 354562 (Metabolite of dimoxystrobin)

Description: Powder, white

Lot/Batch #: 01196-241

Purity: 97.8%

Stability of test compound: The test substance was stable over the study period under the storage conditions and guaranteed until July 2005. The homogeneity of the test substance was guaranteed on account of the high purity. The stability of the test substance in the vehicle DMSO over a period of 4 h was verified analytically.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Solvent/final concentration: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

To demonstrate the efficacy of the S9 mix in this assay, the S9 batch was further characterized with benoz(a)pyrene in the strains TA 100 and TA 98 as required in OECD 471.

3. Activation:

S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg Aroclor 1254 per kg body weight 5 days before sacrifice. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.
Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay:

In the first experiment triplicate plates were prepared for each concentration (neg. control; 20, 100, 500, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay:

In the second experiment the test article / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 20, 100, 500, 2500 and 2500 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains.

B. TEST PERFORMANCE:

1. Dates of experimental work: 04-Feb-2004 to 04-Mar-2004

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates). In the experiments with E. coli the warm soft agar contains 0.5 mM tryptophan instead of histidine + biotin.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.
After incubation in the dark for 48 to 72 hours at 37°C each plate was checked for precipitates and status of the background lawn. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects, the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37°C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.
A bacteriotoxic effect (reduced his^- or trp^- background growth and/or slight decrease in the number of his^+ or trp^+ revertants and/or reduction in the titer) was observed in the standard plate test and in the preincubation test depending on the strain and test conditions from 2500 µg/plate onward.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiment with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table 5.8.1-14). The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Precipitation was not observed up to the maximum concentration.

Table 5.8.1-14: Bacterial gene mutation assay with Reg.No. 354562 - Mean number of revertants

Experiment 1: Plate incorporation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	29 ± 3	27 ± 2	105 ± 6	106 ± 5	18 ± 2	18 ± 2	9 ± 2	10 ± 1	31 ± 5	30 ± 4
Test item										
20 µg/plate	28 ± 4	28 ± 5	100 ± 1	101 ± 6	16 ± 1	17 ± 1	10 ± 1	8 ± 1	32 ± 4	27 ± 4
100 µg/plate	30 ± 2	27 ± 2	108 ± 17	102 ± 3	18 ± 3	16 ± 3	11 ± 2	7 ± 1	31 ± 1	27 ± 2
500 µg/plate	32 ± 3	22 ± 4	116 ± 11	82 ± 8	16 ± 1	15 ± 3	10 ± 1	9 ± 3	25 ± 4	25 ± 1
2500 µg/plate	27 ± 3	19 ± 3	101 ± 7	85 ± 21	15 ± 3	14 ± 5	11 ± 2	9 ± 2	23 ± 3	21 ± 5
5000 µg/plate	20 ± 2	16 ± 2	81 ± 13	87 ± 18	11 ± 1	16 ± 2	6 ± 1	6 ± 2	20 ± 2	19 ± 2
Pos. control[§]	864 ± 17	728 ± 38	1281 ± 295	1372 ± 56	133 ± 10	1087 ± 36	158 ± 11	391 ± 19	207 ± 9	621 ± 35
Experiment 2: Preincubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	32 ± 3	29 ± 4	107 ± 7	107 ± 4	18 ± 2	18 ± 3	9 ± 1	10 ± 2	35 ± 5	33 ± 4
Test item										
20 µg/plate	29 ± 2	24 ± 4	108 ± 5	106 ± 6	15 ± 2	16 ± 1	9 ± 2	9 ± 1	28 ± 7	33 ± 6
100 µg/plate	27 ± 10	24 ±	106 ± 1	101 ± 6	14 ± 1	11 ± 3	6 ± 3	7 ± 2	36 ± 4	27 ± 4
500 µg/plate	25 ± 3	23 ± 1	98 ± 3	102 ± 7	16 ± 1	16 ± 2	7 ± 1	7 ± 1	26 ± 2	26 ± 1
2500 µg/plate	21 ± 3	19 ± 5	104 ± 4	99 ± 3	11 ± 4	15 ± 2	5 ± 3	8 ± 1	20 ± 5	25 ± 5
5000 µg/plate	7 ± 3	7 ± 5	53 ± 9	72 ± 11	4 ± 1	4 ± 2	2 ± 1	2 ± 2	10 ± 2	12 ± 6
Pos. control[§]	621 ± 20	719 ± 27	671 ± 60	791 ± 80	119 ± 19	860 ± 75	123 ± 7	528 ± 22	272 ± 22	610 ± 35

[§] = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSION

According to the results of the present study, the test substance Reg.No. 354562 (Metabolite of dimoxystrobin) is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report: CA 5.8.1/5
Engelhardt G.,Leibold E., 2005a
In vitro gene mutation test with Reg.No. 354 562 (metabolite of BAS 505 F) in CHO cells (HPRT locus assay)
2005/1006707

Guidelines: OECD 476, EEC 2000/32 B.17, EPA 870.5300

GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

Reg. No. 354562 (Metabolite of dimoxystrobin, Batch: 01196-241, Purity: 97.8%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells. Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations of up to 3600 µg/mL were used in main experiment. The treatment intervals for both experiments in the presence and absence of metabolic activation were 4 hours. Ethylmethanesulfonate (EMS) and Methylcholanthrene (MCA) served as positive controls in the experiments without and with metabolic activation, respectively. In both experiments after the incubation period treatment media were replaced by culture medium and the cells were incubated for 6-8 days for expression of mutant cells. At the end of the expression period a part of the cells were further incubated in selection medium containing 6-thioguanine for about 1 week for selection of mutant cells. Cloning efficiency as a marker for cytotoxicity was determined either directly after incubation (CE1, survival) or in parallel to the mutant selection (CE2, viability).

The test substance did result in decreased number of colonies (CE1) at 3600 µg/mL (-S9) and at 900 or 2700 µg/mL (+S9) in the first and second experiment, respectively. Cell density was not reduced. The test substance did not cause any relevant increase in the mutant frequencies either without S9 mix or after the addition of a metabolizing system in two experiments performed independently of each other.

Based on the results of the study it is concluded that under the conditions of the test Reg. No. 354563 (Metabolite of dimoxystrobin) does not induce forward mutations in mammalian cells in-vitro.

(BASF DocID 2005/1006707)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 354562 (Metabolite of dimoxystrobin)
Description: powder, white
Lot/Batch #: 01196-241
Purity: 97.8%
Stability of test compound: The stability of the test substance under storage conditions was guaranteed until July 2005 as indicated by the sponsor.
Solvent used: Dimethyl sulfoxide (DMSO)

2. Control Materials:
Negative control: A negative control was not employed in this study
Solvent control: 1% (v/v) DMSO in culture medium
Positive control -S9: Ethyl methanesulfonate (EMS) 300 µg/mL
Positive control +S9: Methylcholanthrene (MCA) 10 µg/mL

3. Activation: S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg Aroclor 1254 per kg body weight 5 days before sacrifice. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors) in the 1st experiment and 3 volume of S9 fraction was mixed with 7 volumes of S9 supplement in the 2nd experiment. This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	30 mM
$MgCl_2$ x $6H_2O$	10 mM
$CaCl_2$ x $2H_2O$	10 mM

4. Test organism: Chinese hamster CHO cells. They have a high proliferation rate (doubling time about 12-16 h), high plating efficiency (about 90%) and karyotype with a modal number of 20 chromosomes. Stocks of the CHO cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).

5. Culture media:

Culture medium:	Ham's F12 medium with L-glutamine and hypoxanthine supplemented with 10% (v/v) fetal calf serum (FCS).
Pretreatment medium:	("HAT" medium): FCS-supplemented Ham's F12 medium with L-glutamine and hypoxanthine containing per mL 13.6 µg hypoxanthine, 0.18 µg aminopterin and 3.88 µg thymidine.
Selection medium:	("TG" medium): L-Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL
	All media were supplemented with - 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL) - 1% (v/v) amphotericine B (250 µg/mL)

6. Locus examined: hypoxanthine-guanine-phosphoribosyl-transferase (H(G)PRT)

7. Test concentrations:

a) Preliminary toxicity assay:	Nine concentrations ranging from 100 to 3600 µg/mL
b) Mutation assay:	
1st experiment:	225, 450, 900, 1800 and 3600 µg/mL with and without metabolic activation
2nd experiment:	450, 900, 1800, 2700 and 3600 µg/mL with and without metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 12-Nov-2004 to 14-Mar-2005

2. Preliminary cytotoxicity assay:

Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25-cm² flasks with various test substance concentrations in serum-free Ham's F12 medium for about 4 hours (with and without metabolic activation) after an attachment period of 24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS), covered with Ham's F12 and incubated for a further 5 to 7 days. After this incubation period, colonies were fixed, stained and counted. In addition to the cloning efficiency the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

3. Mutation Assay:

Pretreatment of Cells: Cells with a passage number ≥ 2 after thawing from the frozen cells stock were seeded into 75 cm^2-flasks and incubated for 3-4 days with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants. Afterwards, a passage into culture medium followed and the cells were incubated for further 3-4 days.

Cell treatment: For each test group, about $1x10^6$ cells per flask were seeded into flasks containing about 20 mL Ham's F12 medium supplemented with 10% FCS and incubated for about 20 – 24 hours with 5% CO_2 at 37°C and ≥ 90% humidity for cell attachment. Two flasks were used for each test group.

After the cell attachment period the medium was replaced by fresh medium. The test article, dissolved in 200 µL DMSO, was added to the culture medium. Without S9 mix 18/20 mL Ham's F12 medium without FCS and 200 µL test substance preparation were used. With S9 mix 14/16 mL Ham's F12 medium without FCS, 200 µL test substance preparation and 4 mL S9 mix (= 2% and 6% S9 fraction in the 1st and 2nd experiment, respectively.

Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix at 5% CO_2, 37°C and ≥ 90% humidity.

Expression: After incubation for 4 hours, the treatment medium was replaced by 20 mL Ham's F12 medium with 10% FCS after having been rinsed twice with Hanks' balanced salt solution (HBSS). The following 1st passage was carried out after an incubation period of 65 to 72 hours. After an entire expression period of 6 – 8 days the cells were transferred into the selection medium (2nd passage).

Selection: For the mutant selection, six 75-cm^2 flasks each were seeded with $3x10^5$ cells from each treatment group in selection medium (TG medium) and incubated for about 6 to 8 days. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.

Determination of Cytotoxicity: Cloning efficiency 1 (survival):
The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25 cm^2 flasks using 5 mL Ham`s F12 medium with 10% FCS. After about 24 hour attachment period the cells were incubated with vehicle, test substance or the positive control for 4 hours as described above. Following exposure, cells were rinsed several times with HBSS. Finally, cells were cultured in 5 mL Ham's F12 medium incl. 10% (v/v) FCS.

Cloning efficiency 2 (viability):
The viability (cloning efficiency 2; CE_2) was determined after the expression period. About 200 cells were separated during the transfer into selection medium and seeded in two flasks (25 cm²) containing 5 mL Ham's F12 medium incl. 10% (v/v) FCS each.
After seeding of the cells, the flasks were incubated for 5 – 8 days to form colonies. These colonies were fixed, stained and counted.

Calculations:

Mutant frequency: Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{\text{total number of mutant colonies}}{\text{number of seeded cells}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\ absolute}} \times 100$$

Cloning efficiency (CE,%) absolute:

$$CE_{absolute} = \frac{\text{total number of colonies in the test group}}{\text{total number of seeded cells in the test group}} x100$$

relative, in comparison to control:

$$CE_{relative} = \frac{CE_{absolute}\ \text{of the test group}}{CE_{absolute}\ \text{of the vehicle control}} x100$$

4. Statistics:
Due to the clearly negative findings, a statistical evaluation was not carried out.

5. Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies ($MF_{corr.}$) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose-response relationship.

Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose-response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

A test substance is generally considered negative in this test system if:

- The corrected mutation frequency ($MF_{corr.}$) in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance under storage conditions was guaranteed until July 2005 as indicated by the Sponsor. The stability and homogeneity of the test substance at room temperature in the vehicle DMSO and in water over a period of 4 hours was verified analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY

After 4 hours treatment in the absence of S9 mix a slight decrease in the number of colonies (CE) was observed at the two high dose concentrations. In the presence of S9 mix a decrease in the number of colonies (CE) was observed at the top concentration of 3600 µg/mL. Precipitation of the test substance was observed at 2000 µg/mL and above at the end of treatment in the absence of metabolic activation. In the presence of metabolic activation the S9 mix precipitation was observed in all test groups. No marked effect on osmolarity and pH value was observed.
Based on these data the highest concentration tested in the mutagenicity experiments was 3600 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

No increase in the number of mutant colonies was observed either without S9 mix or after the addition of a metabolizing system (see Table 5.8.1-15 and Table 5.8.1-16). The mutant frequencies at any dose were close to the range of that of the concurrent negative control values and within the range of the historical control data.

Without S9 mix, there was a decrease in the number of colonies (CE_1) at 3600 µg/mL in the 1st and 2nd experiment. Cell density was not reduced. With S9 mix, there was a decrease in the number of colonies (CE_1) from about 900 µg/mL onward in the 1st experiment (S9 fraction : cofactors = 1:9) and from about 2700 µg/mL in the 2nd experiment (S9 fraction : cofactors = 3:7). Again, cell density was not reduced.

The pH and osmolarity of the tested concentrations were not altered at the concentrations tested. Precipitation of the test substance was observed at 1800 µg/mL and above at the end of treatment in the absence of metabolic activation. In the presence of metabolic activation S9 mix precipitation was observed in all test groups. No marked effect on osmolarity and pH value was observed.

Treatment with the positive controls EMS and MCA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

Table 5.8.1-15: Gene mutation in mammalian cells - 1st experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	28	7.78	9.56	82.8	100.0	83.5	100.0
Reg.No. 354562							
225 µg/mL	9	2.50	3.06	81.8	98.8	84.7	101.4
450 µg/mL	13	3.61	5.91	81.2	98.1	70.3	84.2
900 µg/mL	13	3.61	4.87	86.0	103.9	73.8	88.4
1800 µg/mL	5	1.39	1.84	84.4	101.9	74.8	89.6
3600 µg/mL	7	1.95	2.98	31.7	38.3	69.1	82.8
Positive control EMS							
300.0 µg/mL	477	132.50	206.80	81.3	98.2	64.2	76.9
With metabolic activation[1]; 4-hour exposure period							
Vehicle (DMSO)	8	2.22	2.92	86.7	100.0	76.2	100.0
Reg.No. 354562							
225 µg/mL	9	2.50	3.50	81.4	93.9	74.5	97.8
450 µg/mL	1	0.28	0.36	80.5	92.8	76.9	100.9
900 µg/mL	1	0.28	0.37	61.5	70.9	76.9	100.9
1800 µg/mL	12	3.34	4.62	27.4	31.6	74.2	97.4
3600 µg/mL	16	4.45	6.16	12.4	14.3	71.0	93.2
Positive control MCA							
10 µg/mL	253	70.28	96.18	77.5	89.4	73.1	95.9

a number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
b correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
1 S9 fraction : cofactors = 1:9

Table 5.8.1-16: Gene mutation in mammalian cells - 2nd experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	Relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	3	0.84	1.10	79.4	100.0	75.3	100.0
Reg.No. 354562							
450 µg/mL	11	3.06	4.50	80.4	101.3	69.4	92.2
900 µg/mL	3	0.84	1.32	76.9	96.9	61.7	81.9
1800 µg/mL	2	0.56	0.78	74.3	93.6	65.4	86.9
2700 µg/mL	4	1.11	2.10	70.1	88.3	53.1	70.5
3600 µg/mL	4	1.11	1.54	61.7	77.7	63.7	84.6
Positive control EMS							
300 µg/mL	341	94.73	134.24	79.5	100.1	70.4	93.5
With metabolic activation[1]; 4-hour exposure period							
Vehicle (DMSO)	6	1.67	2.06	82.0	100.0	79.3	100.0
Reg.No. 3545620.28							
450 µg/mL	1	0.28	0.34	78.2	95.4	77.7	98.0
900 µg/mL	5	1.39	2.17	76.8	93.7	64.1	80.8
1800 µg/mL	3	0.84	1.38	66.5	81.1	57.3	72.3
2700 µg/mL	1	0.28	0.52	58.3	71.1	56.3	71.0
3600 µg/mL	2	0.56	1.10	14.8	18.0	49.7	62.7
Positive control MCA							
10 µg/mL	601	169.72	243.67	69.7	85.0	69.7	87.9

a number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
b correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
1 S9 fraction : cofactors = 3:7

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of the test Reg.No. 354562 (Metabolite of dimoxystrobin) does not induce forward mutations in the HPRT locus in CHO cells in vitro.

Report:	CA 5.8.1/6 Engelhardt G.,Leibold E., 2005c In vitro chromosome aberration assay with Reg.No. 354 562 (metabolite of BAS 505 F) in V79 cells 2005/1004853
Guidelines:	OECD 473, EEC 2000/32 B.10, EPA 870.5375
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

Reg.No. 354562 (metabolite of dimoxystrobin; Batch: 01196-241, Purity: 97.8%) was tested in vitro for the ability to induce chromosome and numerical aberrations in Chinese Hamster V79 cells in one experiment first in the presence and absence of metabolic activation. Based on the cytotoxicity results of a pretest for dose selection, concentrations of 400 µg/mL to 3600 µg/mL were tested for clastogenic effects with and without metabolic activation in experiments with a pulse treatment of 4 hours. Based on the effects observed a repeated experiment was performed using 3000, 3500, 4000 and 4500 µg/mL in the absence of metabolic activation. A 2nd experiment using an 18-hour exposure was performed but not evaluated due to conclusive findings in the first experiment. The cells were prepared 18 h hours post treatment-begin. Vehicle (DMSO) and positive controls (cyclophosphamide (CPP) and ethylmethanesulfonate (EMS) for the experiment with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system.
Prior to cell harvest, addition of colcemid arrested cells in the metaphase. After slide preparation and staining of the cells, at least 200 well spread metaphases per dose and treatment condition were analyzed for chromosomal aberrations, except for the positive control cultures where only 100 metaphases were scored due to clearly increased aberration rates.
At least three doses were evaluated. Among these, the highest concentration scored was 4500 µg/mL. On the basis of the results of the present study, the test substance led to a weak increase in the number of structurally aberrant metaphases including and excluding gaps without S9 mix at extremely high concentrations (> 10 mM) at which evident test substance precipitation occured. No biologically relevant increase in the frequency of cells containing numerical aberrations was demonstrated.
Thus, under the experimental conditions chosen here, the conclusion is drawn that Reg. No. 354562 (Metabolite of dimoxystrobin) is a weak chromosome-damaging (clastogenic) agent under in vitro conditions using V79 cells. However, the clastogenic activity was only observed at extremely high concentrations at which evident test substance precipitation occurred. Therefore, secondary effects resulting from extreme culture conditions cannot be ruled out as the cause for these findings.
(BASF DocID 2005/1004853)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 354562 (Metabolite of dimoxystrobin)

Description: Powder, white

Lot/Batch #: 01196-241

Purity: 97.8%

Stability of test compound: The stability of the test substance under storage conditions was guaranteed by the sponsor. The stability of the test substance at room temperature dissolved in the vehicle DMSO over a period of 4 hours and in water over a period of 96 hours was verified analytically.
The homogeneity of the test substance was ensured by mixing prior to preparation of test substance formulations and on account of the high purity.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: A negative control was not employed in this study.

Solvent control: DMSO

Positive control, -S9: Ethylmethanesulfonate (EMS) 500 µg/mL

Positive control, +S9: Cyclophosphamide (CPP) 0.5 µg/mL

3. Activation: S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg Aroclor 1254 per kg body weight 5 days before sacrifice. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so called S9 mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms: Chinese hamster V79 cells

5. Culture medium: MEM medium with glutamine supplemented with
- 10% (v/v) fetal calf serum (FCS)
- 1% (v/v) penicillin/streptomycin (10000 IU/10000 µg/mL)
- 1% (v/v) amphotericine B (250 µg/mL)

During exposure to the test substance (4-hour treatment), MEM medium was used without FCS supplementation.

6. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 5 to 3600 µg/mL were used in pretests for dose selection for the main experiments. V79 cells were prepared at a sampling time of 18 hours after 4 and 18 hours exposure time without metabolic activation and after 4 hours exposure time with metabolic activation.

b) Mutation assay:

1st experiment: 400, 1200 and 3600 µg/mL with and without metabolic activation (4 hour exposure and 18 h preparation interval)

1st experiment/repeat: 3000, 3500, 4000 and 4500 µg/mL without metabolic activation (4 h exposure and 18 h sampling time)

2nd experiment: 225, 450, 900, 1800 and 3600 µg/mL without metabolic activation (18 h exposure and 18 h preparation interval) (not evaluated)

B. TEST PERFORMANCE:

1. Dates of experimental work: 19-Oct-2004 to 07-Apr-2005

2. Preliminary cytotoxicity assay:

A range-finding cytotoxicity test was conducted with V79 cultures exposed for 4 and 18 hours to test substance concentrations of 5 – 3600 µg/mL both with and without metabolic activation. At the end of the exposure period, cell count, cell attachment, mitotic index and the quality of metaphases were determined in order to derive appropriate test substance concentrations for the main test.

3. Cytogenicity Assay:

Cell treatment: Cells were exposed to the test substance, solvent or positive control in pulse treatment experiments for 4 hours with or without metabolic activation. The cells were incubated in Quadriperm® dishes at 37°C, 5% CO_2 and ≥ 90% humidity. Two chambers of a Quadriperm dish were used for each concentration (=duplicate cultures). The preparation interval was 18 h post treatment-begin. After continuous treatment, i.e. 18 h without S9 mix, cells were treated in culture medium supplemented with 10% FCS and in the case of a sampling time of 28 hours incubated again for another 10 hours.

	For determination of cytotoxicity, additional cell cultures (using 25 cm² plastic flasks) were treated in the same way as in the main experiment. Growth inhibition was estimated by comparing the cell number in the treated groups with the concurrent control.
Spindle inhibition:	100 µL colcemide (stock: 10 µg/mL phosphate buffered saline) was added to the cultures 2 – 3 hours prior to harvesting.
Cell harvest:	At the end of the incubation time the culture medium was completely removed. For hypotonic treatment, 5 mL of a 0.4% KCl solution (37°C) was added for about 20 minutes. The cells were fixed by addition of 5 mL methanol/glacial acetic acid (3:1 v/v). The fixative was changed twice.
Slide preparation:	The slides were removed from the Quadriperm chambers, briefly allowed to drip off and passed through a Bunsen burner flame. After drying, the cells were stained with Giemsa and Titrisol. After rinsing and clarifying in Xylene the cover slips were mounted in Corbit-Balsam.
Metaphase analysis:	Slides were coded prior to analysis. As a rule, the first 100 consecutive well-spread metaphases of each culture were counted for all test groups, and if cells had 20 - 22 chromosomes, they were analyzed for structural chromosome aberrations. Numerical chromosome aberrations were also recorded. If there is a clear increase in chromosomally damaged cells, the number of metaphases to be analyzed is reduced from the planned 200 mitoses/ test group to 100. A mitotic index based on 1000 cells per culture was determined for all evaluated test groups in both experiments.

4. Statistics:

The proportion of metaphases with aberrations was calculated for each group.

A comparison of each dose group with the vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test was Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A statistically significant, dose-related and reproducible increase in the number of cells with structural chromosome aberrations.
- The number of aberrant cells exceeds both the concurrent negative/vehicle control value and the historical negative control data range.

A test substance is generally considered as "negative" if the following criteria are met:

- The number of cells with structural / numerical aberrations in the dose groups is not statistically significant increased above the concurrent negative/vehicle control value and is within the historical negative control data range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours and in water over a period of 96 hours was verified analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY:

A preliminary cytotoxicity assay was performed to determine appropriate test substance concentrations for the main test. Dose selection was based on the cell count, cell attachment, mitotic index and the quality of metaphases. Up to the highest recommended concentration, i.e. 10 mM (ca. 3600 µg/mL), at which distinct test substance precipitation was observed, the test substance did not exhibit any toxic effects after a treatment time of 4 hours. On the basis of these findings, 3600 µg/mL both with and without S9 mix were selected as the top doses.

C. CYTOGENICITY ASSAYS:

<u>1st experiment</u>

With 6.0% aberrant metaphases excl. gaps, there was a marginal increase in the number of structurally damaged cells in the experiment part without S9 mix at the top dose of 3600 µg/mL after an exposure time of 4 hours and a sampling time of 18 hours. However, this value, which is statistically not significant, only slightly exceeds the upper limit of the historical negative control range of 5.5%.

Without metabolic activation no increase in chromosomally damaged metaphases was observed (see Table 5.8.1-17 and Table 5.8.1-18).

<u>1st experiment, repeat</u>

Based on the findings of the 1st study, a repeat experiment, which was limited to the experimental part without S9 mix, was carried out for further clarification. Therefore doses up to 4500 µg/mL were used in order to demonstrate a possible dose-response relationship. Accordingly to the results of this repeat experiment there was an increase in the number of chromosomally damaged cells from about 3500 µg/mL onward with a maximum and significant response at 4000 µg/mL (12.5% excl. gaps, 8.5% exchanges).

Even if statistically not significant in all cases, the values for aberrant metaphases excl. gaps at 3500 µg/mL (6.5% excl. gaps) and 4500 µg/mL (8.5% excl. gaps) also exceed not only the range of the concurrent negative control but also the range of the historical negative control data. The decrease in the aberration rate observed at 4500 mg/mL may be explained by a limited or reduced availability of the test substance due to increasing precipitation and an accumulation of the test substance in this high dose group. In the repeat experiment, a slight increase in polyploidy cells above the historical negative control range (4.2%) was also observed at the lowest and highest dose of 3000 µg/mL (4.8%) and of 4500 µg/mL (4.7%) respectively. However there was no dose response and thus, these findings must be regarded as incidental rather than as an indication of an aneugenic mode of action of the test substance (see Table 5.8.1-19).

2nd experiment

An experiment both with continuous treatment and a sampling time of 18 hours was conducted. However, since after pulse treatment of 4 hours a positive response was demonstrated, no evaluation of the slides was carried out in agreement with the sponsor.

Summary and discussion

In summary, it can be stated that a weak clastogenic activity of the test substance was demonstrated after pulse treatment of 4 hours and a sampling time of 18 hours without metabolic activation. However, the positive response was observed only at extremely high concentrations (> 10 mM) at which evident test substance precipitation occurred. The precipitated test substance particles may be phagocytized, thus leading to high local concentrations within the cell with the possible result of lysosomal damage and a subsequent release of nucleases. Therefore, it is likely that the effect observed is the result of an indirect mechanism (chromosome damage by liberated nucleases), secondary to treatment conditions, rather than a DNA-damaging activity of the test substance. The repeat of the first experiment at the chosen doses is of limited value as the dose chosen for analysis were all clearly beyond solubility of the compound.

The osmolarity and pH values were not influenced by the test substance treatment. Test substance precipitation in culture medium at the end of exposure period was macroscopically observed from 3600 µg/mL onward in both experimental parts in the 1st Experiment and from 3500 µg/mL in the repeated 1st Experiment.
Vehicle and positive controls were all in a range to ensure the validity of the test.

Table 5.8.1-17: Chromosome aberration test with Reg.No. 354562 without metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Metaphases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	N	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	6.7	5	5.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	8.6	4	4.0	2	2.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**7.7**	**9**	**4.5**	**5**	**2.5**	**3**	**3.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Reg.No. 354562																			
400 µg/mL	A	100	6.6	7	7.0	4	4.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	7.2	5	5.0	4	4.0	2	2.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	A + B	**200**	**6.9**	**12**	**6.0**	**8**	**4.0**	**3**	**1.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**
1200 µg/mL	A	100	10.1	3	3.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	13.7	5	5.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**11.9**	**8**	**4.0**	**2**	**1.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
3600µg/mL	A	100	5.4	6	6.0	5	5.0	2	2.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	B	100	8.4	8	8.0	7	7.0	4	4.0	0	0.0	0	0.0	0	0.0	2	2.0	0	0.0
	A + B	**200**	**6.9**	**14**	**7.0**	**12**	**6.0**	**6**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**3**	**1.5**	**0**	**0.0**
Positive control EMS																			
500 µg/mL	A	50	9.1	12	24.0	9	18.0	7	14.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	6.4	8	16.0	7	14.0	4	8.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100#**	**7.8**	**20**	**20.0****	**16**	**16.0****	**11**	**11.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Table 5.8.1-18: Chromosome aberration test with Reg.No. 354562 with metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	12.6	5	5.0	2	2.0	1	1.0	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
	B	100	14.1	1	1.0	1	1.0	0	0.0	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
	A + B	**200**	**13.4**	**6**	**3.0**	**3**	**1.5**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Reg.No. 354562																			
400 µg/mL	A	100	10.0	2	2.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	2	2.0
	B	100	12.7	9	9.0	7	7.0	2	2.0	0	0.0	0	0.0	1	1.0	1	1.0	1	1.0
	A + B	**200**	**11.4**	**11**	**5.5**	**8**	**4.0**	**3**	**1.5**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**1**	**0.5**	**3**	**1.5**
1200 µg/mL	A	100	6.4	6	6.0	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	11.8	6	6.0	6	6.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0
	A + B	**200**	**9.1**	**12**	**6.0**	**9**	**4.5**	**6**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**
3600 µg/mL	A	100	8.0	5	5.0	4	4.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	9.4	6	6.0	4	4.0	2	2.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	A + B	**200**	**8.7**	**11**	**5.5**	**8**	**4.0**	**6**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**
Positive control CPP																			
0.5 µg/mL	A	50	6.7	10	20.0	9	18.0	7	14.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	8.2	11	22.0	10	20.0	9	18.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100#**	**7.5**	**21**	**21.0**	**19**	**19.0**	**16**	**16.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Table 5.8.1-19: Chromosome aberration test with Reg.No. 354562 without metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1-repeat

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	10.5	5	5.0	4	4.0	1	1.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0
	B	100	10.3	5	5.0	4	4.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**10.4**	**10**	**5.0**	**8**	**4.0**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**
Reg.No. 354562																			
3000 µg/mL	A	100	9.5	9	9.0	6	6.0	3	3.0	0	0.0	0	0.0	0	0.0	6	5.7	0	0.0
	B	100	11.8	5	5.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	4	3.8	0	0.0
	A + B	**200**	**10.7**	**14**	**7.0**	**9**	**4.5**	**4**	**2.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**10**	**4.8**	**0**	**0.0**
3500 µg/mL	A	100	11.2	11	11.0	8	8.0	3	3.0	0	0.0	0	0.0	1	1.0	2	1.9	0	0.0
	B	100	9.8	6	6.0	5	5.0	1	1.0	0	0.0	0	0.0	1	0.9	4	3.8	1	0.9
	A + B	**200**	**10.5**	**17**	**8.5**	**13**	**6.5**	**4**	**2.0**	**0**	**0.0**	**0**	**0.0**	**2**	**1.0**	**6**	**2.9**	**1**	**0.5**
4000 µg/mL	A	100	10.3	16	16.0	13	13.0	8	8.0	0	0.0	0	0.0	0	0.0	3	2.9	0	0.0
	B	100	6.9	18	18.0	12	12.0	9	9.0	0	0.0	0	0.0	0	0.0	4	3.8	0	0.0
	A + B	**200**	**8.6**	**34**	**17.0****	**25**	**12.5****	**17**	**8.5****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**7**	**3.4**	**0**	**0.0**
4500 µg/mL	A	100	9.5	12	12.0	11	11.0	5	5.0	0	0.0	0	0.0	0	0.0	5	4.8	0	0.0
	B	100	9.4	7	7.0	6	6.0	2	2.0	0	0.0	0	0.0	1	0.9	5	4.7	0	0.0
	A + B	**200**	**9.5**	**19**	**9.5**	**17**	**8.5**	**7**	**3.5**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**10**	**4.7**	**0**	**0.0**
Positive control EMS																			
500.0 µg/mL	A	50	8.0	8	16.0	7	14.0	5	10.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	8.7	10	20.0	7	14.0	5	10.0	0	0.0	0	0.0	0	0.0	2	3.8	0	0.0
	A + B	**100#**	**8.4**	**18**	**18.0**	**14**	**14.0**	**10**	**10.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**2**	**2.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

III. CONCLUSION

Thus, under the experimental conditions chosen here, the conclusion is drawn that Reg. No. 354562 (Metabolite of dimoxystrobin) is a weak chromosome-damaging (clastogenic) agent under in vitro conditions using V79 cells. However, the clastogenic activity was only observed at extremely high concentrations at which evident test substance precipitation occurred. Therefore, secondary effects resulting from extreme culture conditions are the likely cause for these findings.

Report: CA 5.8.1/7
[redacted] 2005a
Micronucleus assay in bone marrow cells of the mouse with Reg.No. 354 562 (metabolite of BAS 505 F) after a single oral administration
2005/1006738
Guidelines: OECD 474, EEC 2000/32 C.4, EPA 870.5395
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Report: CA 5.8.1/8
[redacted] 2005b
Amendment 1 - Micronucleus assay in bone marrow cells of the mouse with Reg.No. 354562 (metabolite of BAS 505 F) after a single oral application
2005/1006740
Guidelines: OECD 474, EEC 2000/32 C.4, EPA 870.5395
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary
Reg. No. 354562 (Metabolite of dimoxystrobin) (Batch: 01196-241; Purity: 97.8%) was tested for chromosomal damage (clastogenicity) in NMRI mice using the micronucleus test method. For this purpose, the test substance, suspended in CMC, was administered once orally to groups of 6 male mice at dose levels of 500, 1000 and 2000 mg/kg body weight in a volume of 20 mL/kg body weight. The vehicle served as negative and cyclophosphamide as positive control. The animals were sacrificed 24 or 48 (additional high dose group) hours after the administration and the bone marrow of the two femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes occurring per 2000 polychromatic erythrocytes were also recorded.
The oral administration of Reg. No. 354562 (Metabolite of dimoxystrobin) did not lead to any biologically relevant increase in the number of polychromatic erythrocytes containing micronuclei. The rate of micronuclei was mostly close to the concurrent negative control and was within the range of the historical control data. Inhibition of erythropoiesis, determined from the ratio of polychromatic to normochromatic erythrocytes, did not occur. Weak signs of systemic toxicity were observed in the high dose group only, consisting of spontaneous activity and ruffled fur one hour post-treatment. No signs of systemic toxicity were observed in any of the animals treated with the positive control substances, the vehicle or the test substance at the low/mid dose level. The positive control chemical cyclophosphamide led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system.
(BASF DocID 2005/1006738)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg. No. 354562 (Metabolite of dimoxystrobin)

Description:	solid (powder) / white
Density:	NA
Lot/Batch #:	01196-241
Purity/content:	97.8%
Stability of test compound:	The stability of the test item under storage conditions over the study period was guaranteed by the sponsor, and the sponsor holds this responsibility. The stability of the test item in the vehicle was determined indirectly by concentration control analysis.
Vehicle used:	CMC (clean sodium carboxymethylcellulose, 0.5%) in doubly distilled water

2. Control Materials:

Negative:	No negative control was employed in this study.
Vehicle control:	Aqua deionised
Positive control:	Cyclophosphamide (CCP) 40 mg/kg bw for the determination of clastogenic effects

3. Test animals:

Species:	Albino mice
Strain:	Crl:NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 10 weeks
Mean body weight at dosing:	34.3 g
Source:	Harlan Winkelmann GmbH, Germany
Number of animals per dose:	
Range finding study:	3 per sex and dose
Micronucleus assay:	6 males/dose
Acclimation period:	At least 5 days
Diet:	Standardized pelleted feed (Harlan Winkelmann GmbH, Germany)
Water:	Drinking water in bottles, ad libitum
Housing:	During the study the mice were housed individually in Makrolon cages, type I

4. Environmental conditions:

Temperature:	19 - 25 °C
Humidity:	30 - 70%
Air changes:	frequency not indicated (fully air-conditioned rooms)
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	2000 mg/kg
Micronucleus assay:	500, 1000 and 2000 mg/kg The test substance was administered once by oral gavage using an application volume of 20 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 18-Mar-2005 to 06-Apr-2005

2. Preliminary range finding test:

In a pretest for the determination of the acute oral toxicity, 3 male and 3 female animals were treated once by oral gavage with a test substance dose of 2000 mg/kg bw.

3. Micronucleus test:

Treatment and sampling:	Groups of 6 male mice were treated once with the vehicle or 500, 1000 or 2000 mg test substance/kg bw by oral gavage. Additional test groups treated with the vehicle control and the high dose were used for the second sampling period. The application volume was 20 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substance CCP was administered once by oral gavage. The animals were observed for evident clinical signs of toxicity throughout the study. Twenty-four hours after the administration the mice were sacrificed and the two femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 390xg for 10 minutes. The supernatant was discharged and the pellet resuspended in FCS. The sampling of the femora from the additional high dose group was performed as described above 48 h after the treatment.
Slide preparation:	One drop of the suspension was applied on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May-Grünwald solution, rinsed, and finally stained with Giemsa solution. Cover slips were mounted with EUKITT. The slides were coded prior to microscopic evaluation.

Slide evaluation: In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored.
The increase in the number of micronuclei in polychromatic erythrocytes of treated animals as compared to the vehicle control group provides an index of a chromosome-breaking (clastogenic) effect or of a spindle activity (aneugenic) of the substance tested.
The ratio of polychromatic to normochromatic erythrocytes was calculated. An alteration of this ratio indicates a toxic effect on erythropoiesis and thus, that the test substance actually reached the target organ.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the nonparametric Mann-Whitney test.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related or clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group was observed. Statistical methods will be used as an aid in evaluating the results.

A test substance is generally considered negative in this test system if:

- There was no biological relevant increase in the number of micronucleated polychromatic erythrocytes.
- The frequencies of cells containing micronuclei were within the historical control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in the vehicle was verified analytically. The homogeneity of the test substance in the vehicle was guaranteed by constant stirring during the removal and administration of the test substance formulation and by analytical determination.

B. PRELIMINARY RANGE FINDING TEST

In the pretest for the determination of the acute oral toxicity in males and females, the animals were treated with 2000 mg/kg bw. All animals survived with weak signs of toxicity. The clinical signs observed were reduction of spontaneous activity and ruffled fur one hour post-treatment. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

Micronucleus frequencies of the animals treated with the indicated doses of Reg. No. 354562 were near to the concurrent vehicle control values and within the historical control range (see Table 5.8.1-20). The mean values of cells with micronuclei were significantly higher than in the control group (1‰ to 0.3‰). However 1‰ is well within the historical control range (0.1-2.3‰) and the value obtained for the next higher dose was lower and not statistically significant. Thus, the effect was determined to be not biologically relevant. The PCE/NCE ratio was in principle not affected by treatment with the test substance. Thus, there was no indication that erythropoiesis was inhibited.

The clastogenic activity of the positive control substance cyclophosphamide was evident by the increase in the number of PCE with micronuclei (27.7‰). The positive control thus demonstrated the sensitivity of the test system.

The administration of the test substance was well tolerated by all animals with weak clinical signs of toxicity observed only in the high dose group consisting of spontaneous activity and ruffled fur one hour post-treatment. No signs of systemic toxicity were observed in any of the animals treated with the positive control substances, the vehicle or the test substance at the low/mid dose level.

Table 5.8.1-20: Micronucleus test in mice administered Reg. No. 354562 by oral gavage

Test group	Dose mg/kg bw	Sampling time (h)	PCEs with micronuclei (‰)	Range	PCE per 2000 erythrocytes
CMC	0	24	0.30	0-1	1061
Reg. No. 354562	500	24	0.60	1-2	1117
Reg. No. 354562	1000	24	1.00*	1-3	1040
Reg. No. 354562	2000	24	0.80	0-3	1048
CPP	40	24	27.70*	42-98	1001
Reg. No. 354562	2000	48	0.00	0-0	1098

* = $p \leq 0.05$

III. CONCLUSION

Based on the result of this study Reg. No. 354562 does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study, i.e. is devoid of clastogenic activity in vivo.

This conclusion is further supported by a mechanistic study (BASF DocID 2004/1006526; study summary can be found in chapter 5.1). It was shown that metabolite 505M08 was found in significant amounts in urine and faeces samples of rats treated with dimoxystrobin by the oral route. This is supportive to prove bioavailability of metabolite 505M08 after oral administration of parent. Dimoxystrobin was shown to be devoid of a mutagenic concern (chapter 5.1). By weight of evidence this then applies also to 505M08.

Studies on metabolite 505M09

Report: CA 5.8.1/9
[redacted] 2004b
Reg.No. 354 563 (metabolite of BAS 505 F) - Acute oral toxicity study in rats
2004/1021193
Guidelines: OECD 423, EEC 2004/73 B.1 tris, EPA 870.1100
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report: CA 5.8.1/10
[redacted] 2004a
Amendment No. 1 to the report: Reg.No. 354 563 (metabolite of BAS 505 F) - Acute oral toxicity study in Wistar rats
2004/1025739
Guidelines: OECD 423, EEC 2004/73 B.1 tris, EPA 870.1100
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an acute oral toxicity study groups of 3 fasted female Wistar rats were administered single doses of 2000 mg/kg bw of Reg.No. 354563 (metabolite of dimoxystrobin, batch 01196-245) in 0.5% aqueous CMC-solution. The diluted suspensions were administered at a volume of 10 mL/kg bw. The observation period was 14 days.
Administration of 2000 mg/kg bw of Reg.No. 354563 (metabolite of dimoxystrobin) resulted in no mortality and no clinical signs and findings. Accordingly, the oral LD_{50} was greater than 2000 mg/kg bw in rats.

LD_{50} > 2000 mg/kg bw

No macroscopic pathologic abnormalities were noted in the animals examined at the end of the observation period. The amendment no. 1 corrects the wrong total page number from the main report.
(BASF DocID 2004/1021193)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 354563 (metabolite of dimoxystrobin)

Description:	solid (powder), white
Lot/Batch #:	01196-245
Purity:	99.6%
Stability of test compound:	The stability of the test substance in the vehicle for the maximum application period was confirmed indirectly by analysis of the homogeneity. The homogeneity of the test substance preparation in the vehicle used for the first administration was confirmed by analysis.

2. Vehicle: 0.5% carboxymethylcellulose (CMC) in doubly distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar, HanBrl:WIST(SPF)
Sex:	female
Age:	8 - 12 weeks
Weight at dosing (mean):	180 - 181 g
Source:	RCC Ltd Laboratory Animal Services, CH-4414 Füllinsdorf, Switzerland
Acclimation period:	At least 5 days
Diet:	Kliba Laboratory Diet (Maus / Ratte Haltung "GLP"), Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water, ad libitum
Housing:	Single housing in stainless steel wire mesh cages, type DK-III (Becker&Co, Castrop-Rauxel, Germany)
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70%
Air changes:	Fully air-conditioned rooms; number of air-changes not indicated in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 23-Aug-2004 - 23-Sep-2004

2. Animal assignment and treatment:
Groups of 3 female rats received single doses of 2000 mg/kg bw (two groups) of the test substance by oral gavage. Prior to administration the animals were deprived of food for 16 hours, however had free access to water.
The dose was administered with an administration volume of 10 mL/kg bw. The aqueous dilutions were prepared shortly before administration. Homogeneity was ensured by constant stirring with a magnetic stirrer.
Animals were observed for signs of toxicity and behavioral changes several times on the day of administration, and at least once each workday for the remainder of the 14-day observation period. A check for mortality/morbidity was performed twice each workday and once on Saturdays, Sundays and on public holidays.
Body weights were recorded at day 0 (prior to dosing), weekly thereafter and at the end of the observation period.
Necropsy with gross-pathology examination was performed on the last day of the observation period. The animals were sacrificed by CO_2-inhalation.

II. RESULTS AND DISCUSSION

A. MORTALITY
Administration of 2000 mg/kg bw of Reg.No. 354563 resulted in no death during the whole observation period.

B. CLINICAL OBSERVATIONS
No clinical observations were observed during clinical examination.

C. BODY WEIGHT
The mean body weights of the administration groups increased throughout the study period.

D. NECROPSY
No macroscopic pathologic abnormalities were noted in the animals examined at termination of the study.

III. CONCLUSION
Under the conditions of this study the median lethal dose of Reg.No. 354563 (metabolite of dimoxystrobin) after oral administration was found to be greater than 2000 mg/kg bw in rats.

Report:	CA 5.8.1/11 Engelhardt G.,Leibold E., 2004b Salmonella typhimurium / Escherichia coli reverse mutation assay (standard plate test and preincubation test) with Reg.No. 354 563 (metabolite of BAS 505 F) 2004/1027669
Guidelines:	OECD 471, EEC 2000/32 B.13/B.14, EPA 870.5100
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

S. typhimurium and E. coli were exposed to Reg.No. 354563 (Metabolite of dimoxystrobin; Batch: 01196-245, Purity: 99.6%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment. In the plate incorporation assay, Reg.No. 354563 was tested in concentrations of 20 to 5000 µg/plate. In the preincubation test Reg.No. 354563 was tested in concentrations of 4 to 2500 µg/plate. In both, the plate incorporation assay and in the preincubation assay a weak bacteriotoxic effect was observed occasionally depending on the strain and test conditions at concentrations from about 500 - 2500 µg/plate onward. Precipitation of the test substance did not occur up to the highest tested concentration.

A biologically relevant increase in the number of revertant colonies was not noticed in any of the strains tested in the presence or absence of metabolic activation in any of the experiments. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.

According to the results of the study, test substance Reg.No. 354563 was not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions of the study.

(BASF DocID 2004/1027669)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg.No. 354563 (Metabolite of dimoxystrobin)
Description: Powder, white
Lot/Batch #: 01196-245
Purity: 99.6%
Stability of test compound: The test substance was stable over the study period under the storage conditions and guaranteed until August 2016. The homogeneity of the test substance was guaranteed on account of the high purity. The stability of the test substance in the vehicle DMSO over a period of 4 h was verified analytically.
Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:
Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control).
Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.
Solvent/final concentration: 100 µL/plate

Positive control compounds tested without addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

To demonstrate the efficacy of the S9 mix in this assay, the S9 batch was further characterized with benoz(a)pyrene in the strains TA 100 and TA 98 as required in OECD 471.

3. Activation:

S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg Aroclor 1254 per kg body weight 5 days before sacrifice. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537
E. coli: WP2 uvrA
Salmonella typhimurium:
The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity. Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay:

In the first experiment triplicate plates were prepared for each concentration (neg. control; 20, 500, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and conditions (i.e. with and without S9) for all tester strains indicated above.

Pre-incubation assay:

In the second experiment the test article / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 4, 20, 100, 500 and 2500 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for all tester strains.

B. TEST PERFORMANCE:

1. Dates of experimental work: 30-Mar-2004 to 22-Apr-2004

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar (containing 0.5 mM histidine + 0.5 mM biotin), 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel-Bonner agar plates (minimal glucose agar plates). In the experiments with E. coli the warm soft agar contains 0.5 mM tryptophan instead of histidine + biotin.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.
After incubation in the dark for 48 to 72 hours at 37°C each plate was checked for precipitates and status of the background lawn. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects, the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37°C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9 mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.
The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.
A bacteriotoxic effect (reduced his^- or trp^- background growth and/or slight decrease in the number of his^+ or trp^+ revertants and/or reduction in the titer) was observed in the standard plate test and in the preincubation test depending on the strain and test conditions from about 500 µg - 2500 µg/plate onward.

C. MUTATION ASSAYS

Neither in the plate incorporation nor in the preincubation experiment with and without metabolic activation was a biologically relevant increase in number of revertants observed in any strain tested (see Table 5.8.1-21). The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Precipitation was not observed up to the maximum concentration.

Table 5.8.1-21: Bacterial gene mutation assay with Reg.No. 354563 - Mean number of revertants

Experiment 1: Plate incorporation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	34 ± 2	30 ± 6	117 ± 9	107 ± 5	15 ± 2	17 ± 2	10 ± 3	9 ± 1	38 ± 6	34 ± 7
Reg.No. 354563										
20 µg/plate	39 ± 9	25 ± 2	140 ± 2	110 ± 8	17 ± 1	14 ± 4	10 ± 2	9 ± 3	34 ± 7	27 ± 3
100 µg/plate	36 ± 4	29 ± 1	103 ± 13	108 ± 12	19 ± 1	20 ± 3	8 ± 2	9 ± 4	40 ± 9	42 ± 7
500 µg/plate	36 ± 4	26 ± 2	103 ± 8	116 ± 18	15 ± 6	19 ± 6	9 ± 3	7 ± 2	24 ± 3	36 ± 5
2500 µg/plate	33 ± 6	26 ± 1	78 ± 12	109 ± 19	16 ± 2	17 ± 6	4 ± 2	5 ± 2	18 ± 3	42 ± 10
5000 µg/plate	16 ± 4	14 ± 5	79 ± 11	97 ± 13	15 ± 6	16 ± 4	5 ± 2	5 ± 2	20 ± 9	41 ± 4
Pos. control§	867 ± 112	687 ± 77	1043 ± 307	1033 ± 97	128 ± 22	1189 ± 251	146 ± 28	416 ± 22	236 ± 15	630 ± 38
Experiment 2: Preincubation assay										
Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Metabol. activation	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9
Neg. control (DMSO)	30 ± 3	33 ± 4	107 ± 5	108 ± 4	18 ± 2	16 ± 1	11 ± 2	9 ± 1	30 ± 3	31 ± 3
Reg.No. 354563										
4 µg/plate	35 ± 5	33 ± 4	113 ± 8	102 ± 4	15 ± 2	14 ± 4	9 ± 3	9 ± 2	29 ± 7	33 ± 4
20 µg/plate	30 ± 4	32 ± 4	110 ± 12	97 ± 7	14 ± 2	13 ± 4	8 ± 1	9 ± 1	26 ± 1	26 ± 1
100 µg/plate	30 ± 3	27 ± 1	104 ± 8	100 ± 7	16 ± 2	14 ± 2	10 ± 1	8 ± 3	28 ± 3	27 ± 2
500 µg/plate	37 ± 4	23 ± 2	124 ± 6	83 ± 11	13 ± 1	13 ± 2	6 ± 3	9 ± 4	25 ± 5	26 ± 5
2500 µg/plate	24 ± 4	18 ± 2	83 ± 6	78 ± 7	14 ± 1	14 ± 2	4 ± 2	6 ± 1	16 ± 6	33 ± 2
Pos. control§	536 ± 32	901 ± 75	917 ± 55	1133 ± 131	108 ± 2	1470 ± 333	119 ± 23	393 ± 36	223 ± 36	607 ± 23

§ = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSION

According to the results of the present study, the test substance Reg.No. 354563 (Metabolite of dimoxystrobin) is not mutagenic in the Salmonella typhimurium / Escherichia coli reverse mutation assay under the experimental conditions chosen here.

Report:	CA 5.8.1/12 Engelhardt G.,Leibold E., 2005b In vitro gene mutation test with Reg.No. 354 563 (metabolite of BAS 505 F) in CHO cells (HPRT locus assay) 2005/1006706
Guidelines:	OECD 476, EEC 2000/32 B.17, EPA 870.5300
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

Reg. No. 354563 (Metabolite of dimoxystrobin, Batch: 01196-245, Purity: 99.6%) was tested in vitro for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells. Two independent experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations of up to 3600 µg/mL were used in the main experiment. The treatment intervals for both experiments in the presence and absence of metabolic activation were 4 hours. Ethylmethanesulfonate (EMS) and Methylcholanthrene (MCA) served as positive controls in the experiments without and with metabolic activation, respectively. After the incubation period treatment media were replaced by culture medium in both experiments and the cells were incubated for 6-8 days for expression of mutant cells. This was followed by incubation of cells in selection medium containing 6-thioguanine for about 1 week.
The test substance did not exhibit any pronounced toxicity up to the highest recommended concentration (3600 µ/mL), at which distinct test substance precipitation was observed.
The test substance did not cause any relevant increase in the mutant frequencies either without S9 mix or after the addition of a metabolizing system in two experiments performed independently.
Based on the results of the study it is concluded that under the conditions of this test Reg. No. 354563 (Metabolite of dimoxystrobin) does not induce forward mutations in mammalian cells in vitro.

(BASF DocID 2005/1006706)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg. No. 354563 (Metabolite of dimoxystrobin)

Description: powder, white

Lot/Batch #: 01196-245

Purity: 99.6%

Stability of test compound: The stability of the test substance under storage conditions was guaranteed until August 2006 as indicated by the sponsor. The homogeneity of the test substance was guaranteed on account of the high purity and ensured by mixing prior to preparation of test substance solutions.
The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: A negative control was not employed in this study

Solvent control: 1% (v/v) DMSO in culture medium

Positive control -S9: Ethylmethanesulfonate (EMS) 300 µg/mL

Positive control +S9: Methylcholanthrene (MCA) 10 µg/mL

3. Activation: S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg Aroclor 1254 per kg body weight 5 days before sacrifice. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors) in the 1st experiment and 3 volume of S9 fraction was mixed with 7 volumes of S9 supplement in the 2nd experiment. This preparation, the so-called S9-mix, was kept on ice until used.
The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	30 mM
$MgCl_2 \times 6H_2O$	10 mM
$CaCl_2 \times 2H_2O$	10 mM

4. Test organism:	Chinese hamster CHO cells. They have a high proliferation rate (doubling time about 12-16 h), high plating efficiency (about 90%) and karyotype with a modal number of 20 chromosomes. Stocks of the CHO cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).
5. Culture media:	
Culture medium:	Ham's F12 medium with L-glutamine and hypoxanthine supplemented with 10% (v/v) fetal calf serum (FCS).
Pretreatment medium:	("HAT" medium): FCS-supplemented Ham's F12 medium with L-glutamine and hypoxanthine containing per mL 13.6 µg hypoxanthine, 0.18 µg aminopterin and 3.88 µg thymidine.
Selection medium:	("TG" medium): L-Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL
	All media were supplemented with - 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL) - 1% (v/v) amphotericine B (250 µg/mL)
6. Locus examined:	hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)
7. Test concentrations:	
a) Preliminary toxicity assay:	Nine concentrations ranging from 100 to 3600 µg/mL
b) Mutation assay:	
1st experiment:	225, 450, 900, 1800 and 3600 µg/mL with and without metabolic activation
2nd experiment:	450, 900, 1800, 2700 and 3600 µg/mL with without metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 25-Nov-2004 to 14-Mar-2005

2. Preliminary cytotoxicity assay:

Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25-cm² flasks with various test substance concentrations in serum-free Ham's F12 medium for about 4 hours (with and without metabolic activation) after an attachment period of 24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS), covered with Ham's F12 and incubated for a further 5 to 7 days. After this incubation period, colonies were fixed, stained and counted. In addition to the cloning efficiency the following parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

3. Mutation Assay:

Pretreatment of Cells: Cells with a passage number ≥ 2 after thawing from the frozen cells stock were seeded into 75 cm^2-flasks and incubated for 3-4 days with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants. Afterwards, a passage into culture medium followed and the cells were incubated for further 3-4 days.

Cell treatment: For each test group, about $1x10^6$ cells per flask were seeded into flasks containing about 20 mL Ham's F12 medium supplemented with 10% FCS and incubated for about 20 – 24 hours with 5% CO_2 at 37°C and ≥ 90% humidity for cell attachment. Two flasks were used for each test group.

After the cell attachment period the medium was replaced by fresh medium. The test article, dissolved in 200 µL DMSO, was added to the culture medium. Without S9 mix 18/20 mL Ham's F12 medium without FCS and 200 µL test substance preparation were used. With S9 mix 14/16 mL Ham's F12 medium without FCS, 200 µL test substance preparation and 4 mL S9 mix (= 2% and 6% s9 fraction in the 1st and 2nd experiment, respectively.
Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix at 5% CO_2, 37°C and ≥ 90% humidity.

Expression: After incubation for 4 hours, the treatment medium was replaced by 20 mL Ham's F12 medium with 10% FCS after having been rinsed twice with Hanks' balanced salt solution (HBSS). The following 1st passage was carried out after an incubation period of 65 to 72 hours. After an entire expression period of 6 – 8 days the cells were transferred into the selection medium (2nd passage).

Selection: For the mutant selection, six 75-cm^2 flasks each were seeded with $3x10^5$ cells from each treatment group in selection medium (TG medium) and incubated for about 6 to 8 days. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.

Determination of Cytotoxicity: Cloning efficiency 1 (survival):
The survival (cloning efficiency 1; CE_1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25 cm^2 flasks using 5 mL Ham`s F12 medium with 10% FCS. After about 24 hour attachment period the cells were incubated with vehicle, test substance or the positive control for 4 hours as described above. Following exposure, cells were rinsed several times with HBSS. Finally, cells were cultured in 5 mL Ham's F12 medium incl. 10% (v/v) FCS.

Cloning efficiency 2 (viability):
The viability (cloning efficiency 2; CE_2) was determined after the expression period. About 200 cells were separated during the transfer into selection medium and seeded in two flasks (25 cm^2) containing 5 mL Ham's F12 medium incl. 10% (v/v) FCS each.
After seeding of the cells, the flasks were incubated for 5 – 8 days to form colonies. These colonies were fixed, stained and counted.

Calculations:

Mutant frequency: Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{\text{total number of mutant colonies}}{\text{number of seeded cells}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\ absolute}} \times 100$$

Cloning efficiency (CE, %) absolute:

$$CE_{absolute} = \frac{total\ number\ of\ colonies\ in\ the\ test\ group}{total\ number\ of\ seeded\ cells\ in\ the\ test\ group} x100$$

relative, in comparison to control:

$$CE_{relative} = \frac{CE_{absolute}\ of\ the\ test\ group}{CE_{absolute}\ of\ the\ vehicle\ control} x100$$

4. Statistics:
Due to the clearly negative findings, a statistical evaluation was not carried out.

5. Evaluation criteria:
The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies ($MF_{corr.}$) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose-response relationship.

Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose-response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

A test substance is generally considered negative in this test system if:

- The corrected mutation frequency ($MF_{corr.}$) in all dose groups is within the historical control range and is not significantly above the concurrent negative control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance under storage conditions was guaranteed until August 2006 as indicated by the Sponsor. The stability of the test substance at room temperature in the vehicle DMSO and in water over a period of 4 hours was verified analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY

After 4 hours treatment in the absence and presence of S9 mix cytotoxicity indicated by reduced relative cloning efficiency of about or below 20% relative survival was observed at 3600 µg/mL. Precipitation of the test substance was observed at 2000 µg/mL and above at the end of treatment in the absence of metabolic activation. In the presence of metabolic activation the S9 mix precipitation was observed in all test groups. No marked effect on osmolarity and pH value was observed.
Based on these data the highest concentration tested in the mutagenicity experiments was 3600 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

No increase in the number of mutant colonies was observed ether without S9 mix or after the addition of a metabolizing system (see Table 5.8.1-22 and Table 5.8.1-23). The mutant frequencies at any dose were close to the range of that of the concurrent negative control values and within the range of the historical control data.
Without S9 mix, there was a slight decrease in the number of colonies (CE_1) at 3600 µg/mL in the 1st experiment and from about 2700 µg/mL onward in the 2nd experiment.

Cell density was only slightly reduced in the 2nd experiment at 3600 µg/mL. With S9 mix, there was a decrease in the number of colonies (CE_1) from about 1800 µg/mL onward in the 1st experiment (S9 fraction: cofactors = 1:9) and at 3600 µg/mL in the 2nd experiment (S9 fraction : cofactors = 3:7). Cell density was only slightly reduced in the 2nd experiment at 3600 µg/mL.
The pH and osmolarity of the tested concentrations were not altered at the concentrations tested. In the 1st and 2nd experiment in the absence and presence of S9 mix, test substance precipitation was observed in culture medium 4 hours after start of treatment at 1800 µg/mL and above. Besides in the presence of S9 mix precipitates in culture medium occurred at 450 µg/mL and above in the 2nd experiment only. Cell attachment was not influenced at any dose evaluated.
Treatment with the positive controls EMS and MCA resulted in a marked increase in the number of mutant colonies as well as of mutant frequencies in all experiments, thus demonstrating the sensitivity of the test.

Table 5.8.1-22: Gene mutation in mammalian cells - 1st experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded) Cloning efficiency (%)		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded) Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	7	1.95	2.31	100.2	100.0	85.9	100.0
Reg.No. 354563							
225 µg/mL	8	2.23	2.83	102.4	102.2	77.6	90.3
450 µg/mL	6	1.67	1.89	103.4	103.2	80.4	93.6
900 µg/mL	9	2.50	3.55	98.9	98.7	71.6	83.4
1800 µg/mL	1	0.28	0.33	78.6	78.4	82.7	96.3
3600 µg/mL	3	0.84	1.24	47.3	47.2	67.9	79.0
Positive control EMS							
300.0 µg/mL	545	151.39	180.68	84.4	84.2	84.1	97.9
With metabolic activation[1]; 4-hour exposure period							
Vehicle (DMSO)	11	3.06	3.56	96.0	100.0	85.7	100.0
Reg.No. 354563							
225 µg/mL	2	0.56	0.70	99.0	103.1	78.3	91.4
450 µg/mL	10	2.78	4.16	94.8	98.8	69.7	81.3
900 µg/mL	3	0.84	1.12	86.1	89.7	74.9	87.4
1800 µg/mL	8	2.23	2.80	62.7	65.3	78.3	91.4
3600 µg/mL	19	5.28	9.70	35.4	36.9	52.8	61.6
Positive control MCA							
10 µg/mL	280	77.78	125.33	93.7	97.6	64.0	74.7

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
[1] S9 fraction : cofactors = 1:9

Table 5.8.1-23: Gene mutation in mammalian cells - 2nd experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded) Cloning efficiency (%)		CE_2 (viability) (at the end of the expression period; approx. 200 cells/flask seeded) Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	Relative
Without metabolic activation; 4-hour exposure period							
Vehicle (DMSO)	5	1.39	1.62	84.3	100.0	85.9	100.0
Reg.No. 354563							
450 µg/mL	5	1.39	1.64	70.4	83.5	88.6	103.1
900 µg/mL	3	0.84	1.17	66.2	78.5	74.4	86.6
1800 µg/mL	6	1.67	2.06	65.4	77.6	81.2	94.5
2700 µg/mL	9	2.50	2.91	48.4	57.4	84.2	98.0
3600 µg/mL	0	0.00	0.00	10.2	12.1	76.5	89.1
Positive control EMS							
300 µg/mL	530	147.22	198.70	68.7	81.5	74.1	86.3
With metabolic activation[1]; 4-hour exposure period							
Vehicle (DMSO)	12	3.34	3.92	84.2	100.0	87.2	100.0
Reg.No. 354563							
450 µg/mL	5	1.39	1.56	73.6	87.4	86.4	99.1
900 µg/mL	6	1.67	1.83	79.8	94.8	91.5	104.9
1800 µg/mL	26	7.22	8.08	62.1	73.8	90.1	103.3
2700 µg/mL	0	0.00	0.00	63.7	75.7	99.2	113.8
3600 µg/mL	29	8.06	9.02	12.3	14.6	89.2	102.3
Positive control MCA							
10 µg/mL	1197	332.50	408.32	66.3	78.7	81.5	93.5

[a] number of colonies 7 days after seeding 3 x 10^5 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
[1] S9 fraction : cofactors = 3:7

III. CONCLUSION

Based on the results of the study it is concluded that under the conditions of this test Reg.No. 354563 (Metabolite 505M09) does not induce forward mutations in the HPRT locus in CHO cells in vitro.

Report: CA 5.8.1/13
Engelhardt G.,Leibold E., 2005d
In vitro chromosome aberration assay with Reg.No. 354 563 (metabolite of BAS 505 F) in V79 cells
2005/1004854
Guidelines: OECD 473, EEC 2000/32 B.10, EPA 870.5375
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

Reg.No. 354563 (metabolite of dimoxystrobin; Batch: 01196-245, Purity: 99.6%) was tested in vitro for the ability to induce chromosome and numerical aberrations in Chinese Hamster V79 cells in two independent experiments in the presence and absence of metabolic activation. Based on the cytotoxicity results of a pretest for dose selection, concentrations of 400 µg/mL to 3600 µg/mL were tested for clastogenic effects with and without metabolic activation in experiments with a pulse treatment of 4 hours. In the 2nd experiment additionally an 18-hour exposure was done. The cells were prepared 18 h or 28 hours after start of the treatment. Vehicle (DMSO) and positive controls (cyclophosphamide (CPP) and ethylmethanesulfonate (EMS) for the experiment with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system. Prior to cell harvest, addition of colcemid arrested cells in the metaphase. After slide preparation and staining of the cells, at least 200 well spread metaphases per dose and treatment condition were analyzed for chromosomal aberrations, except for the positive control cultures where only 100 metaphases were scored due to clearly increased aberration rates.
At least three doses were evaluated. Among these, the highest concentration scored was 3600 µg/mL. On the basis of the results of the present study, the test substance did not cause any relevant increase in the number of structurally aberrant metaphases including and excluding gaps at both sampling times either without S9 mix or after adding a metabolizing system in several experiments performed independently. No increase in the frequency of cells containing numerical aberrations was demonstrated either.
Based on the results of this study, Reg.No. 354563 (metabolite of dimoxystrobin) is considered to be neither a clastogenic nor an aneugenic agent under in vitro conditions in V79 cell in the presence or absence of metabolic activation.

(BASF DocID 2005/1004854)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 354563 (Metabolite of dimoxystrobin)

Description: Powder, white

Lot/Batch #: 01196-245

Purity: 99.6%

Stability of test compound: The stability of the test substance under storage conditions was guaranteed by the sponsor. The stability of the test substance at room temperature dissolved in the vehicle DMSO over a period of 4 hours and in water over a period of 96 hours was verified analytically.
The homogeneity of the test substance was ensured by mixing prior to preparation of test substance formulations and on account of the high purity.

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Negative control: A negative control was not employed in this study.

Solvent control: DMSO

Positive control, -S9: Ethylmethanesulfonate (EMS) 500 µg/mL

Positive control, +S9: Cyclophosphamide (CPP) 0.5 µg/mL

3. Activation: S9 was produced from the livers of induced male Sprague-Dawley rats. The rats received a single intraperitoneal injection of 500 mg Aroclor 1254 per kg body weight 5 days before sacrifice. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so called S9 mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	Concentration
Phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms: Chinese hamster V79 cells

5. Culture medium: MEM medium with glutamine supplemented with
- 10% (v/v) fetal calf serum (FCS)
- 1% (v/v) penicillin/streptomycin (10000 IU/10000 µg/mL)
- 1% (v/v) amphotericine B (250 µg/mL)

During exposure to the test substance (4-hour treatment), MEM medium was used without FCS supplementation.

6. Test concentrations:

a) Preliminary toxicity assay: Eight concentrations ranging from 5 to 3600 µg/mL were used in pretests for dose selection for the main experiments. V79 cells were prepared at a sampling time of 18 hours after 4 and 18 hours exposure time without metabolic activation and after 4 hours exposure time with metabolic activation.

b) Mutation assay:

1st experiment: 400, 1200 and 3600 µg/mL with and without metabolic activation (4 hour exposure and 18 h preparation interval)

1st experiment/repeat: 500 (not evaluated), 1000, 1500 and 2000 µg/mL with metabolic activation (4 h exposure and 18 h sampling time)

2nd experiment: 250 (not evaluated), 500, 1000, 1500 and 2000 (evaluation not possible) µg/mL without metabolic activation (18 h exposure and 18 h preparation interval)

1000, 1500 and 2000 µg/mL without metabolic activation (18 h exposure and 28 h sampling time): none of the concentration could be evaluated because no or only a few metaphases for evaluation.

900, 1800 and 3600 with metabolic activation (4 h exposure and 28 h sampling time)

2nd experiment/repeat: 250, 500, 750 and 1000 µg/mL without metabolic activation (18 h exposure and 28 h sampling time) - only 1000 µg/mL was evaluated

1000 (was not evaluated), 1500, 2000 and 2500 µg/mL with metabolic activation (4 h exposure and 28 h sampling time)

B. TEST PERFORMANCE:

1. Dates of experimental work: 19-Oct-2004 to 29-Jun-2005

2. Preliminary cytotoxicity assay:

A range-finding cytotoxicity test was conducted with V79 cultures exposed for 4 and 18 hours to test substance concentrations of 5 – 3600 µg/mL both with and without metabolic activation. At the end of the exposure period, cell count, cell attachment, mitotic index and the quality of metaphases were determined in order to derive appropriate test substance concentrations for the main test.

3. Cytogenicity Assay:

Cell treatment: Cells were exposed to the test substance, solvent or positive control in pulse treatment experiments for 4 hours with or without metabolic activation. The cells were incubated in Quadriperm® dishes at 37°C, 5% CO_2 and ≥ 90% humidity. Two chambers of a Quadriperm dish were used for each concentration (=duplicate cultures). The preparation interval was 18 h post treatment-begin. After continuous treatment, i.e. 18 h without S9 mix, cells were treated in culture medium supplemented with 10% FCS and in the case of a sampling time of 28 hours incubated again for another 10 hours.
For determination of cytotoxicity, additional cell cultures (using 25 cm² plastic flasks) were treated in the same way as in the main experiment. Growth inhibition was estimated by comparing the cell number in the treated groups with the concurrent control.

Spindle inhibition: 100 µL colcemide (stock: 10 µg/mL phosphate buffered saline) was added to the cultures 2 – 3 hours prior to harvesting.

Cell harvest: At the end of the incubation time the culture medium was completely removed. For hypotonic treatment, 5 mL of a 0.4% KCl solution (37°C) was added for about 20 minutes. The cells were fixed by addition of 5 mL methanol/glacial acetic acid (3:1 v/v). The fixative was changed twice.

Slide preparation: The slides were removed from the Quadriperm chambers, briefly allowed to drip off and passed through a Bunsen burner flame. After drying, the cells were stained with Giemsa and Titrisol. After rinsing and clarifying in Xylene the cover slips were mounted in Corbit-Balsam.

Metaphase analysis: Slides were coded prior to analysis. As a rule, the first 100 consecutive well-spread metaphases of each culture were counted for all test groups, and if cells had 20 - 22 chromosomes, they were analyzed for structural chromosome aberrations. Numerical chromosome aberrations were also recorded. If there is a clear increase in chromosomally damaged cells, the number of metaphases to be analyzed is reduced from the planned 200 mitoses/ test group to 100.

A mitotic index based on 1000 cells per culture was determined for all evaluated test groups in both experiments.

4. Statistics:

The proportion of metaphases with aberrations was calculated for each group.

A comparison of each dose group with the vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test was Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one-sided.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A statistically significant, dose-related and reproducible increase in the number of cells with structural chromosome aberrations.
- The number of aberrant cells exceeds both the concurrent negative/vehicle control value and the historical negative control data range.

A test substance is generally considered as “negative” if the following criteria are met:

- The number of cells with structural / numerical aberrations in the dose groups is not statistically significant increased above the concurrent negative/vehicle control value and is within the historical negative control data range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours and in water over a period of 96 hours was verified analytically.

B. PRELIMINARY CYTOTOXICITY ASSAY:

A preliminary cytotoxicity assay was performed to determine appropriate test substance concentrations for the main test. Dose selection was based on the cell count, cell attachment, mitotic index and the quality of metaphases. Up to the highest recommended concentration, i.e. 10 mM (ca. 3600 µg/mL), at which distinct test substance precipitation was observed, the test substance did not exhibit any toxic effects after a treatment time of 4 hours. On the basis of these findings, 3600 µg/mL both with and without S9 mix were selected as the top doses.

C. CYTOGENICITY ASSAYS:

1st experiment

After pulse treatment of 4 hours and a sampling time of 18 hours, no increase in the number of chromosomally damaged cells was observed either without S9 mix or after the addition of a metabolizing system up to the highest dose of 3600 µg/mL when compared with the current negative (solvent) control groups (see Table 5.8.1-24 and Table 5.8.1-25). However, a value of 6.0% aberrant metaphases excl. gaps found at the intermediate dose of 1200 µg/mL in the experimental part with S9 mix (see Table 5.8.1-25) slightly exceeds the upper limit of the historical negative control range, i.e. 5.5%. For clarification of these findings, a repeat study, which was limited to the experimental part with metabolic activation, was carried out to clarify whether the findings of the 1st experiment are incidental or an indication of a clastogenic activity of the test chemical. For this purpose, closer doses were selected, i.e. 1000, 1500 and 2000 µg/mL. The result obtained demonstrates that Reg.No. 354563 (Metabolite of dimoxystrobin) is not clastogenic under the experimental conditions chosen (see Table 5.8.1-26).

2nd experiment

After continuous treatment and a sampling time of 18 hours, no increase in the number of aberrant metaphases was observed without S9 mix up to the highest analyzable dose of 1500 µg/mL (see Table 5.8.1-27). After continuous treatment at the later sampling time of 28 hours without S-9 mix, none of the selected doses, i.e. 1000, 1500 and 2000 µg/mL could be analyzed due to severe cytotoxicity. In the experimental part with S-9 mix after pulse treatment of 4 hours and a sampling time of 28 hours, an increase in aberrant metaphases both incl. and excl. gaps was observed at the low and intermediate doses of 900 µg/mL and 1800 µg/mL, respectively, but not at the top dose of 3600 µg/mL. However, with 13.5% incl. gaps and 7.5% excl. gaps, there was also an increase in the number of chromosomally damaged metaphases in the negative (solvent) control group (see Table 5.8.1-28) which significantly exceeds the upper limit of the negative historical control range. Therefore, this experiment must be regarded as not valid and thus as unsuitable for an assessment concerning a clastogenic mode of action.

Due to severe cytotoxicity after continuous treatment with 1000 – 2000 µg/mL and a sampling time of 28 hours in the 2nd experiment without S9 mix, this experimental part was repeated selecting lower doses, i.e. 250 -1000 µg/mL. In this repeat experiment, the top dose of 1000 µg/mL could be analyzed and did not show any clastogenicity (see Table 5.8.1-29).

Since the experimental part with S9 mix and a sampling time of 28 hours must be considered invalid due to a substantial amount of aberrant metaphases in the current negative (solvent) control, this experimental part was repeated. However, even if the invalid experiment cannot be used for an assessment of clastogenicity, the doses for the repeat experiment, i.e. 1500 µg/mL - 2500 µg/mL, were selected on the basis of the fact that an increased aberration rate was found in the invalid 2nd experiment at 900 µg/mL and 1800 µg/mL but not at 3600 µg/mL. In this repeat experiment, no clastogenicity was demonstrated (see Table 5.8.1-30).
The osmolarity and pH values were not influenced by the test substance treatment. Test substance precipitation in culture medium at the end of exposure period was macroscopically observed from 3600 µg/mL onward in both experimental parts in the 1st Experiment and from 1800 µg/mL in the 2nd Experiment with metabolic activation.
Vehicle and positive controls were all in a range to ensure the validity of the test.

III. CONCLUSION

Thus, under the experimental conditions chosen here, the conclusion is drawn that Reg. No. 354563 (Metabolite 505M09) is neither a clastogenic (chromosome-damaging) nor an aneugenic agent under in vitro conditions using V79 cells.

Table 5.8.1-24: Chromosome aberration test with Reg.No. 354563 without metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	
Vehicle DMSO	A	100	12.1	4	4.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
	B	100	9.9	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	
	A + B	**200**	**11.0**	**7**	**3.5**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**	
Reg.No. 354563																				
400 µg/mL	A	100	9.3	5	5.0	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
	B	100	10.2	7	7.0	3	3.0	3	3.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	
	A + B	**200**	**9.8**	**12**	**6.0**	**6**	**3.0**	**5**	**2.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**	
1200 µg/mL	A	100	8.5	7	7.0	4	4.0	2	2.0	1	1.0	0	0.0	2	1.9	2	1.9	0	0.0	
	B	100	11.3	4	4.0	2	2.0	2	2.0	1	1.0	0	0.0	0	0.0	1	1.0	0	0.0	
	A + B	**200**	**9.9**	**11**	**5.5**	**6**	**3.0**	**4**	**2.0**	**2**	**1.0**	**0**	**0.0**	**2**	**1.0**	**3**	**1.5**	**0**	**0.0**	
3600µg/mL	A	100	10.2	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
	B	100	10.9	3	3.0	1	1.0	1	1.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	
	A + B	**200**	**10.6**	**5**	**2.5**	**2**	**1.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	
Positive control EMS																				
500 µg/mL	A	50	8.2	10	20.0	10	20.0	7	14.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
	B	50	9.1	8	16.0	7	14.0	6	12.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
	A + B	**100#**	**8.7**	**18**	**18.0****	**17**	**17.0****	**13**	**13.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	

*: p ≤ 0.05, **: p ≤ 0.01 (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Table 5.8.1-25: Chromosome aberration test with Reg.No. 354563 with metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	11.4	6	6.0	4	4.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0
	B	100	13.6	11	11.0	6	6.0	3	3.0	0	0.0	0	0.0	0	0.0	1	1.0	1	1.0
	A + B	**200**	**12.5**	**17**	**8.5**	**10**	**5.0**	**4**	**2.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**2**	**1.0**
Reg.No. 354563																			
400 µg/mL	A	100	11.7	3	3.0	2	2.0	0	1.0	0	0.0	0	0.0	1	1.0	1	1.0	0	0.0
	B	100	10.8	7	7.0	2	2.0	1	0.0	0	0.0	0	0.0	0	0.0	2	1.9	1	1.0
	A + B	**200**	**11.3**	**10**	**5.0**	**4**	**2.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**3**	**1.5**	**1**	**0.5**
1200 µg/mL	A	100	11.2	6	6.0	5	5.0	4	4.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	14.0	7	7.0	7	7.0	6	6.0	1	1.0	0	0.0	0	0.0	1	1.0	0	0.0
	A + B	**200**	**12.6**	**13**	**6.5**	**12**	**6.0**	**10**	**5.0**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**
3600 µg/mL	A	100	9.6	4	4.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	9.9	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0
	A + B	**200**	**9.8**	**6**	**3.0**	**2**	**1.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**
Positive control CPP																			
0.5 µg/mL	A	50	8.4	11	22.0	10	20.0	4	8.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	9.1	13	26.0	10	20.0	7	17.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100**	**8.8**	**24**	**24.0****	**20**	**20.0****	**11**	**11.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: p ≤ 0.05, **: p ≤ 0.01 (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Table 5.8.1-26: Chromosome aberration test with Reg.No. 354563 with metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1/Repeat

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	12.8	11	11.0	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	3	2.9	1	1.0
	B	100	16.8	4	4.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**14.8**	**15**	**7.5**	**4**	**2.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**3**	**1.5**	**1**	**0.5**
Reg.No. 354563																			
1000 µg/mL	A	100	8.0	7	7.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	B	100	8.8	5	5.0	3	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**8.4**	**12**	**6.0**	**7**	**3.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**
1500 µg/mL	A	100	10.3	11	11.0	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	2	2.0	0	0.0
	B	100	9.2	7	7.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	A + B	**200**	**9.8**	**18**	**9.0**	**6**	**3.0**	**3**	**1.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**3**	**1.5**	**0**	**0.0**
2000 µg/mL	A	100	12.1	15	15.0	7	7.0	5	5.0	0	0.0	0	0.0	0	0.0	3	2.9	0	0.0
	B	100	13.8	4	4.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**13.0**	**19**	**9.5**	**9**	**4.5**	**5**	**2.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**3**	**1.5**	**0**	**0.0**
Positive control CPP																			
0.5 µg/mL	A	50	6.3	10	20.0	9	18.0	4	8.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	7.0	12	24.0	12	24.0	6	12.0	1	2.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100**	**6.7**	**22**	**22.0****	**21**	**21.0****	**10**	**10.0****	**1**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: p ≤ 0.05, **: p ≤ 0.01 (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)

#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Table 5.8.1-27: Chromosome aberration test with Reg.No. 354563 without metabolic activation (18 hours treatment, harvest after 18 hours) - Experiment 2

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	10.4	5	5.0	4	4.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	9.6	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**10.0**	**8**	**4.0**	**5**	**2.5**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Reg.No. 354563																			
500 µg/mL	A	100	10.5	4	4.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	11.8	5	5.0	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**11.2**	**9**	**4.5**	**5**	**2.5**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
1000 µg/mL	A	100	9.9	3	3.0	2	2.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	7.5	9	9.0	5	5.0	2	2.0	1	1.0	0	0.0	1	1.0	0	0.0	0	0.0
	A + B	**200**	**8.7**	**12**	**6.0**	**7**	**3.5**	**3**	**1.5**	**2**	**1.0**	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**
1500 µg/mL	A	100	4.2	9	9.0	6	6.0	5	5.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	3.7	6	6.0	5	5.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**4.0**	**15**	**7.5**	**11**	**5.5**	**7**	**3.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Positive control EMS																			
500.0 µg/mL	A	50	9.5	15	30.0	13	26.0	10	20.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	7.1	10	20.0	10	20.0	8	16.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100#**	**8.3**	**25**	**25.0****	**23**	**23.0****	**18**	**18.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: p ≤ 0.05 , **: p ≤ 0.01 (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Table 5.8.1-28: Chromosome aberration test with Reg.No. 354563 with metabolic activation (4 hours treatment, harvest after 28 hours) - Experiment 2

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	12.8	18	18.0	12	12.0	6	6.0	0	0.0	0	0.0	0	0.0	2	2.0	0	0.0
	B	100	13.3	9	9.0	3	3.0	1	1.0	0	0.0	0	0.0	1	1.0	1	1.0	0	0.0
	A + B	**200**	**13.1**	**27**	**13.5**	**15**	**7.5**	**7**	**3.5**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**3**	**1.5**	**0**	**0.0**
Reg.No. 354563																			
900 µg/mL	A	100	11.9	19	19.0	10	10.0	5	5.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	13.4	9	9.0	3	3.0	0	0.0	0	0.0	0	0.0	1	1.0	3	2.9	0	0.0
	A + B	**200**	**12.7**	**28**	**14.0**	**13**	**6.5**	**5**	**2.5**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**3**	**1.5**	**0**	**0.0**
1800 µg/mL	A	100	9.5	23	23.0	13	13.0	3	3.0	0	0.0	0	0.0	0	0.0	4	4.0	0	0.0
	B	100	9.6	14	14.0	7	7.0	3	3.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	A + B	**200**	**9.6**	**37**	**18.5**	**20**	**10.0**	**6**	**3.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**5**	**2.4**	**0**	**0.0**
3600 µg/mL	A	100	7.8	8	8.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0
	B	100	10.0	13	13.0	6	6.0	3	3.0	0	0.0	0	0.0	1	1.0	4	3.8	0	0.0
	A + B	**200**	**8.9**	**21**	**10.5**	**8**	**4.0**	**4**	**2.0**	**0**	**0.0**	**0**	**0.0**	**1**	**0.5**	**5**	**2.4**	**0**	**0.0**
Positive control CPP																			
0.5 µg/mL	A	50	10.7	11	22.0	10	20.0	9	18.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	14.7	14	28.0	13	26.0	11	22.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100#**	**12.7**	**25**	**25.0***	**23**	**23.0****	**20**	**20.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Table 5.8.1-29: Chromosome aberration test with Reg.No. 354563 without metabolic activation (18 hours treatment, harvest after 28 hours) - Experiment 2/Repeat

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	14.3	4	4.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	6	6.0	0	0.0
	B	100	14.3	6	6.0	2	2.0	0	0	0	0.0	0	0.0	0	0.0	3	3.0	0	0.0
	A + B	**200**	**14.3**	**10**	**5.0**	**4**	**2.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**9**	**4.3**	**0**	**0.0**
Reg.No. 354563																			
1000 µg/mL	A	100	13.1	8	8.0	4	4.0	3	3.0	0	0.0	0	0.0	0	0.0	3	2.9	0	0.0
	B	100	13.0	7	7.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	4	3.8	0	0.0
	A + B	**200**	**13.1**	**15**	**7.5**	**5**	**2.5**	**4**	**2.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**7**	**3.4**	**0**	**0.0**
Positive control EMS																			
500.0 µg/mL	A	50	10.5	12	24.0	11	22.0	9	18.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	8.4	13	26.0	11	22.0	8	16.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100#**	**9.5**	**25**	**25.0****	**22**	**22.0****	**17**	**17.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Table 5.8.1-30: Chromosome aberration test with Reg.No. 354563 with metabolic activation (4 hours treatment, harvest after 28 hours) - Experiment 2/Repeat

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	21.3	4	4.0	3	3.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	18.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**19.7**	**6**	**3.0**	**4**	**2.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Reg.No. 354563																			
1500 µg/mL	A	100	16.1	3	3.0	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	15.3	4	4.0	3	3.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**15.7**	**7**	**3.5**	**5**	**2.5**	**3**	**1.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
2000 µg/mL	A	100	15.6	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	10.2	2	2.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**12.9**	**4**	**2.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
2500 µg/mL	A	100	15.3	2	2.0	1	1.0	1	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	11.0	5	5.0	2	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**	**13.2**	**7**	**3.5**	**3**	**1.5**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**
Positive control CPP																			
0.5 µg/mL	A	50	15.0	15	15.0	11	11.0	6	6.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	14.5	10	10.0	10	10.0	5	5.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100#**	**14.8**	**25**	**12.5****	**21**	**10.5****	**11**	**5.5****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**

*: $p \leq 0.05$, **: $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferroni-Holm corrections)
#: Due to a clear increase in chromosomally damaged cells, the number of metaphases analyzed was reduced from the planned 200 mitoses to 100 cells.

Report: CA 5.8.1/14
[redacted] 2005c
Micronucleus assay in bone marrow cells of the mouse with Reg.No. 354 563 (metabolite of BAS 505 F) after a single oral application
2005/1006737
Guidelines: OECD 474, EEC 2000/32 C.4, EPA 870.5395
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Report: CA 5.8.1/15
[redacted] 2005d
Amendment - Micronucleus assay in bone marrow cells of the mouse with Reg.No. 354563 (metabolite of BAS 505 F) after a single oral application
2005/1006739
Guidelines: OECD 474, EEC 2000/32 C.4, EPA 870.5395
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary

Reg.No. 354563 (505M09, metabolite of dimoxystrobin; Batch: 01196-245, Purity: 99.6%) was tested in NMRI mice in order to investigate the potential to induce micronuclei in polychromatic erythrocytes (PCEs) using the micronucleus test method. For this purpose, the test substance, dissolved in 0.5% CMC, was administered once orally to groups of 6 male mice at dose levels of 500, 1000 and 2000 mg/kg body weight in a volume of 20 mL/kg body weight. The vehicle served as negative and cyclophosphamide as positive control. The animals were sacrificed 24 or 48 hours after the administration and the bone marrow of the two femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. To describe a cytotoxic effect due to the treatment with the test substance the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and reported as the number of normochromatic erythrocytes (NCEs) per 2000 PCEs.
The oral administration of Reg.No. 354563 did not lead to any biologically relevant increase in the number of polychromatic erythrocytes. The rate of micronuclei was mostly close to the concurrent negative control and was within the range of the historical control data. After treatment with the test substance the ratio of NCE:PCE was not relevantly altered as compared to the vehicle control. Weak signs of systemic toxicity were observed in the high dose groups in the first hour after administration consisting of ruffled fur and reduction of spontaneous activity.
The positive control chemical, i.e. cyclophosphamide, led to the expected increase in the rate of polychromatic erythrocytes containing micronuclei, thus demonstrating the sensitivity of the test system.

(BASF DocID 2005/1006737)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 354563 (Metabolite of dimoxystrobin)

Description:	Solid powder, white
Lot/Batch #:	01196-245
Purity:	99.6%
Stability of test compound:	The stability of the test substance under storage conditions throughout the study period was guaranteed by the sponsor. Homogeneity of the test substance was guaranteed on account of the high purity and was ensured by mixing before test substance preparation.
Vehicle used:	0.5% carboxymethylcellulose (CMC) in doubly distilled water

2. Control Materials:

Negative:	No negative control was employed in this study.
Vehicle control:	0.5% CMC
Positive control:	Cyclophosphamide (CPA) 40 mg/kg bw in aqua deionized

3. Test animals:

Species:	mice
Strain:	NMRI
Sex:	Male for the main study; male and female for the range finding study
Age:	8 - 10 weeks
Mean body weight at dosing:	33.8 ± 2.3 g
Source:	Harlan Winkelmann GmbH, 33178 Borchen, Germany
Number of animals per dose:	
Range finding study:	3 animals/sex and test group
Micronucleus assay:	6 males/dose
Acclimation period:	At least 5 days
Diet:	Standardized pelleted feed ad libitum (Harlan Winkelmann GmbH, 33178 Borchen, Germany)
Water:	tap water, ad libitum
Housing:	During the study the mice were housed individually in Makrolon cages, type I with wire mesh top (EHRET GmbH, 79302 Emmendingen)

4. Environmental conditions:

Temperature:	22 ± 3°C
Humidity:	28 - 70%
Air changes:	frequency not indicated (fully air-conditioned rooms)
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound doses:

Range finding test:	2000 mg/kg bw
Micronucleus assay:	500, 1000 and 2000 mg/kg bw The test substance was administered once by oral gavage using an application volume of 20 mL/kg bw.

B. TEST PERFORMANCE

1. Dates of experimental work: 29-Mar-2005 to 11-Apr-2005

2. Preliminary range finding test:

Male and female NMRI mice were treated once by oral gavage with a test substance dose of 2000 mg/kg bw.

3. Micronucleus test:

Treatment and sampling:	Groups of 6 male mice were treated once with the vehicle or 500, 1000 or 2000 mg test substance/kg bw by oral gavage. Additional test groups treated with the high dose were used for the second sampling period. The application volume was 20 mL/kg bw and the volume to be applied was calculated based on actual weight on the day of administration. The positive control substance CPA was administered once by oral gavage. The animals were observed for evident clinical signs of toxicity throughout the study. Twenty-four hours after the administration the mice were sacrificed and the two femora were removed. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 390xg for 10 minutes. The supernatant was discarded and the pellet resuspended. The sampling of the femora from the additional high dose and vehicle control group was performed as described above 48 h after the treatment.
Slide preparation:	One drop of the suspension was applied on a clean microscopic slide and smears were prepared. After air drying the smears were stained with May Grünwald solution. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample.
Slide evaluation:	In general, 2000 polychromatic erythrocytes (PCEs) from each male animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored. To describe the cytotoxic effect the ratio between the NCEs and PCEs were determined in the same sample and expressed in NCEs per 2000 PCEs. Five males per test group were evaluated as described. The remaining 6th animal in the respective test group is usually evaluated in case an animal dies in its test group spontaneously.

4. Statistics:

For statistical analysis the nonparametric Mann-Whitney test was used if the mean micronucleus frequency was above the vehicle control value.

5. Evaluation criteria:

The test chemical is classified as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group. Statistical methods will be used as an aid in evaluating the results. However, the primary point of consideration is the biological relevance of the results.

A test substance that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes is considered non-mutagenic in this system.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance in water was verified analytically by the sponsor. The used concentrations of the test substance in vehicle were analysed by the sponsor in a separate study.

B. PRELIMINARY RANGE FINDING TEST

In the pretest for the determination of the acute oral toxicity in males and females, the animals were treated with 2000 mg/kg bw. All animals survived with weak signs of toxicity. The clinical signs observed were reduction of spontaneous activity and ruffled fur. There were no distinct differences between male and female animals. Thus, only male animals were used for the main experiment.

C. MICRONUCLEUS ASSAY

After oral treatment with Reg.No. 354563 the bone marrow of these mice showed no relevant altered ratio of NCE/PCE as compared to the vehicle control. In comparison to the corresponding vehicle controls there was no biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test substance. The mean values of micronuclei observed after treatment with Reg.No. 354563 were either below or near to the value of the vehicle control group (see Table 5.8.1-31).

40 mg/kg bw cyclophosphamide administered orally was used as positive control which showed a statistically significant increase of induced micronucleus frequency.

The administration of the test substance was well tolerated by all animals with weak clinical signs of toxicity observed in only in the high dose group consisting of reduction of spontaneous activity and ruffled fur.

Table 5.8.1-31: Micronucleus test in mice administered Reg.No. 354563 by oral gavage

Test group	Dose mg/kg bw	Sampling time (h)	PCEs with micronuclei (‰)	range	NCEs per 2000 PCEs
Vehicle	0	24	1.10	1 – 4	1694
Reg.No. 354563	500	24	0.60	1 – 2	1754
Reg.No. 354563	1000	24	1.10	0 – 4	1556
Reg.No. 354563	2000	24	1.00	0 – 4	1661
cyclophosphamid	40	24	28.30**	28 – 112	1885
Reg.No. 354563	2000	48	0.70	0 - 2	1808

** $p \leq 0.01$ (non-parametric Mann-Whitney test)
PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes

III. CONCLUSION

Based on the result of this study Reg.No. 354563 (505M09, metabolite of dimoxystrobin) does not induce the formation of micronuclei in mouse polychromatic erythrocytes under the conditions of the study.

Report: CA 5.8.1/16
[redacted] et al., 2005a
BF 505-7, BF 505-8, BAS 505 F - Determination of iron in serum and duodenal weights of male Wistar rats after oral administration in the diet over 7 days
2005/1006725
Guidelines: <none>
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary
The aim of this study was to determine the level of iron in serum and duodenum weights of male Wistar rats after oral administration of dimoxystrobin and its metabolites BF 505-7 (505M08) and BF 505-8 (505M09). The test substances were administered to groups of 10 male Wistar rats at dietary concentrations of 500 ppm over a period of 7 days. Body weight and food consumption were determined. Blood from all non-fasted animals were taken at the end of the administration period. Terminal body weights as well as duodenum weights were determined.
No animal died during the study. Food consumption and body weights were not significantly changed in all groups.
Serum iron concentration was decreased (-23%) only after treatment with dimoxystrobin but not with its metabolites. Furthermore, an increased absolute and relative duodenum weight (30%) was observed also only after treatment with dimoxystrobin but not with its metabolites BF 505-7 and BF 505-8). Thus, the metabolites of dimoxystrobin do not influence the serum iron concentrations as well as duodenum weights of male Wistar rats after 7 days of treatment.
(BASF DocID 2005/1006725)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 354562 = BF 505-7 (Metabolite of dimoxystrobin)
Reg.No. 354653 = BF 505-8 (Metabolite of dimoxystrobin)
BAS 505 F (dimoxystrobin)

Description: Powder / white
Lot/Batch #: 01196-241
01196-245
N15
Purity: 97.8%
99.6%
98.4%
Stability of test compound: The test substance was stable at room temperature over the study period (Expiry date July 2005 for BF 505-7 and August 2006 for BF 505-8 and January 2007 for dimoxystrobin).

2. Vehicle and/or positive control: normal diet

3. Test animals:

Species:	Rat
Strain:	Wistar (CrlGlxBrlHan:WI)
Sex:	Male
Age:	about 7 weeks
Weight at dosing:	mean 221 g
Source:	Charles River, Sulzfeld, Germany
Acclimation period:	6 days
Diet:	basic maintenance diet for rat/mouse/hamster, meal from Provimi KLIBA SA, Kaiseraugust, Switzerland, ad libitum
Water:	water, ad libitum
Housing:	single housing in type DK III stainless steel wire mesh cages, floor area about 800 cm²

Environmental conditions:

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

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 21-Sep-2004 - 28-Oct-2004
(In life dates: 27-Sep-2004 (start of administration) to 04-Oct-2004 (necropsy)

2. Animal assignment and treatment:
The test substances were administered to groups of 10 male rats at a dietary concentration 500 ppm for up to 7 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

3. Test substance preparation and analysis:
The test substances were weighed out and thoroughly mixed with a small amount of diet. The food was added in order to obtain the desired concentrations, and mixing was carried out for about 10 minutes in a laboratory mixer. The mixtures were prepared once before the start of the study. No analysis of the test substance preparations were carried out for this study.

4. Statistics:
Means and standard deviations (S.D.) of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Statistics of clinical examinations, clinical pathology and pathology

Parameter	Statistical test
Food consumption, body weight	A comparison of the dose group with the control group was performed using DUNNET test (two-sided) for the hypothesis of equal means.
Blood chemistry	Pair-wise comparison of each substance with a control group was performed using Wilcoxon-test (two-sided) for the equal medians
Weight of the anesthetized animals and absolute and relative organ weights	Wilcoxon test

C. METHODS

1. Observations:

The animals were examined for evident signs of toxicity or mortality twice daily on working days and once daily on weekends and public holidays. Observations for general clinical signs of toxicity were performed once daily.

2. Body weight:

Body weight was determined before the start of the administration period in order to randomize the animals. Thereafter the body weight was determined at the start of the administration period and at the end of the study.

3. Food consumption and compound intake:

Individual food consumption was determined at the end of the study and calculated as mean food consumption (g/animal/day).
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$Substance\ intake\ for\ day\ x = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm, and BW_x as body weight on day x of the study (in g).

4. Water consumption:

Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume.

5. Ophthalmoscopy:

Not performed in this study.

6. Hematology and clinical chemistry:

Blood samples were taken from the retroorbital venous plexus in the morning (day 7) from non-fasted animals. The animals were anesthetized using isoflurane. For all animals serum iron was determined.

7. Urinalysis:

Not performed in this study

8. Sacrifice and pathology:

The animals were sacrificed by decapitation under carbon dioxide anesthesia. The weights of the anesthetized animals and the duodenum were determined. All gross lesions and the duodenum were fixed in 4% formaldehyde.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of the parent substance (dimoxystrobin) in the diet over a period of 49 days at room temperature was determined in a previous study. No analyses of the test substance preparations were carried out for this study.

B. OBSERVATIONS

1. Clinical signs of toxicity

There was nothing abnormal detected in all animals.

2. Mortality

No animal died during the study.

3. Ophthalmoscopy

Not performed in this study.

C. BODY WEIGHT AND BODY WEIGHT GAIN

No significant changes in body weight were observed.

D. FOOD CONSUMPTION AND COMPOUND INTAKE

No significant changes in food consumption were observed.

The approximate mean daily test substance intake in male rats was calculated to be 39.6, 41.6 and 41.2 mg/kg bw/day at a dietary dose level of 500 ppm for dimoxystrobin, BF 505-7 and BF 505-8, respectively.

E. BLOOD ANALYSIS

1. Serotological findings

Only animals of the group treated with dimoxystrobin showed a significant reduction of iron concentration in serum (-23%) compared to control animals. [see Table 5.8.1-32].

Table 5.8.1-32: Iron concentration (µmol/L) in the serum of male rats after administration of 500 ppm dimoystrobin, BF 505-7 and BF 505-8 for 7 days

Sex	Blood sampling		control	Dimoxystrobin	BF 505-7	BF 505-8
Males	Day 7	Mean % Dev.	45.3±7.3	**35.0±6.1**** **-23**	45.6±7.8 0	47.5±8.9 5

* p ≤ 0.05; ** p ≤ 0.01 (Wilcoxon-test, two-sided)

2. Clinical chemistry findings

Not performed in this study.

3. Urinalysis

Not performed in this study.

F. NECROPSY

No abnormalities were observed during the macroscopic observation.
The absolute and relative duodenum weights of animals treated with dimoxystrobin were found significantly increased (30%) compared to control animals (see Table 5.8.1-33).

Table 5.8.1-33: Terminal body weights and duodenum weights

	Terminal body weight		duodenum	
	Absolute weight [g]	Relative weight [%]	Absolute weight [g]	Relative weight [%]
Control	245.6±9.1	100.0	0.49±0.03	0.20±0.01
Dimoxystrobin	245.2±14.6	100.0	**0.63±0.05****	**0.26±0.02****
BF 505-7	239.3±0.48	100.0	0.48±0.04	0.20±0.01
BF 505-8	245.2±9.1	100.0	0.5±0.04	0.20±0.01

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis H and Wilcoxon test, two-sided)

III. CONCLUSIONS

Only the parent substance dimoxystrobin at a concentration of 500 ppm reduces serum iron concentration as well as increases duodenum weight of male Wistar rats after 7 days of treatment. The metabolites BF 505-7 (505M08) and BF 505-8 (505M09) showed no abnormal effects.

CA 5.8.2 Supplementary studies on the active substance

Studies submitted in the original Annex I Dossier (2003):
Dimoxystrobin (BAS 505 F) has been tested in various testing models to gather information on the mode of action. All studies are scientifically valid. The studies listed in Table 5.8.2-1 have been evaluated and peer reviewed during the previous Annex I inclusion process.

Table 5.8.2-1: Summary of already peer-reviewed mechanistic studies with dimoxystrobin

Category of test	Dose range	Results	Reference (BASF DocID)
Studies conducted in mice			
S-phase response study Mice, B6C3F1 (Rj), male, dietary study 2-day, 2- and 4-week treatment	0, 25, 1000 ppm 0, 6.25, 250 mg/kg bw/day	25 ppm: no effects 1000 ppm: - minimal/slight thickening of duodenal mucosa - increased cell proliferation - no effect on apoptosis	2001/1014892 2002/1012807
S-phase response study Mice, B6C3F1 (Rj), male, dietary study 2-day and 2-week treatment; w/wo 1- and 2-week recovery	0, 200, 1000 ppm 0, 150, 200 mg/kg bw/day	200 ppm: no effects 1000 ppm: - minimal/slight thickening of duodenal mucosa - increased cell proliferation - reversible within 1- or 2-week recovery	2001/1014893 2002/1012809
Studies conducted in rats			
5-week feeding study (effects on clinical chemistry) Wistar rats, recovery groups included	0, 4500 ppm 0, 232/264 mg/kg bw/day	4500 ppm: - reduced food consumption and body weight gain - evidence of hypochromic microcytic anaemia - reversible changes in clinical chemistry (serum iron reduced by 51% after 1 day or 74% after 5 days)	2002/1005354
7-day feeding study (effects on serum iron) Wistar rats	0, 50, 250, 500 ppm 0, 1, 4, 20 and 40 mg/kg bw/day (calculated)	NOAEL 50 ppm (equivalent to 4 mg/kg bw/day)	2002/1014245
14-day (males) or 7-day (females) feeding study (Effect of administration of additional iron on dimoxystrobin-induced changes in duodenum of Wistar rats)	Males: 0, 4500 ppm 0, 291/235 mg/kg bw/day Females: 0, 500, 4500 ppm 0, 38/18, 191/85 mg/kg bw/day	Dimoxystrobin induced duodenal effects (increased weight and mucosal thickening) were partly reduced by administration of additional iron	2002/1013984
In vitro studies			
In vitro study for testing the formation of dimoxystrobin complexes with iron		Some evidence that dimoxystrobin can form complexes with Fe(III) ions, but not with Fe(II) ions, in vitro	2002/1012887

Studies submitted in this supplementary dossier (not yet peer-reviewed):
For further mechanistic evaluation of dimoxystrobin the following studies were performed after Annex I inclusion (see Table 5.8.2-2).

Table 5.8.2-2: Summary of not yet peer-reviewed mechanistic studies with dimoxystrobin

Category of test	Dose range	Results	Reference (BASF DocID)
Studies conducted in mice			
7-day feeding study, B6C3F1 mice	0, 4000, 8000 ppm (no data on substance intake)	- downward trend in haemoglobin and serum iron levels (not significant) - increased duodenum weights with histopathological correlate (thickening of the mucosa) - decrease of ferroportin, Dcytb, ferritin, Hif2 mRNA levels and H-ferritin protein levels in the duodenum - increase of transferrin receptor mRNA levels in the duodenum - decrease of liver hepcidin mRNA (by 50%, only at 4000 ppm) - small increase in liver non-hem iron (8000 ppm compared to 4000 ppm)	2011/1001622
Luciferase reporter assay (hepcidin) in HepG2 cells	25, 50, 100 µM	- reduced hepcidin promotor activity (about 50% of control value at all concentrations)	2011/1001622
Iron binding affinity of dimoxystrobin	up to 2.2 mM	- no relevant iron binding properties	2011/1001622
Amendment to the S-phase response study B6C3F1 mice male dietary study 2-day, 2- or 4-week treatment (2001/1014892) Additional pathological examinations	1000 ppm 250 mg/kg bw/day	lower levels of iron (stained as Fe^{2+}/Fe^{3+}) in the duodenum after 2 and 4 weeks of treatment	2012/1221002
Studies conducted in rats			
7-day feeding study (effects on serum iron) Wistar rats male (age : 3 or 10 weeks)	0, 250, 500 ppm 0, 33.8, 65.3 mg/kg bw/day (3 weeks old) 0, 500 ppm 0, 33.4 mg/kg bw/day (10 weeks old)	- NOEL <250 ppm for serum iron concentration - decrease of serum iron in all dose and animal groups, no difference between young and adult animals - thickening of the duodenum in 7/10 animals (at 500 ppm in 10 week old animals)	2005/1004845
7-day feeding study (effects on serum iron and transferrin) Wistar rats	0, 6, 11, 22 ppm 0, 0.95, 1.71, 3.42 mg/kg bw/day	NOAEL considered to be 22 ppm (equivalent to about 4 mg/kg bw/day)	2010/1026748

A detailed discussion on the mode of action of duodenal toxicity induced by dimoxystrobin can be found in chapter M-CA 5.5 in this dossier.

For the convenience of the reviewer summaries of the already peer-reviewed, as well as detailed study summaries of the new, not peer-reviewed studies are provided below. Studies will be arranged logically combining peer-reviewed and not peer-reviewed studies (studies conducted in mice followed by studies conducted in rats) to give a clear picture on the mode of action of dimoxystrobin.

Studies conducted in mice:

Effects of dimoxystrobin on cell proliferation and apoptosis in mouse duodenum

These studies with male mice were designed to investigate the mode of action responsible for the induction of duodenal tumours in the mouse carcinogenicity study. The studies used the same strain of mice as in the carcinogenicity study and included the dose (1,000 ppm) at which duodenal tumours were seen in male mice. Expected no observed effect concentrations (25 ppm in the first study, 200 ppm in the second study) were included.

Dimoxystrobin, S-phase response study (mouse 4-week dietary administration) (2001/1014892; 2002/1012807) – peer-reviewed

Guidelines: No test guideline exists for this type of study.
Deviations: NA
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and methods
Dimoxystrobin (Batch/purity: N 15/98.4%) was administered to groups of 10 male B6C3F1 Rj mice at dietary concentrations of 0, 25 ppm (about 6.25 mg/kg bw/day) and 1,000 ppm (about 250 mg/kg bw/day) for 2 days, 2 weeks and 4 weeks. The animals were examined for signs of toxicity or mortality at least once a day.

The influence of treatment on DNA synthesis (S-phase response), an indicator of cell proliferation, in the duodenum was determined using bromodeoxyuridine (BrdU), which is incorporated into the DNA if DNA synthesis and cell proliferation are induced.
Animals were necropsied 1 day after the end of exposure. Two hours prior to necropsy, BrdU (100 mg/kg body weight) was administered intraperitoneally. Cell proliferation in the duodenal epithelium (S-phase response) was determined in all animals by measuring BrdU incorporation immunohistologically (identified by red pigment over nuclei). The overall mean number of S-phase nuclei per crypt/villi in the treated groups was expressed as a percentage of the control value. Further histolopathological examination of the duodenum has been performed (Amendment No. 1; 2002/1012807). Apoptosis in duodenal epithelium was determined in high dose and control animals treated for 4 weeks using the Tunel immunohistological technique.

Findings

The stability of the test substance over the study period was proven. The stability of the test substance in the diet was confirmed by analysis. The correctness of the concentration and its homogeneity were analytically confirmed.

One control mouse died incidentally.
At 1000 ppm a macroscopically visible slight thickening of duodenal wall occurred in all mice after 2 and 4 weeks of treatment (one mouse also had erosion/ulcer in the glandular stomach at 2 weeks). On microscopic examination, thickening of the mucosal wall of the duodenum (minimal-slight) was seen in all animals after 2 days and 2 weeks and in 9 animals after 4 weeks.
The proliferation of epithelial cells in duodenal crypts (S-phase response) was statistically significantly increased after 2 days (219% of control), 2 weeks (208% of control) and 4 weeks (149% of control) (see Table 5.8.2-3).
At 25 ppm no substance-related findings were seen at any time point. Thus, dimoxystrobin clearly enhances cell proliferation in the duodenum at a concentration of 1000 ppm.

After four weeks of treatment, the number of apoptotic epithelial cells in the duodenum seemed comparable between control males and males treated with 1000 ppm of the test-substance.

Table 5.8.2-3: Cell proliferation in mouse duodenal cell after administration of dimoxystrobin for 2 days, 2 weeks and 4 weeks.

Dose level [ppm]	**Cell proliferation (S-Phase response)**					
	2 days		2 weeks		4 weeks	
0	10.18		8.65		8.07	
25	8.95	88%	8.05	93%	7.22	895
1000	22.27*	219%	18.01*	208%	12.06*	149%

*$p \leq 0.05$

Conclusion

Dimoxystrobin induced cell proliferation (with minimal or slight thickening of the duodenal wall) at a high dose level of 1000 ppm. No proliferating activity or thickening of the mucosa was detected in the duodenum at 25 ppm. No influence on the apoptotic process was observed at 1000 ppm.

Dimoxystrobin, S-phase response study (mouse 2-day or 2-week dietary administration with recovery period) (2001/1014893; 2002/1012809) – peer-reviewed

Guidelines: No test guideline exists for this type of study.
Deviations: NA
GLP: Yes
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and methods
Dimoxystrobin (Batch/purity: N 15/98.4%) was administered to groups of 8 male B6C3F1Rj mice at dietary concentrations of 0 ppm, 200 ppm (equivalent to about 50 mg/kg bw/day) and 1000 ppm (equivalent to about 250 mg/kg bw/day). One untreated control group and four test substance groups per concentration were used. Treatment was for 2 days (with or without one week recovery) or for 2 weeks (with or without 2 weeks recovery). The animals were examined for signs of toxicity or mortality at least once a day. Two hours prior to necropsy, BrdU (100 mg/kg bw) was administered intraperitoneally. Cell proliferation in duodenal epithelium was determined as described in the mechanistic study before (Dimoxystrobin, S-phase response study; mouse 4-week dietary administration; 2001/1014892). The histopathological examination of the duodenum can be found in the Amendment No 1 of the report (2002/1012809).

Findings
The stability of the test substance over the study period was proven. The stability of the test substance in the diet was confirmed by analysis. The correctness of the concentration and its homogeneity were analytically confirmed.

No mice died during the study. No gross lesions were noted at necropsy.
At 1000 ppm cell proliferation in the duodenal epithelium was statistically significantly increased after exposure for 2 days (79% above control) and 2 weeks (38% above control). The effect was fully reversible after 1 or 2 weeks of recovery (see Table 5.8.2-4).
On histopathological examination, minimal to slight thickening of the mucosa of the duodenum was seen in 7 animals after treatment for 2 days and in 5 animals after treatment for 2 weeks. No histopathological changes were noted in the recovery groups.
At 200 ppm no increased cell proliferation or histopathological changes in the duodenum were observed.

Table 5.8.2-4: Cell proliferation in mouse duodenum after administration of dimoxystrobin for 2 day, or 2 weeks including recovery.

Dose level [ppm]	**Cell proliferation (S-Phase response)**							
	2 days		2 days + 1 week recovery		2 weeks		2 weeks + 2 weeks recovery	
0	9.80		9.85		9.87		9.81	
200	10.09	103%	9.06	92%	8.59**	87%	8.83*	90%
1000	17.63**	179%	8.38**	85%	13.61**	138%	9.91	101%

*p ≤ 0.05; ** p ≤ 0.01

Conclusion
Cell proliferation and increased thickening of the mucosa was induced in the duodenum after treatment with 1000 ppm (about 250 mg/kg bw/day) for 2 days or 2 weeks. The increase in cell proliferation and thickening of the mucosa was fully reversible after one or two weeks of recovery. No increase in cell proliferation or thickening of the mucosa was seen in the duodenum after 2-day or 2-week feeding of 200 ppm (about 50 mg/kg bw/day). The NOAEL for this finding was 200 ppm (50 mg/kg bw/day).

Note: Additional histopathological investigations (Amendment BASF DocID 2012/1221002 to S-phase response study described before BASF DocID 2001/1014892) on the iron content in the duodenal epithelial cells of B6C3F1 mice after 2 days, 2 and 4 weeks of treatment with dimoxystrobin were performed with samples of the S-phase response study described before (not peer-reviewed).

Report:	CA 5.8.2/1 [redacted] 2012a BAS 505 F (Dimoxystrobin) - S-Phase-response study (duodenum) in male B6C3F1-mice - Administration via the diet up to 4 weeks - Experimental report 2012/1221002
Guidelines:	none
GLP:	no

Executive Summary

An immunohistochemical staining of relevant receptors involved in iron absorption and transport in the body was performed. In addition the status of iron storage was determined. For the present investigations tissue was taken from the original report (see BASF DocID 2001/1014892) and further examined.

The iron content in the enterocytes was specifically stained and evaluated. Iron staining was performed with Perl´s and Turnbull stain. Turnbull and Perl´s staining for iron within the duodenal epithelial cells revealed distinct differences between treated and untreated animals. There is evidence, that treatment with the test substance led to lower levels of iron (stained as Fe^{2+} and Fe^{3+}) in the duodenum cells of B6C3F1 mice after 2 and 4 weeks of treatment.

For elucidating the mechanism of lower iron uptakes it was intended to stain three different iron receptors/transporters (DMT1, Dcytb and ferroportin) immunohistochemically, but no staining could be established successfully.

(BASF DocID 2012/1221002)

Results and discussion

Turnbull and Perl´s staining for iron within the duodenal epithelial cells revealed distinct differences between treated and untreated animals. After 2 days of treatment there were still single iron-positive cells (Turnbull) within the lamina propria of the duodenal villi. After 2 and 4 weeks of treatment there were almost no iron-positive cells observed in contrast to control animals. In the control animals at all three time points at least in one duodenal section and in addition occasionally in the lamina propria iron-positive cells were observed. There is evidence, that treatment with the test substance led to lower levels of iron (stained as Fe^{2+} and Fe^{3+}) in the duodenum of B6C3F1 mice after 2 and 4 weeks of treatment.

For elucidating the mechanism of lower iron uptakes it was intended to stain three different iron receptors/transporters (DMT1, Dcytb and ferroportin) immunohistochemically, but no staining could be established successfully.

Conclusion
After treatment of male B6C3F1 mice with dimoxystrobin (1000 ppm) for 2 days, 2 weeks or 4 weeks, respectively, there is evidence, that treatment with the test substance led to lower levels of iron (stained as Fe^{2+} and Fe^{3+}) in the duodenum of B6C3F1 mice after 2 and 4 weeks of treatment. No evaluation of mouse duodenum receptors related to iron uptake and transport was possible.

To further elucidate the hypothesized local mode of action of interference of dimoxystrobin with iron uptake in the duodenum a new mechanistic study in mice was conducted (not peer-reviewed).

Report: CA 5.8.2/2
McKie A.T., 2012a
Summary report - Effect of Dimoxystrobin on iron metabolism
2011/1001622
Guidelines: none
GLP: no

Report: CA 5.8.2/3
Grauert E., 2011a
Summary of results - Stability analysis of BAS 505 F (Dimoxystrobin) in Kiba lab diet mose/rat GLP
2011/1292075
Guidelines: none
GLP: no

In the context of toxicological studies the concentration and homogeneity of the test item dimoxystrobin (Batch No. 13; purity 98.5%) in the vehicle Kliba lab diet mouse/rat “GLP” was determined.

Report: CA 5.8.2/4
Grauert E., 2012c
Amendment No. 1 - Summary of results - Stability analysis of BAS 505 F (Dimoxystrobin) in Kiba lab diet mouse/rat GLP
2012/1267647
Guidelines: none
GLP: no

Executive Summary

The aim of this study was to determine the effect of dimoxystrobin on iron metabolism after oral administration of dimoxystrobin for 7 days. Dimoxystrobin was administered to groups of 4 male B6C3F1 mice at dietary concentrations of 4000 and 8000 ppm. Clinical pathology, necropsy and histopathology (duodenum) findings were investigated. Furthermore gene expression analysis was performed using duodenum and liver mRNA. In addition, the influence of dimoxystrobin on hepcidin transcription using promotor assays in HepG2 cells was investigated and the affinity constant of dimoxystrobin for the binding of iron (Fe^{3+}) was determined. Hepcidin is an iron regulatory hormone, controlling iron uptake in the gastrointestinal tract and placenta and iron release from the reticulo-endothelial system as well as iron mobilization from hepatic stores.
The administration of dimoxystrobin to male B6C3F1 mice for one week revealed a thickening of the duodenal mucosa. There was no clear difference between the two test groups (4000 and 8000 ppm). The thickening of the mucosa was well in accordance with the observed increase in duodenal weights. This finding is regarded to be related to the treatment with the test substance. Haemoglobin and serum iron were slightly, but not statistically significantly decreased. There was no evidence that dimoxystrobin increased the levels of hepcidin or would function as an effective iron chelator. Marked changes in iron metabolism genes (namely Dcytb and Ferroportin) in the duodenum were observed.

(BASF DocID 2011/1001622)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Dimoxystrobin (BAS 505 F, 06/0629-3)
Description:	solid / beige
Lot/Batch #:	OP-No. 13
Purity:	98.5%
Stability of test compound:	The test substance was stable and homogenously distributed in the diet at room temperature for a period of 10 and 19 days, as determined before the start of the study
2. Vehicle and/or positive control:	None
3. Test animals:	
Species:	Mouse
Strain:	B6C3F1
Sex:	Male
Age:	42 - 44 days (start of administration)
Weight at dosing:	♂: 48.17 – 48.69 g
Source:	Charles River Laboratories, Sulzfeld, Germany
Acclimation period:	7 days
Diet:	NA
Water:	water, ad libitum
Housing:	in groups (4 animals per cage) in standard open cages (tape 1284), TECNIPLAST, Italy, floor area about 2065 cm²
Environmental conditions:	
Temperature:	19 - 23°C
Humidity:	45 - 65%
Air changes:	18 / hour
Photo period:	12 h light / 12 h dark (07:00 - 19:00 / 19:00 - 07:00)
4. Test cell line:	
Name:	HepG2
Properties:	Transfected with a plasmid containing 2.7 kb of the human hepcidin promoter sub-cloned into the pGL3-basic luciferase reporter vector.
Culture medium:	DMEM supplemented with 10% heat inactivated fetal bovine serum, penicillin-streptomycin, and glutamine.
Culture conditions:	37°C, 95% air / 5% CO_2

B. STUDY DESIGN (IN VIVO STUDY)

1. Dates of experimental work: 25-Jan-2011 - 20-June-2011
(In life dates: 01-Feb-2011 (start of administration) to 08-Feb-2011 (necropsy)

2. Animal assignment and treatment:
Dimoxystrobin was administered to groups of 4 male mice at dietary concentrations of 0, 4000 and 8000 ppm for up to 7 days. These dose groups were chosen based on the 90-day mouse study (see Chapter MCA 5.3), where duodenum effects were seen at doses ≥ 4000 ppm.

3. Test substance preparation and analysis:
The test substance was applied via the diet. The substance preparations were prepared once before the beginning of the study and stored at room temperature. 20 g of the food were placed in each cage in glass food hoppers. After each 24 hour period the food remaining was removed and weighed and fresh food given.
The stability of the test substance in the diet at room temperature for a period of 10 and 19 days was determined before the start of the study. No homogeneity and concentration control analyses in the carrier were carried out in this study.

4. Statistics:
Means and standard deviations (S.D.) of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Statistics of weight parameters and microarray analysis

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians.
Microarray analysis	The genes regulated by the test substance will be identified by applying a Students' t-test to the normalized data, comparing to the untreated control samples in each case and genes will be ranked by t-test p-value of ≤0.01. Unsupervised Principal component of Analysis (PCA) will be performed by Qlucore Omics Explorer. Venn diagram using differentially expressed genes will be employed to identify unique and common sequences between treatment groups. Pathway analysis will be performed using GeneGO software.

C. METHODS

In vivo study

1. Observations:

The animals were examined for evident signs of toxicity or mortality once daily on working days and on weekends and public holidays. Observations for general clinical signs of toxicity were performed once daily.

2. Body weight:

Body weight was determined before the start of the administration period and during the administration period the body weight will be determined on day 0-7. The difference between the body weight on the respective day of weighing and the body weight on day 0 were calculated as body weight change.

3. Food intake:

Individual food consumption was determined on each study days 1-7 (as representative value over 1 day) and calculated as mean food consumption in grams per day.

4. Food efficiency:

Food efficiency was calculated for each animal at weekly intervals on the basis of the body weight change and the total weekly food consumption.

5. Hematology and clinical chemistry:

Blood samples were taken from by cardiac puncture on study day 7. For all animals haemoglobin and serum iron was determined.

6. Sacrifice and pathology:

The animals were sacrificed at the end of the 7 day period under Isoflurane anaesthesia. Liver samples were removed and snap frozen before determination of non-haem iron and extraction of RNA for determination of hepcidin mRNA levels by RT PCR. Duodenum weights were determined and the duodenum was further fixed in paraformaldehyde (2.5%) for histological processing. After 24 hours the samples were transferred to 70% ethanol for further processing. Examination by light microscopy was performed after staining with Hematoxylin and Eosin.

7. Gene expression analyses:

Duodenal mucosa was scraped off the underlying serosa using a glass slide and homogenized using a glass homogenizer. Total RNA from duodenal epithelial cells of 4 individual mice per group were extracted using TRIzol® following manufacturer´s instructions. Gene expression analysis was conducted using Affymetrix Mouse Gene 1.0 ST array containing 750000 probe set representing all approximately 28853 genes in the mouse genome. Total RNA (500 ng) was amplified and 2.5 µg of fragmented and biotin-labelled cDNA was used to prepare the hybridization cocktail, which was injected into the array cartridge.

In vitro studies

8. Reporter gene assay experiment in HepG2 cells:

Cells (2.5 x 10^5 cells/mL) were seeded onto 24 well plates for transfections. Recombinant human BMP4 was obtained from R&D Systems. The pGL3-hepcidin reporter construct was co-transfected into cells with a TK-renilla control reporter at a 3:1 ratio using Fugene-6 according to manufacturer's instructions. Dimoxystrobin (dissolved in DMSO) was added to cells at 25, 50 and 100 µM. Control cells received an equivalent volume of the DMSO. As a positive control the cells were treated with human BMP-4 (25ng/mL, 16-18 h). All experiments were performed in triplicate. Luminescence was detected using Dual-Luciferase Reporter Assay system and measured by a luminometer. Relative luciferase activity was calculated and expressed as the ratio of the signal of firefly luciferase to TK-renilla.

9. Determination of iron binding by dimoxystrobin:

The automatic titration system used in this study comprised of an autoburette and pH meter with pH electrode and reference electrode. 0.1M KCl electrolyte solution was used to maintain the ionic strength. The temperature of the test solutions was maintained in a thermostatic jacketed titration vessel at 25.0 ± 0.1°C using a temperature controller. The solution under investigation was stirred vigorously during the experiment. A pump with speed capability (20 mL/min) was used to circulate the test solution through a Hellem quartz 10 mm flow cuvette which was mounted on a UV-visible spectrophotometer. All the instruments were interfaced to a computer and controlled by a Visual Basic program. Automatic titration and spectral scans adopted the following strategy: The pH of a solution was increased by 0.1 pH unit by the addition of KOH (0.1M) from the autoburette, when pH readings varied by <0.001 pH unit over a 3 second period, an equilibration period of 1 minute was adopted. The spectrum of the solution was then recorded and analysed.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

No homogeneity and concentration control analyses in the carrier were carried out in the study.

B. OBSERVATIONS

1. Clinical signs of toxicity

No clinical signs of toxicity were reported.

2. Mortality

No animal died during the study.

C. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight of the mice decreased significantly in both 4000 and 8000 ppm groups within the first day of the feeding period. The body weight data are summarized in the Table below:

Table 5.8.2-5: Body weight data collected from day 0 to day 7 of dietary dimoxystrobin administration to 4 male mice

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Dose groups	Body weight [g] (SD)							
0 ppm	26.00 (0.57)	26.00 (0.66)	26.10 (0.74)	25.93 (0.88)	26.10 (1.15)	25.88 (0.95)	26.15 (1.16)	26.10 (1.15)
4000 ppm	25.48 (0.57)	24.35 (0.44)	23.78 (1.18)	23.38 (0.85)	23.85 (1.16)	24.35 (1.18)	24.25 (0.99)	24.25 (0.77)
8000 ppm	26.95 (0.87)	25.08 (1.11)	23.73 (0.85)	23.50 (0.93)	23.03 (0.88)	22.55 (0.72)	22.93 (0.94)	22.93 (0.90)

D. FOOD CONSUMPTION

Daily food intakes were comparable over the 7 day period with the exception of day one where mice of the 8000 ppm diet consumed only 1.7 g compared to 15 g for the 4000 ppm group and 14 g for the controls. By day 2 however food consumption was comparable in all groups and remained so for the rest of the 7 day period. The food intake data are given below:

Table 5.8.2-6: Food intake data collected from day 0 to day 7 of dietary dimoxystrobin administration to 4 male mice

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Dose groups	Food intake [g]						
0 ppm	13.86	17.09	16.15	16.67	15.58	17.71	20
4000 ppm	15.1	15.34	19.14	19.42	18.59	18.37	18.89
8000 ppm	1.71	18.47	20	19.3	18.11	18.18	18.67

E. BLOOD ANALYSIS

1. Clinical chemistry findings

A downward trend in haemoglobin and serum iron levels in the substance-treated groups was observed, although not statistically significant.

Table 5.8.2-7: Iron and Haemoglobin concentration in the serum of male mice after administration of dimoxystrobin for 7 days

Sex		0 ppm	4000 ppm	8000 ppm
		Serum iron		
Males	Conc. [µM]	62 ± 14	54 ± 10	52 ± 8
	Conc. [µg/dL]	346 ± 79	301 ± 27	289 ± 24
		Hemoglobin		
	Conc. [g/dL]	16.21 ± 0.86	15.83 ± 1.62	15.21 ± 0.36

F. NECROPSY

1. Organ weight

When compared to the control group (set to 100%) the mean absolute duodenum weights were significantly changed (see Table 5.8.2-8).

Table 5.8.2-8: Absolute duodenum weights after dimoxystrobin administration

Sex		0 ppm	4000 ppm	8000 ppm
Males	Absolute weight [mg ± SD]	0.085 ± 0.017	0.230 ± 0.038*	0.222 ± 0.021*
	Deviation from controls [%]	100	270*	260*

* = p≤0.05

There was no difference between the 4000 and the 8000 ppm dose group.

2. Gross lesions

No macroscopic lesions were recorded.

3. Histopathology

The duodenum showed treatment-related lesions. The table below gives an overview of the relevant histopathological findings.

Table 5.8.2-9: Selected histopathological findings of mice administered dimoxystrobin for 7 days

ppm	0	4000	8000
No. of animals	4	4	4
Thickening of mucosa	0	4	4
Grade 2	-	3	2
Grade 3	-	1	2

All treated animals revealed a thickening of the duodenal mucosa. All parts of the mucosa (villi and crypts) showed an increase of thickness. All cells revealed an increase of cytoplasmic basophilia. The villi were undulating and no longer straight as in control animals. At the tip of the villi the cells revealed an increased number of small, clear vacuoles.

4. Liver – non-haem iron

Liver non-haem iron levels were significantly increased in the 8000 ppm group ($p<0.05$) compared to the 4000 ppm group, but not when compared to controls.

Table 5.8.2-10: Iron (non-haem) concentration in the liver (nmol/mg wet weight) of male mice after administration of dimoxystrobin for 7 days

Sex		0 ppm	4000 ppm	8000 ppm
Males	Concentration [nmol/mg wet weight]	1.18 ± 0.20	1.02 ± 0.15	1.44 ± 0.20

G. EFFECT OF DIMOXYSTROBIN ON HEPCIDIN PROMOTOR ACTIVITY

Treatment with BMP-4 increased luciferase activity as expected. Treatment with dimoxystrobin for 16-18 hours significantly reduced hepcidin promoter activity at all concentrations tested (25-100 µM). This result accords with the decrease in hepcidin levels seen *in vivo* with dimoxystrobin, which is however only seen at the lower dose of 4000 ppm.

Table 5.8.2-11: Hepcidin promotor activity after dimoxystrobin treatment

Test group	**Luciferase activity (fold control) [mean ± SD]**
Control	1 ± 0.06
Bmp4	2.66 ± 0.11*
DMSO	
0.25 µL	1.01 ± 0.03
0.5 µL	1.03 ± 0.05
1 µL	0.99 ± 0.06
Dimoxystrobin	
25 µM	0.50 ± 0.10*
50 µM	0.42 ± 0.12*
100 µM	0.41 ± 0.17*

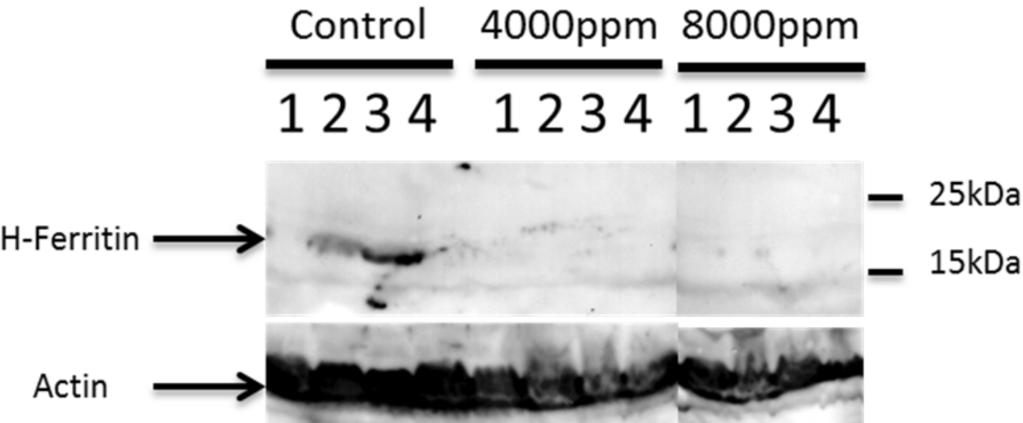
* $p<0.01$ (compared to DMSO; Student´s t-test)

H. EFFECT OF DIMOXYSTROBIN ON GENE EXPRESSION

The numbers of differentially expressed genes (in the duodenum compared to control were 414 (43%↑/57%↓) and 394 (30%↑/70%↓) for the 4000 and 8000 ppm dose group, respectively. Differentially expressed genes were selected based on the criterion that statistical significance higher than 0.017 or 0.02 for the 4000 and 8000 ppm dose group. 181 genes were commonly expressed different to the control for the 4000 and 8000 ppm dose group. With respect to the iron genes there was a marked significant decrease in iron transporter ferroportin mRNA (1.5 and 1.9-fold for the 4000 and 8000 ppm dose group). The apical ferric reductase Dcytb was also downregulated 2.7 and 3.2-fold for the 4000 and 8000 ppm dose group. No change was observed for the basolateral ferroxidase hephaestin mRNA levels. The apical ferrous iron transporter DMT1 mRNA levels were unchanged. The iron storage protein ferritin was slightly down regulated by both treatments. In contrast Transferrin receptor mRNA levels were increased in duodenum of treated mice. The transcription factor Hif2 was also decreased 1.6 and 1.3-fold.

To investigate mucosal ferritin at the protein level a Western blot was performed with duodenal lysates from control mice and mice treated with 4000 and 8000 ppm. At both dose levels H-Ferritin protein levels were decreased.

Figure 5.8.2-1: Effects of dimoxystrobin on mucosal H ferritin levels in B6C3F1 mice (4 mice per group)



Liver hepcidin mRNA levels were decreased around 2-fold by treatment with 4000 ppm, although not statistically significant. No effect was observed in the 8000 ppm dose group.

Table 5.8.2-12: Regulation of iron genes after treatment with dimoxystrobin

	Gene expression (fold control) [mean]	Gene expression (fold control) [mean]
Gene name	**4000 ppm**	**8000 ppm**
Hif-2	0.61**	0.77**
Dcytb	0.37**	0.31**
Ferroport	0.65**	0.53**
Hephaestin	0.92	0.79**
HCP1	0.56**	0.54**
Zip14	0.66**	0.60**
H-Ferritin	0.91*	0.90
L-Ferritin	0.76*	0.96
TfR1	1.98**	1.55*
DMT1	1.12	0.80

* p<0.05; ** p<0.01

I. DETERMINATION OF IRON BINDING BY DIMOXYSTROBIN

pKa determination

The pKa spectrophotometric titration of the dimoxystrobin revealed only a dilution effect during the entire titration (pH range 1.8 to 11.8). To confirm this observation, a potentiometric titration was also carried out. Although the titration solution was cloudy due to the low solubility of the sample, no buffer capacity was observed. Finally, a prediction by Marvin software predicts the pKa of the $NOCH_3$ function to be 1.12 but this was not supported by a second spectrophotometric titration carried out between pH 2 and pH 0.8. These experiments confirm that dimoxystrobin has no titratable group within the pH range 0.8-12 at the concentrations of dimoxystrobin tested. The solubility of dimoxystrobin was estimated to be less than 100 µM in water by monitoring light scattering when the sample was added in a stepwise fashion.

Iron(III) affinity determination

When dimoxystrobin was added to Fe^{3+} (35 µM) in a 50% DMSO/water solution in a stepwise fashion, there was no evidence of complex formation. dimoxystrobin was added to a final concentration of 2.2 mM and there was no sign of complex formation. It was conclude that there is no interaction between dimoxystrobin and iron(III) at concentrations less than 2 mM.

Ligand stability
The ligand undergoes rapid hydrolysis at pH 7.4 as indicated by spectral changes of the solution. The likely mechanism of hydrolysis is outlined below (see Table 5.8.2-13) and the predicted product is unlikely to coordinate iron(III).

Table 5.8.2-13: Hydrolysis scheme of Dimoxystrobin

III. CONCLUSIONS

The administration of dimoxystrobin to male B6C3F1 mice for one week revealed a thickening of the duodenal mucosa. There was no clear difference between the two test groups (4000 and 8000 ppm). The thickening of the mucosa was well in accordance with the observed increase in duodenal weights. This finding is regarded to be related to the treatment with the test substance.

A downward trend in hemoglobin levels and serum iron concentrations was observed in treated animals of both dose groups. Liver non-hem iron levels were significantly increased in the 8000 ppm group compared to the 4000 ppm group, but not compared to the controls. Liver hepcidin mRNA levels were decreased around 2-fold by treatment with 4000 ppm. There was no effect on liver hepcidin in the 8000 ppm dose group. Treatment of HepG2 cells transfected with a hepcidin promoter luciferase reporter plasmid with dimoxystrobin in vitro reduced hepcidin promotor activity.

The gene array experiments showed that oral dimoxystrobin treatment led to marked changes in iron metabolism genes in the duodenum. In general there was down-regulation of genes involved in iron absorption. In particular, both Dcytb and ferroportin mRNA were decreased in duodenum which would be expected to reduce the iron absorption. Since ferroportin controls the amount of iron which enters the body the effects of dimoxystrobin on iron metabolism could be explained by this observation alone. A decrease was also observed in the transcription factor Hif2α which has been shown to directly regulate the transcription of both Dcytb and ferroportin genes.
The data suggest that dimoxystrobin induces iron deficiency in duodenum possibly showing maximum effect at 4000 ppm. This is supported by the data showing increased levels of the transferrin receptor (TfR1) and decreased levels of ferritin.

No relevant iron binding properties could be observed.

Studies in rats:

Effects on clinical chemistry in rats

This study was designed to investigate short-term effects of the test compound on biochemical parameters in the serum of Wistar rats in order to correlate observed duodenal findings with hematological changes. For this purpose several enzymes and chemical substrates were examined after administration of 4,500 ppm (the highest dose level used in the subchronic rat study, at which slight to moderate thickening of the duodenal mucosa was seen).

Dimoxystrobin, 5-week rat feeding study (effects on clinical chemistry parameters) (2002/1005354) – peer-reviewed
Guidelines: No test guideline exists for this type of study
Deviations: NA
GLP: No
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and methods
Dimoxystrobin (batch/purity: N 15: 98.4%) was administered to groups of 10 male Wistar rats (CrlGlxBrlHan:Wi), aged 11 weeks at the start of administration, at dietary concentrations of 0 and 4500 ppm (232 mg/kg bw/day) for 3 weeks. The study then continued for a further 2 weeks with all rats dosed as before (mean intake of test substance over this period was 264 mg/kg bw/day) except that 5 rats from the test group were fed control food to determine the reversibility of effects. Test diets were prepared once and used over a period within the known stability of the test material in diet. Homogeneity of the dietary test substance preparation and correctness of the concentrations were investigated analytically. The animals were examined for evident signs of toxicity or mortality at least once a day. Body weight was determined before the start of the administration period, and then weekly. Food consumption was determined weekly. Blood sampling was performed on study days –5, 1, 2, 5, 15, 30 and 36. Blood was taken from all rats (non-fasted) except on day 30 when blood was taken from 5 fasted control rats. Haematology was investigated on day 30 only. Clinical-chemical parameters were determined in serum from day –5 to day 30, except for iron (also on day 36), transferrin (only on day 30), and unsaturated iron binding capacity (only on day 36).

Findings

General observations
There were no deaths or clinical signs of toxicity.

Body weight and body weight gain

Body weight was depressed (by 19% at Day 33, which equated to slight body weight loss) in the group feed 4,500 ppm continuously but returned to normal by Day 33 in the recovery group (see Table 5.8.2-14 and Figure 5.8.2-2). The body weight gain development is consequently decreased in animals treated with the test substance until Day 36, but started to increase in the recovery group after weaning of the test item, and thus no statistical difference compared with the control group was determined on Day 33 in animals treated only till Day 19.

Figure 5.8.2-2: Body weight development of rats administered dimoxystrobin for 36 days or 19 days with a recovery period of 14 days

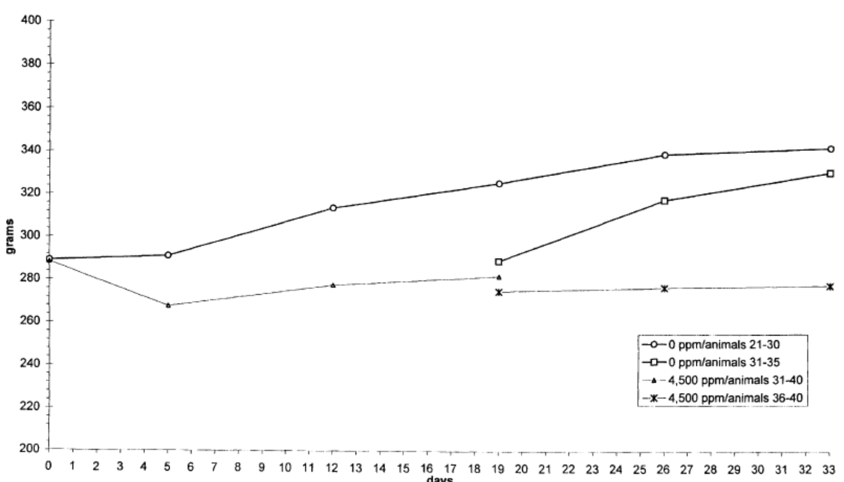


Table 5.8.2-14: Body weight development in rat administered dimoxystrobin for 36 days or 19 days with a recovery period of 14 days

Dose level [ppm]	0 (n = 10)	4,500 (n = 10)	
Body weight [g]			
Day 0	289.0 ± 8.5	288.3 ± 9.9	
Day 5 Δ% (compared to control)	291.2 ± 8.5	**267.7**** ± 13.2 (-8.1)	
Day 12 Δ% (compared to control)	313.8 ± 13.2	**277.8**** ± 24.2 (-11.5)	
Day 19 Δ% (compared to control)	325.8 ± 15.6	**282.2**** ± 24.8 (-13.4)	
		Treatment group (n = 5)	Recovery group (n = 5)
Day 19 Δ% (compared to control)	325.8 ± 15.6	**275.1**** ± 19.9 (-15.6)	**289.4*** ± 29.4 (-11.2)
Day 26 Δ% (compared to control)	339.4 ± 17.4	**277.0**** ± 24.3 (-18.4)	318.0 ± 21.7 (-6.3)
Day 33 Δ% (compared to control)	342.3 ± 17.2	**277.9**** ± 20.6 (-18.8)	330.9 ± 22.4 (-3.3)
Overall body weight gain [g]			
Day 5 Δ% (compared to control)	2.2 ± 4.1	**-20.5**** ± 6.6	
Day 12 Δ% (compared to control)	24.8 ± 9.1	**-10.5**** ± 17.2	
Day 19 Δ% (compared to control)	36.8 ± 12.2	**-6.0**** ± 18.2	
		Further treatment (n = 5)	Recovery period (n = 5)
Day 19 Δ% (compared to control)	36.8 ± 12.2	**-9.9**** ± 18.8	**-2.1**** ± 18.8
Day 26 Δ% (compared to control)	50.5 ± 13.9	**-8.0**** ± 23.6	**26.5**** ± 10.8 (-47.5)
Day 33 Δ% (compared to control)	53.4 ± 13.6	**-7.2**** ± 19.6	39.5 ± 10.6 (-26.0)

* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001 (Welch t-test, two sided)

Food and compound intake

Food consumption and efficiency were depressed (by 23 and 58% at Day 33, respectively) in the group feed 4,500 ppm continuously. In the recovery group, food consumption returned to normal by Day 33. Furthermore, food efficiency increased markedly after weaning of the test substance and was approximately 350% compared with the control group by Day 33 (see Table 5.8.2-15).
The mean substance intake on Day 19 was 266.3 ± 21.9 mg/kg bw (calculated for all animals treated with 4,500 ppm) and 266.1 ± 9.4 mg/kg bw (only animals continuously treated with 4,500 ppm in a period of Day 1 to 33) on Day 33, respectively.

Table 5.8.2-15: Average daily food consumption of rats administered dimoxystrobin for 36 days or 19 days with a recovery period of 14 days

Dose level [ppm]	0 (n = 10)	4,500 (n = 10)	
Food consumption [g/anima/day]			
Day 5 Δ% (compared to control)	20.7 ± 2.0	**10.6**** ± 1.7 (-48.9)	
Day 12 Δ% (compared to control)	21.7 ± 2.0	**15.7**** ± 3.0 (-27.6)	
Day 19 Δ% (compared to control)	21.6 ±1.7	**16.7**** ± 2.3 (-22.3)	
		Treatment group (n = 5)	Recovery group (n = 5)
Day 19 Δ% (compared to control)	21.6 ± 1.7	**16.8*** ± 2.8 (-22.0)	**16.7**** ± 2.0 (-22.7)
Day 26 Δ% (compared to control)	21.5 ± 1.7	**15.7**** ± 2.5 (-27.1)	**23.3*** ± 1.0 (8.5)
Day 33 Δ% (compared to control)	21.4 ± 1.9	**16.4**** ± 1.0 (-23.3)	21.7 ± 1.2 (1.6)
Food efficiency [g]			
Day 5 Δ% (compared to control)	1.8 ± 4.5	**-41.2**** ± 18.3	
Day 12 Δ% (compared to control)	14.7 ± 3.0	7.2 ± 12.0 (-51.0)	
Day 19 Δ% (compared to control)	7.9 ± 2.5	**3.5*** ± 4.6 (-55.7)	
		Further treatment (n = 5)	Recovery period (n = 5)
Day 19 Δ% (compared to control)	7.9 ± 2.5	3.6 ± 6.1 (-54.4)	**3.4*** ± 3.3 (-57.0)
Day 26 Δ% (compared to control)	9.0 ± 2.5	1.3 ± 6.9 (-85.6)	**17.5*** ± 6.0 (94.4)
Day 33 Δ% (compared to control)	1.9 ± 1.7	0.8 ± 5.5 (-57.9)	**8.5**** ± 3.0 (347.4)

$* p \leq 0.05$; $** p \leq 0.01$; $*** p \leq 0.001$ (Welch t-test, two sided)

Clinical chemistry

As a consequence of the reduced food consumption, parameters like alanine aminotransferase, alkaline phosphatase, glutamate dehydrogenase, glucose, and triglycerides were reduced for most of the exposure period in the study (starting at Days 1-2) (see Table 5.8.2-16).

Table 5.8.2-16: Selected clinical chemistry findings in rats administered dimoxystrobin for 36 days or 19 days with a recovery period of 11 days

	Study day	ALT [µkat/L]	ALP [µkat/L]	GLDH [nkat/L]	UREA [mmol/L]	Gluc. [mmol/L]	TBil [µmol/L]	TG [g/L]
Control	Day 1	0.8 ± 0.1	8.8 ± 1.6	90 ± 12	8.0 ± 0.5	7.4 ± 0.4	2.6 ± 0.6	1.5 ± 0.5
	Day 2	0.8 ± 0.1	8.1 ± 1.4	111 ± 15	7.4 ± 0.7	7.9 ± 0.6	1.4 ± 0.5	1.4 ± 0.4
	Day 5	0.8 ± 0.1	8.5 ± 1.1	108 ± 19	8.3 ± 0.8	7.4 ± 0.5	1.3 ± 0.5	1.5 ± 0.4
	Day 15	0.8 ± 0.1	9.4 ± 1.3	116 ± 17	8.0 ± 0.8	7.3 ± 0.8	1.4 ± 0.5	1.8 ± 0.7
	Day 30	0.8 ± 0.2	10.1 ± 1.2	93 ± 18	8.4 ± 1.1	7.8 ± 0.9	1.5 ± 0.2	1.7 ± 0.3
4,500 ppm (30 days)$	Day 1	0.8 ± 0.2	**5.3**** ± 0.9	**83*** ± 20	**5.7**** ± 0.8	**6.4**** ± 0.4	**3.4**** ± 0.5	**0.6**** ± 0.2
	Day 2	**0.6**** ± 0.1	**4.8**** ± 0.9	**97*** ± 12	**5.5**** ± 0.5	**6.8**** ± 0.5	**1.8*** ± 0.3	1.0 ± 0.3
	Day 5	**0.6**** ± 0.2	**4.1**** ± 1.3	**97*** ± 17	8.1 ± 1.0	**6.7**** ± 0.5	**2.0*** ± 0.8	**0.9**** ± 0.3
	Day 15	**0.6**** ± 0.1	**4.3**** ± 1.1	**92*** ± 19	8.1 ± 0.6	**6.1**** ± 0.3	**2.4**** ± 0.6	**0.9**** ± 0.2
	Day 30$	0.7 ± 0.1	**5.2**** ± 1.9	81 ± 10	8.1 ± 0.4	**5.9**** ± 0.7	2.3 ± 1.0	1.1 ± 0.4
	Day30#	0.9 ± 0.3	9.6 ± 2.3	110 ± 39	7.5 ± 0.3	7.4 ± 0.8	1.4 ± 0.3	1.6 ± 0.2

* $p \leq 0.05$, ** $p \leq 0.01$, (Wilcoxon-tests, two-sided)

$ = the animals (n = 5) received the test substance continuously from Day 1 to Day 30.

\# = the animals received the test substance from Day 1 to Day 19 and ground diet thereafter.

Serum iron was also reduced for most of the exposure period in the study (from Day 1) (see Table 5.8.2-17).

Table 5.8.2-17: Serum iron in rats administered dimoxystrobin for 36 days or 19 days with a recovery period of 11 days

		Serum iron concentration (µmol/L)								
Dose [ppm]		**5 d before admin**	**Admin for 1 d**	**Admin for 2 d**	**Admin for 5 d**	**Admin for 15 d**	**Admin for 30 d**	**admin for 36 d**	**Admin for 19 d + 11 d recov**	**Admin for 19 d + 17 d recov**
Control	Mean	42.2 ± 8.6	52.8 ± 15.5	46.8 ± 6.0	57.3 ± 11.4	45.9 ± 8.7	37.2 ± 4.3	43.0 ± 6.2	37.2 ± 4.3	43.0 ± 6.2
4500	Mean	48.5 ± 9.4	**25.6**** ± 4.8	**25.4**** ± 6.7	**14.7**** ± 3.6	**16.2**** ± 3.5	**24.4*** ± 10.7	**13.4**** ± 5.0	55.9 ± 23.4	54.9 ± 26.0
	Δ%	(15)	(-52)	(-46)	(-74)	(-65)	(-34)	(-69)	(50)	(28)

Already one day after test substance administration the iron level in the serum of the treated animals was decreased by 51% compared to the corresponding control. On day 5, serum iron concentration of the treatment group was decreased by about 74% of the respective control. There was no effect on the serum level of transferrin (the carrier of iron in plasma) when measured on day 30.
As a consequence of the iron deficiency, the iron reserves depleted, which is indicated by an increase in unsaturated iron binding capacity in the serum of treated animals. Unsaturated iron binding capacity on Day 36 was significantly increased ($p \leq 0.01$) in animals that were continuously treated with the test item and was 82% higher than in the control group. Animals of the recovery group (19 days treatment + 17 days recovery) did not show a statistically significant difference to the control group in iron binding capacity.

Due to a sufficiently high content of iron in the diet, the decrease in serum iron concentration in this study was mainly related to an interaction of dimoxystrobin with iron kinetics rather than to the decreased food consumption.
Since it is known that iron deficiency causes thickening of the duodenum in rats (Smith et al., 2000; BASF DocID 2000/1024126), it is assumed that the thickening of the duodenum induced by dimoxystrobin is also correlated to the decrease in serum iron concentration.

Haematology
After exposure for 30 days, there were statistically significant decreases in haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. There were also significant increases in white blood cells, platelets and reticulocytes. In animals exposed for 19 days followed by a 11 day recovery period, there were still some statistically significant reductions in some parameters (haemoglobin, haematocrit, MCV and MCH) but the magnitude of the reductions was clearly less than in animals exposed for 30 days, i.e. there was some but not complete recovery in haematological effects (see Table 5.8.2-18).

Table 5.8.2-18: Selected hematology findings in rats administered dimoxystrobin for 36 days or 19 days with a recovery period of 11 days

	Study day	MCV [fL]	MCH [fmol]	MCHC [mmol/L]	WBC [giga/L]	HGB [mmol/L]	HCT [L/L]	PLT [giga/L]	RETI [%]
Control	Day 30	56.3 ± 0.8	1.17 ± 0.02	20.79 ± 0.21	5.29 ± 0.55	9.4 ± 0.4	0.455 ± 0.019	693 ± 72	19.4 ± 13.2
4,500 ppm (30 days)$^{\$}$	Day 30	**44.3**** ± 4.4	**0.89**** ± 0.10	**20.02**** ± 0.43	**7.92*** ± 1.80	**7.6**** ± 0.9	**0.377**** ± 0.043	**1036**** ± 187	**33.8*** ± 9.7
Δ% (compared to control)		(-21.3)	(-23.9)	(-3.7)	(49.7)	(-19.1)	(-17.1)	(49.5)	(74.2)
4,500 ppm (19 d + 11 d recovery)$^{\#}$	Day 30	**48.7**** ± 0.9	**1.01**** ± 0.04	20.67 ± 0.47	5.54 ± 0.60	**8.7*** ± 0.3	**0.422*** ± 0.015	809 ± 179	24.0 ± 5.6
Δ% (rel. to control)		(-13.5)	(-13.7)	(-0.6)	(4.7)	(-7.4)	(-7.3)	(16.7)	(23.7)

* $p \leq 0.05$; ** $p \leq 0.01$; (Wilcoxon-test, two sided)

$ = the animals (n = 5) received the test substance continuously from Day 1 to Day 30.

= the animals received the test substance from Day 1 to Day 19 and ground diet thereafter.

Conclusion

Dietary administration of dimoxystrobin at a dose level of 4500 ppm caused reduced food consumption and body weight gain, and a number of clinical chemistry changes (fully reversible). Many of the clinical chemistry changes were probably caused by reduced food consumption.
Dietary administration of dimoxystrobin at a dose level of 4500 ppm further caused hematological changes (statistically significant decreases in haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration), that were partly reversible within an 11 day recovery period. The changes in the hematological parameters are indicative of an iron deficiency microcytic hypochromic anaemia.

With what is known from the mechanistic gene array studies a local and direct interaction of dimoxystrobin with the duodenal receptors is considered plausible. Thus the distinct decreases in serum iron are considered to be caused as a consequence of a reversible interaction with the duodenal receptors. The decrease in serum iron resulted in an iron deficiency anaemia (iron deficiency is a known cause of microcytic hypochromic anaemia, see Howard and Hamilton, 1997). The adaptive thickening of the duodenal mucosa, occurring rapidly after dimoxystrobin exposure is a further consequence of the local interaction and the lower iron status, in order to increase the absorption of iron. Status. Duodenal mucosa thickening is a known response to iron deficiency (see Smith et al., 2000; BASF DocID 2000/1024126).

A further study had been conducted to determine the NOAEL for the decrease in serum iron after oral administration of dimoxystrobin in rats.

Determination of NOEL for lowering serum iron concentration in rats (short-term screening study) (2002/1014245) – peer-reviewed

Guidelines: No test guideline exists for this type of study
Remarks: Administration of test substance commenced on the day of arrival of the rats at the laboratory (only minimal acclimatisation period)
GLP: No
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and methods

Dimoxystrobin (batch/purity: N15/98.4%) was administered to groups of 6 male Wistar rats (CrlGlxBrlHan:Wi), aged 10 weeks, at dietary concentrations of 0, 10, 50, 250 and 500 ppm for 7 days. Body weights and food intake were not investigated. Therefore intake of dimoxystrobin has been calculated approximately based on intakes for rats of similar age (but female and therefore probably of lower body weight) administered 500 ppm for 7 days in the study described below (BASF DocID 2002/1013984). On this basis intakes of dimoxystrobin in the present study are calculated to be approximately: 0, 1, 4, 20 and 40 mg/kg bw/day.
Test diets were prepared once and used over a period within the known stability of the test material in diet. No analysis of the test diets for homogeneity, stability or test substance concentrations were performed. The test diet was basic maintenance diet, the same as used in other rat studies, and therefore is expected to have contained 250 mg iron/kg. Animals were observed for clinical signs of toxicity. Blood was taken from non-fasted rats on the morning of days 2 and 6. Serum iron was measured by the guanidine/ferrozine method without deproteinisation. Animals were killed on day 7. No further investigations were perfomed.

Findings

There were no deaths or clinical signs of toxicity.

After 2 and 6 days of test substance administration statistically significantly decreased iron concentrations were found in the serum of the animals receiving 250 and 500 ppm [see Table 5.8.2-19]. The values were in the range of about 30% below control. Marginal decreases (9.66 and 4.07% below control) were seen in the rats at 50 ppm. This was, however, statistically not significant, and not considered to be toxicologically relevant. No deviations from control were seen at 10 ppm.

Table 5.8.2-19: Serum iron levels in a 7-day feeding study in rats

		Serum iron concentration (µmol/L)				
Blood sampling		0 ppm	10 ppm	50 ppm	250 ppm	500 ppm
Day 2	Mean	52.65	52.58	47.57	**37.35***	**34.25****
	% dev.		-0.13	-9.66	-29.06	-34.95
Day 6	Mean	58.15	61.73	55.78	**39.62***	**37.92****
	% dev.		6.16	-4.07	-31.87	-34.80

*p ≤ 0.05; ** p ≤ 0.01(Kruskal Wallis + Wilcoxon-test)

Conclusion

The NOAEL for decreased serum iron is 50 ppm (equivalent to about 4 mg/kg bw/day) in this study based on effects at 250 ppm (about 20 mg/kg bw/day). The extent of serum iron depression at 250 and 500 ppm was similar after 2 and 6 days.

Another mechanistic study was conducted to determine the serum iron levels in young and adult rats after oral administration of dimoxystrobin (summarized in the Addendum to DAR, July 2005).

Report:	CA 5.8.2/5 [redacted] et al., 2005b BAS 505 F - Determination of serum iron concentration in young and adult male Wistar rats after oral administration in the diet over 7 days 2005/1004845
Guidelines:	none
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The aim of this study was to determine the level of iron in serum after dietary administration of dimoxystrobin. Dimoxystrobin was administered to groups of 10 young (3 weeks old) and adult (10 weeks old) male Wistar rats at dietary concentrations of 0, and 500 ppm over a period of 7 days. Due to the low body weights and the higher basal metabolic rate of young rats as compared to adult animals, the animals had a higher test substance intake (65.3 mg/kg bw/day) during the treatment period as compared to adults (33.4 mg/kg bw/day). Thus, an additional group of young rats was included in the study receiving a test substance concentration of 250 ppm in the diet (33.8 mg/kg bw/day) in order to achieve a similar test substance intake at the end of the 7 days of administration.
Food consumption was impaired in all treatment groups. Body weight was slightly reduced in young animals treated with 250 ppm of test substance.
Serum iron concentrations were decreased in all treated animals. The reduction was -42% and -37.9% in young animals and -20.8% and -18.3% in adult animals at study day 2 and 7, respectively. Since iron levels in young animals were distinctly higher than in adult animals at the start of the study, a comparison between serum iron reductions between young and adult animals may be misleading. Therefore, young animals are not more sensitive to the test substance compared to adult animals. During macroscopic examination a thickening of the duodenum was observed only in adult animals after treatment with the test substance. No duodenum findings were observed in young animals treated with 250 or 500 ppm of dimoxystrobin.

In conclusion, dimoxystrobin reduced serum iron concentration in young and adult Wistar rats with no higher susceptibility in young rats. The RMS did not agree with this conclusion by the notifier.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Dimoxystrobin (BAS 505 F)

Description:	solid / powder / white
Lot/Batch #:	N15
Purity:	98.4%
Stability of test compound:	Stable - Expiry date January 2007

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Wistar (CrlGlxBrlHan:WI)
Sex:	Male
Age:	3 - 10 weeks
Weight at dosing:	35.6 – 47.2 (young), 230.5 – 252.8 (adult)
Source:	Charles River, Sulzfeld, Germany
Acclimation period:	6 days
Diet:	basic maintenance diet for rat/mouse “GLP”, meal from Provimi KLIBA SA, Kaiseraugust, Switzerland, ad libitum
Water:	water, ad libitum
Housing:	single housing in type DK III stainless steel wire mesh cages, floor area about 800 cm²

Environmental conditions:

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

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 21-Sep-2004 - 28-Oct-2004

2. Animal assignment and treatment:

Dimoxystrobin was administered to groups of 10 male rats (3 weeks old) at dietary concentrations of 0, 250, and 500 for 7 days. In a second experiment, Dimoxystrobin was administered to groups of 10 male rats (10 weeks old) at dietary concentrations of 0 and 500 for 7 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

3. Test substance preparation and analysis:

For each preparation, the test substance was weighed out and thoroughly mixed with a smaller amount of diet. Then food was added in order to obtain the desired concentrations, and mixing was carried out for about 10 minutes in a laboratory mixer. The mixtures were prepared once before the start of the study.

No analyses of the test substance preparations were carried out for this study.

4. Statistics:

Means and standard deviations (S.D.) of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Statistics of clinical examinations, clinical pathology and pathology

Parameter	Statistical test
Food consumption, body weight	A comparison of the dose group with the control group was performed using Welch t-test (two-sided) for the hypothesis of equal means.
Blood chemistry	Pair-wise comparison of each substance with a control group was performed using Wilcoxon-test (two-sided) for the equal means.

C. METHODS

1. Observations:

The animals were examined for evident signs of toxicity or mortality twice daily on working days and once daily on weekends and public holidays. Observations for general clinical signs of toxicity were performed once daily.

2. Body weight:

The body weight of the animals was determined on the day of randomization and at the end of the study.

3. Food consumption and compound intake:

Individual food consumption was determined at the end of the study and calculated as mean food consumption in grams per animal and day.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$Substance\ intake\ for\ day\ x = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in mg/kg, and BW_x as body weight on day x of the study (in g).

4. Clinical pathology:

Non-fasted animals were anesthetized with Isoflurane anesthesia. The blood sampling procedure and the subsequent analysis of the serum samples were carried out in a randomized sequence. For all animals iron levels were determined.

5. Urinalysis:

Not determined in the study.

6. Sacrifice and pathology:

The animals were sacrificed by decapitation under carbon dioxide anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The stability of dimoxystrobin in the diet over a period of 49 days at room temperature was determined in a previous study (08B0264/966003; BASF DocID 1997/1008341). No further analyses of the test substance were carried out for this study.

B. OBSERVATIONS

1. Clinical signs of toxicity

There was nothing abnormal detected in any animal.

2. Mortality

Two control animals (group 4) died during the blood sampling.

C. BODY WEIGHT AND BODY WEIGHT GAIN

Body weight of animals treated with 250 ppm of dimoxystrobin was found slightly reduced (-6%) but statistically significant at the end of the study (see Table 5.8.2-20).

Table 5.8.2-20: Body weight (g) of male rats (3 and 10 weeks old) at day 0 and day 7 of the experiment

	Males (3 weeks)		Males (10 weeks)	
Day	0	7	0	7
Dose level [ppm]				
Experiment 1				
0	39.3 ± 2.7	73.9 ± 4.0	240.6 ± 7.1	277.2 ± 16.1
500	39.2 ± 2.7	70.5 ± 4.3	240.1 ± 6.2	276.7 ± 9.9
	-0.4% vs control	-4.6% vs control	-0.2% vs control	-0.2% vs control
Experiment 2				
0	42.1 ± 3.3	78.3 ± 4.1	-	-
250	41.2 ± 2.9	73.6 ± 4.8*	-	-
	-2.2% vs control	-6.0% vs control	-	-

*: $p \leq 0.05$

D. FOOD CONSUMPTION AND COMPOUND INTAKE

An impairment of food consumption (group 1: -8.8%; group 3: -9.4%; group 5: -8.5%) was observed in all animal groups treated with dimoxystrobin (see Table 5.8.2-21).

The approximate mean daily test substance intake was calculated to be 33.8 and 65.3 mg/kg bw/day in young males (3 weeks old) in the 250 and 500 ppm test group. The mean daily test substance intake for the older males (10 week old) in the 500 ppm test group was 33.4 mg/kg bw/day.

Table 5.8.2-21: Daily food consumption (g/animal per day) of male rats (3 and 10 weeks old) after administration of dimoxystrobin for 7 days

	Males (3 weeks)	Males (10 weeks)
day	7	7
Dose level [ppm]		
Experiment 1		
0	10.1 ± 0.7	20.4 ± 1.9
500	9.2 ± .6**	18.5 ± 1.0*
	-8.8% vs control	-9.4% vs control
Experiment 2		
0	10.9 ± 0.6	-
250	10.0 ± 0.8*	-
	-8.5% vs control	-

*: $p \leq 0.05$, **: $p \leq 0.01$

E. BLOOD ANALYSIS

1. Clinical chemistry

All animals showed significant reduction of iron concentration in serum after 2 and 7 days of dimoxystrobin treatment compared to control animals [see Table 5.8.2-22]. The serum iron concentrations determined in the young control animals (92.6 and 95.3 µmol/L at days 2 and 7) were roughly factor 2 higher than the respective control values in 10 week old rats (43.1 and 44.0 µmol/L at days 2 and 7). The determined serum iron concentrations were decreased by 42.0 and 37.9 % at 33.8 mg/kg bw in young animals, compared to reductions of only -20.8 and -18.0% in the 10 week old animals, however the measured serum iron concentrations were higher in young 56.0 and 59.7 µmol/L) compared to the adult animals (34.2 and 36.0 µmol/L) at equivalent dose levels. Thus the apparent higher susceptibility of young animals to show decreased serum iron levels after dimoxystrobin treatment is not plausible.

Table 5.8.2-22: Serum iron concentration (µmol/L) of male rats (3 and 10 weeks old) after administration of dimoxystrobin for 2 and 7 days

Animal s	Blood sampling		0 ppm	250 ppm (33.8 mg/kg bw)	500 ppm (65.3 mg/kg bw
Males (3 weeks)	Day 2	Mean % Dev.	92.6	56.0** -42.0	35.8** -61.3
	Day 7	Mean % Dev.	95.3	59.7** -37.9	33.6** -64.8
			0 ppm		500 ppm (33.4 mg/kg bw)
Males (10 weeks)	Day 2	Mean % Dev.	43.1	-	34.2** -20.8
	Day 7	Mean % Dev.	44.0	-	36.0** -18.0

** $p \leq 0.01$

F. NECROPSY

A thickening of the duodenum was observed during macroscopic examination in seven rats from group 3 (10 weeks old animals receiving 500 ppm of dimoxystrobin). This finding is considered as being related to treatment. No duodenum findings were observed in the other animal groups.

III. CONCLUSION

Dietary administration of dimoxystrobin to male rats at dose levels of 250 and 500 ppm (young, 3 weeks old corresponding to 33.8 and 65.3 mg/kg bw) or 500 ppm (adult, 10 weeks old, corresponding to 33.4 mg/kg bw) over a period of 7 days resulted in a decrease of serum iron concentrations in all treated animals. The percent decrease of serum iron levels was about -40% in young animals and about -20% in adult animals, however the iron levels in young control animals were factor 2 higher compared to the respective control values in the adults (95.3 vs 44.0 µmol/L at day 7 after administration). Thus, regarding the decrease of serum iron concentration in all treated animals, the no observed effect level was < 250 ppm.
Based on the existing age-related differences in hematologic and iron status it cannot be assumed, that young animals are more sensitive to the test substance than adult animals.
This assessment is further supported by the NOAEL of roughly 4 mg/kg bw determined for decreased serum iron levels in young (3 week old) rats in the new study (see BASF DocID 2010/1026748). 4 mg/kg bw was also the NOAEL determined for adult rats (see BASF DocID 2002/1014245).

In order to derive a NOAEL for effects of dimoxystrobin on serum iron in young rats, a further mechanistic study was performed to determine serum iron levels in young (3-week old) rats after oral administration of dimoxystrobin.

Report:	CA 5.8.2/6 [redacted] 2010a BAS 505 F (Dimoxystrobin) - Determination of serum iron concentration of young male Wistar rats after oral administration via the diet over 7 days 2010/1026748
Guidelines:	none
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The aim of this study was to determine the level of iron and transferrin in serum after oral administration of dimoxystrobin. Dimoxystrobin was administered to groups of 10 young, male Wistar rats at dietary concentrations of 6, 11 and 22 ppm over a period of 7 days. A target dose of 22 ppm (4 mg/kg body weight/day) has been chosen, as this dose was found to be the NOAEL for adult rats with respect to iron concentrations in blood serum.
Based on clinical findings the oral administration of dimoxystrobin caused no signs of general systemic toxicity. With regard to clinical pathology findings, no treatment-related findings or differences were observed after gross examination. In summary, oral administration of Dimoxystrobin via dietary incorporation at levels of 6, 11 and 22 ppm, over a period of 7 days caused no test substance-related adverse effects. The study did not reveal any toxicologically relevant difference.

(BASF DocID 2010/1026748)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Dimoxystrobin (BAS 505 F, 06/0629-2)

Description:	solid / white
Lot/Batch #:	01171-55
Purity:	99.7% (±1.0%)
Stability of test compound:	The test substance was stable at room temperature over the study period.

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	Crl:WI (Han)
Sex:	Male
Age:	21 days (start of administration)
Weight at dosing:	♂: 48.17 – 48.69 g
Source:	Charles River Laboratories, Sulzfeld, Germany
Acclimation period:	8 days
Diet:	basic maintenance diet for rat/mouse, meal from Provimi KLIBA SA, Kaiseraugust, Switzerland, ad libitum
Water:	water, ad libitum
Housing:	individually during administration period in Makrolon, type M III cages, floor area about 800 cm²

Environmental conditions:

Temperature:	20 - 24°C
Humidity:	30 - 70%
Air changes:	10 / hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 06-Oct-2009 - 04-Feb-2010
(In life dates: 06-Oct-2009 (start of administration) to 21-Oct-2009 (necropsy)

2. Animal assignment and treatment:
Dimoxystrobin was administered to groups of 10 male rats at dietary concentrations of 0, 6 (low dose), 11 (mid dose) and 22 ppm (high dose) for up to 7 days. The animals were assigned to the treatment groups by means of a computer generated randomization list based on body weights.

3. Test substance preparation and analysis:
For each preparation, the test substance was weighed out and mixed with a small amount of food. Then, corresponding amounts of food, depending on test group, were added to this premix in order to obtain the desired concentrations. Mixing was carried out for about 10 minutes in a laboratory mixer. Details of the mixers used are retained with the raw data. The mixtures were prepared once before the start of the study.
The animals received dietary concentrations of dimoxystrobin based on control data for food consumption and body weight of a previous study (48C0360/96072; BASF DocID 2005/1004845).
Mean daily food consumption males, age 21 to 28 days: 10 g.
Mean body weight males, age 21/28 days: 56 g.
The dietary concentrations of dimoxystrobin were calculated using the following formula:

$$ppm = \frac{BW_x \times D}{FC_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, D as the dose in mg/kg bw, and BW_x as mean body weight on day x of the study (in g).

The stability of the test substance in the diet over a period of up to 49 days at room temperature was proven earlier. Homogeneity was verified from the low and high concentration at the beginning of the study (was used as a concentration control at the same time). Concentration control analysis was carried out from the mid concentration.

4. Statistics:

Means and standard deviations (S.D.) of each test group were calculated for several parameters. Further statistical analyses were performed according to following table:

Statistics of clinical examinations, clinical pathology and pathology

Parameter	Statistical test
Body weight, body weight change	A comparison of the dose group with the control group was performed using DUNNETT´s test (two-sided) for the hypothesis of equal means.
Clinical phathology	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians.

C. METHODS

1. Observations:

The animals were examined for evident signs of toxicity or mortality twice daily on working days and once daily on weekends and public holidays. Observations for general clinical signs of toxicity were performed once daily.

2. Body weight:

The body weight of the animals was determined on day 0 (start of administration period), 2 and 7. The difference between the consecutive days of weighing was calculated as body weight change.

3. Food consumption and compound intake:

Individual food consumption was determined on study days 2 and 7 and calculated as mean food consumption in grams per animal and day.
The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$Substance\ intake\ for\ day\ x = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm, and BW_x as body weight on day x of the study (in g).

4. Water consumption:

Water consumption was observed daily by visual inspection of the water bottles for any changes in volume.

5. Hematology and clinical chemistry:

Blood samples were taken from the retroorbital venous plexus in the morning (day 7) from fasted animals. For all animals iron and transferrin was determined.

6. Sacrifice and pathology:

The animals were sacrificed using carbon dioxide, necropsied and assessed by gross pathology. No further examinations were carried out.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

The concentration control analysis revealed that the values were in the expected range of the target concentration (96.2 – 111.4% of the nominal concentration; Part III, Supplement)

B. OBSERVATIONS

1. Clinical signs of toxicity

There was nothing abnormal detected in dams and test animals.

2. Mortality

No animal died during the study.

C. BODY WEIGHT AND BODY WEIGHT GAIN

No test group showed significant deviations in comparison to the control animals [see Table 5.8.2-23].

Table 5.8.2-23: Body weight and body weight gain data after administration of dimoxystrobin

Sex	Blood sampling		0 ppm	6 ppm	11 ppm	22 ppm
Males	Day 0	Mean±SD [µmol/L]	48.62 ± 2.89	48.63 ± 3.03	48.69 ± 2.63	48.17 ± 2.82
	Day 2	Mean±SD [µmol/L]	53.86 ± 5.00	52.57 ± 4.46	53.50 ± 3.42	51.51 ± 3.83
		deviation vs control		0.02	0.14	-0.93
	Day 7	Mean±SD [µmol/L]	79.52 ± 5.70	77.11 ± 5.85	79.04 ± 4.39	77.68 ± 3.87
		deviation vs control		-3.03	-0.60	-2.31

D. FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption was not influenced by dosing with the test substance during the study (see Table 5.8.2-24).

Table 5.8.2-24: Food consumption after administration of dimoxystrobin

Sex	Blood sampling		0 ppm	6 ppm	11 ppm	22 ppm
Males	Day 2	Mean ± SD [µmol/L]	6.96 ± 1.32	7.14 ± 1.36	7.38 ± 1.03	6.92 ± 0.91
		deviation vs control		2.66	6.11	-0.43
	Day 7	Mean ± SD [µmol/L]	10.52 ± 0.64	10.72 ± 0.91	10.61 ± 0.77	10.46 ± 0.73
		deviation vs control		1.90	0.89	-0.53

The approximate mean daily test substance intake was calculated to be 0.95, 1.71 and 3.42 mg/kg bw/day at dietary dose levels of 6, 11 and 22 ppm, respectively.

E. BLOOD ANALYSIS

1. Clinical chemistry findings

No treatment-related changes of the iron and transferrin values were measured (see Table 5.8.2-25 and Table 5.8.2-26)

Table 5.8.2-25: Iron concentration (µmol/L) in the serum of male rats after administration of dimoxystrobin for 2 and 7 days

Sex	Blood sampling		0 ppm	6 ppm	11 ppm	22 ppm
Males	Day 2	Mean ± SD [µmol/L]	107.8 ± 14.4	102.8 ± 12.7	102.2 ± 16.2	102.5 ± 19.6
	Day 7	Mean ± SD [µmol/L]	77.3 ± 12.7	77.7 ± 10.3	73.1 ± 18.0	82.2 ± 9.7

Table 5.8.2-26: Transferrin concentration (g/L) in the serum of male rats after administration of dimoxystrobin for 2 and 7 days

Sex	Blood sampling		0 ppm	6 ppm	11 ppm	22 ppm
Males	Day 2	Mean ± SD [µmol/L]	6.52 ± 0.38	6.30 ± 0.41	6.34 ± 0.39	6.67 ± 0.50
	Day 7	Mean ± SD [µmol/L]	5.02 ± 0.39	5.21 ± 0.48	5.05 ± 0.37	4.96 ± 0.35

F. NECROPSY

No test substance-related findings were observed during macroscopically assessment.

III. CONCLUSIONS

The administration of dimoxystrobin in a dose up to 22 ppm (about 4 mg/kg bw/day) via the diet did not lead to dose-dependent changes of serum iron and transferrin values in young (21 to 28 days old) male Wistar rats when fed for two and seven days.
The NOAEL concerning both clinical pathology parameters was at least 22 ppm (about 4 mg/kg bw/day) in young male Wistar rats.

As the NOAEL for decreased serum iron levels in rats (determined in 7-day dietary studies; BASF DocID 2002/1014245) was determined to be 4 mg/kg bw, the result of this study confirm, that young rats are not susceptible to show decreased serum iron levels after dimoxystrobin treatment.

Conclusion on effects on decreased serum iron concentrations and relevance for reference dose setting

Dimoxystrobin causes decreased serum iron concentrations in rats. Clear NOAELs of 4 mg/kg bw for the induction of this effect were derived for adult (7-day study in 10 week old rats, BASF DocID 2002/1014245), for young (7-day study in 3-week old rats, BASF DocID 2010/1026748), for dams and offspring (enhanced one generation toxicity study, BASF DocID 2011/1211676). Only a slight tendency of decreased serum iron in blood was detected in the new mechanistic study in rats at high doses (BASF DocID 2011/1001622, see below). Thus the 4 mg/kg bw is considered to represent the lowest relevant NOAEL identified in the most sensitive species after administration of dimoxystrobin and should be used for reference dose setting.
For humans average nutrient requirements (ANR) for iron are known. When recommended iron intakes are compared, differences in the iron demands in humans depending on sex, and age are described. Comparing the recommended intakes for males, $\text{females}_{\text{premenopausal}}$, and $\text{females}_{\text{postmenopausal}}$ a factor of about 2 can be derived for differences in dietary iron requirement over the different human population groups (Doets et al., 2011, BASF DocID 2011/1297651).

The aim of the following study was to determine if an intramuscular administration of iron can prevent an increase in duodenal weights (indicative of duodenal thickening and cell proliferation) induced by oral administration of dimoxystrobin in rats.

Effect of administration of additional iron on dimoxystrobin-induced changes in the duodenum of rats (2002/1013984, 2003/1009197, 2004/1010601) – peer-reviewed

Guidelines: No test guideline exists for this type of study
Remarks: NA
GLP: Not fully in compliance with GLP. No analysis of test substance preparations was performed and the study report was not audited by QAU.
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material and methods

Dimoxystrobin (batch/purity: N15/98.4%) was administered to groups of 10 male Wistar rats at dietary concentrations of 0 ppm and 4,500 ppm over a period of 14 days and to groups of 10 female Wistar rats at dietary concentrations of 0 ppm, 500 ppm and 4,500 ppm over a period of 7 days. Simultaneously, additional groups received an iron complex (Myofer®100, iron (III)-hydroxoid-dextran-complex) intramuscularly (males once daily at a dose level of 100 mg/kg body weight on study days 0, 7, 11 and 13, and females twice daily at a dose level of 50 mg/kg body weight from day 2 to day 6).
Test diets (prepared once at the start for males and once at the start for females) were used over a period within the known stability of the test material in diet. The test diet was a basic maintenance diet, the same as used in other rat studies.
Clinical signs of toxicity were noted. Food consumption and body weights were determined weekly. In males, blood was sampled on days 3, 7, 13 and 14 for measurement of serum levels of iron (by the guanidine/ferrozine method without deproteinisation), transferrin (by turbimentry) and unsaturated iron binding capacity (by the ferrozine method).
A gross necropsy was performed (day 7 in females, day 14 in males) and terminal body weights and duodenal weights were determined. The duodenum and all gross lesions were preserved but no histopathological examination was performed.

Findings

Clinical chemistry
Serum iron (males):
Administration of 4500 ppm dimoxystrobin caused significant decreases in serum iron concentrations. Compared to the concurrent control value the iron levels were decreased by up to 51% (day 14). In the animals given the iron complex without or with dimoxystrobin markedly increased serum iron concentrations were measured on day 14, only. No clear treatment-related effects on serum iron were seen on days 3, 7 and 13 in these animals given the iron complex.

Serum iron (females):
Serum iron levels were decreased by about 13% in the 500 ppm group and by about 26% in the 4500 ppm group. Animals receiving the iron complex twice a day showed marked increases in serum iron concentrations at the end of the study. Administration of dimoxystrobin in combination with the iron complex did not result in reduced serum iron levels in these animals. This might be due to a massive iron overload in the serum of these animals given the iron complex which could not be affected by the oral administration of dimoxystrobin.

Unsaturated iron binding capacity (males only):
On day 13 and 14 unsaturated iron binding capacity was increased statistically significantly in the serum of the animals given 4500 ppm dimoxystrobin by 38% compared to the respective control. The unsaturated iron binding capacity in the serum of animals receiving the iron complex with or without dimoxystrobin was decreased compared to the control group by 20-60% on days 3 and/or 7.

Transferrin (males only):
Throughout the study decreased transferrin concentrations were found in the serum of the animals which received the iron complex. The effects in the group also receiving dimoxystrobin were more pronounced than in the group receiving no dimoxystrobin. No consistent treatment-related effects were seen in serum transferrin levels of the animals receiving 4500 ppm dimoxystrobin alone.

Gross Pathology
Duodenum:
Thickening of the duodenum was observed only in males treated with 4500 ppm dimoxystrobin. It was seen in 8 males without iron complex administration but in only 2 males with iron complex administration.

Duodenal weight:
Duodenal weights (absolute) were statistically significantly increased after exposure to 500 ppm (females) and 4500 ppm (both sexes) dimoxystrobin, for females in a dose dependent manner. Additional treatment with the iron complex led to a lower increase in all treatment groups (only a marginal difference at 500 ppm) compared to dimoxystrobin alone.
However, these are difficult to assess because both dimoxystrobin alone and iron complex alone caused reduced body weight.

Conclusion

Compared to the untreated controls, serum iron levels were increased in all groups receiving the iron complex (with and without dimoxystrobin), and decreased in the groups receiving only dimoxystrobin.

However, no reduction of increased serum iron was seen in females receiving dimoxystrobin and iron complex compared with dimoxystrobin alone. Administration of the iron complex and dimoxystrobin did result in a lower increase in absolute duodenal weight compared to dimoxystrobin alone (notably at 4500 ppm dimoxystrobin, but only marginally at 500 ppm dimoxystrobin). The incidence of duodenal thickening (as determined grossly) was also reduced in males administered 4500 ppm dimoxystrobin plus iron complex compared with 4500 ppm dimoxystrobin alone.

These findings therefore suggest that the decrease in serum iron levels induced by dimoxystrobin administration might be a causative factor in the increased thickening of the duodenal mucosa seen in repeated dose studies with rats and mice.

However this study does not provide evidence of a completely consistent relationship between reduced serum iron and increased duodenal thickness. Therefore, the supplementation of iron did not prevent effects caused by oral administration of dimoxystrobin (reduction in serum iron, duodenal thickening, and increase in duodenal weights).

This study was performed to investigate the ability of dimoxystrobin to form complexes with ferrous and ferric iron in vitro.

In vitro study of ability of dimoxystrobin to form complexes with ferrous and ferric ions (2002/1012887) – peer-reviewed

Guidelines: NA
Remarks: NA
GLP: Not conducted according to GLP. Details of bach number and purity of dimoxystrobin were not provided, but assumed to be of analytical grade.
Acceptance: The study was considered acceptable in the EU registration process 2003.

Material/methods and findings:

Ferrous ions, Fe^{2+}

Solutions of dimoxystrobin and dimoxystrobin / $FeCl_2$ in deuterated acetonitrile were analysed by one dimensional 1H NMR spectroscopy, using TMS (Tetramethylsilan) as a reference compound. The results show that the presence of $FeCl_2$ has no interpretable effect on the spectrum indicating that Fe^{2+} does not form complexes with dimoxystrobin.

Ferric ions, Fe^{3+}

Solutions of dimoxystrobin and dimoxystrobin / $FeCl_3$ in deuterated acetonitrile were analysed by both one and two dimensional (COSY and HSQC) NMR spectroscopy. Deuterated acetonitrile was used as a reference in each analysis. The ^{1}H NMR spectra were obtained at ratios of dimoxystrobin to $FeCl_3$ ranging from 1:1 to 500:1.
The results show that in the presence of $FeCl_3$ broadening of the spectral lines and changes in the chemical shifts occurs. Broadening of the spectral lines, associated with the methyl protons of the biophore group (α methoxyimino-N-methyl-acetamide function), occurs even at the lowest concentration of Fe^{3+} indicating that there is localised interaction with this region of the molecule. The chemical shifts observed are also more pronounced for these protons. The magnitude of the chemical shifts is less than might be expected for a strong interaction

Conclusion

The study results showed that Fe^{2+} does not form complexes with dimoxystrobin. Furthermore, complexation between dimoxystrobin and Fe^{3+} is considered to be not very strong.

Overall, these later studies do not provide sufficient evidence for strong iron (Fe3+) complexation properties, which would explain the lower iron absorption, lower serum iron levels and duodenal mucosa thickening. Also an additional intramuscular dosing of iron does not prevent rats from showing duodenum toxicity after dietary dimoxystrobin treatment. This observation is in line with the negative result of the complexation experiment conducted in the McKie study (BASF DocID 2011/1001622). A local mode of action as suggested in Chapter M-CA 5.5 based on studies conducted in mice is much more likely.

CA 5.8.3 Endocrine disrupting properties

Introduction

According to Regulation (EC) No 1107/2009, "an active substance shall only be approved, if it is not considered to have endocrine disrupting properties that may cause adverse effect in humans."

It is further stated, that "By 14 December 2013, the Commission shall present to the Standing Committee on the Food Chain and Animal Health a draft of the measures concerning specific criteria for the determination of endocrine disrupting properties to be adopted in accordance with the regulators procedure with scrutiny referred to in Article 79(4)."
As the adoption of these criteria is still pending, it is currently stated that "substances that are or have to be classified as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine disrupting properties."

With its current legal classification with Carc. Cat. 2 (H351) and Repr. Cat. 2 (H361d) dimoxystrobin would legally fall under the interim criteria.
In this chapter a detailed discussion will be presented proving that dimoxystrobin does not have endocrine disrupting properties. A clear description of the mechanism of action leading to duodenum tumors in mice (effects leading to classification with Carc. Cat. 2) and effects in the offspring animals in the multi-generation study (leading to the current legal classification with Repr. Cat. 2) is provided in the respective chapters (M-CA 5.5 and M-CA 5.6) of this dossier.
Furthermore, new data are available supporting that a classification of dimoxystrobin for developmental toxicity is not warranted. With this dimoxystrobin would no longer trigger the currently applied interim criteria.

Definition of an endocrine disruptor

There are several definitions of an endocrine active substance available. The definition mostly used is the definition of the WHO/IPCS (2002) stating that an endocrine active substance is "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations"

Classification with Carc. Cat. 2 (H351)

The classification of dimoxystrobin with Carcinogenicity Category 2 is based on increased incidences of duodenum adenoma and adenocarcinoma in mice at the highest dose level tested only. Reduced iron uptake in the duodenum leads to an increased proliferation of duodenal mucosa to increase the surface area for absorption of iron.

The postulated mode of action is a local interference of dimoxystrobin with duodenal receptors for iron uptake/metabolism leading to a reduced absorption of iron into duodenal enterocytes. To compensate for the decreased local iron absorption an increased cell proliferation of enterocytes (seen by an increased lengthening and broadening of villi) is mediated resulting in a thickening of the duodenal mucosa, which is the main route for iron absorption in the body. The ultimate effect seen in mice after long-term treatment with dimoxystrobin are slightly increased incidences in duodenal adenoma and adenocarcinoma at high dose levels only.
The cell proliferation in the duodenum in mice is reversible and shows a clear threshold.
The mode of action of duodenal toxicity caused by dimoxystrobin is described in detail in chapter M-CA 5.5 of this dossier.

In conclusion, the effects leading to the classification with Carc. Cat. 2 are clearly not related to endocrine disruption.

Classification with Repr. Cat. 2 (H361d)

The current classification with Reproduction Toxicity Category 2 was based on the offspring findings observed in the 2-generation toxicity study in rats (microcytic hypochromic anemia, reduced body weights of the offspring, cardiomegaly of the offspring).
Dimoxystrobin reduces the iron uptake and thus leads to the development of an iron deficiency microcytic hypochromic anemia in both parental and offspring animals, with no higher susceptibility of the offspring. This is its general toxic effect and not a specific developmental effect.
It is known that reduced availability of iron leads to depressed body weight development in growing animals. Transient cardiomegaly is a consequence of hypoxia caused by the anemia in pups. Therefore, the effects on body weights and the heart are observed only in the presence of other toxic effects (iron deficiency/anemia). These effects are considered secondary, specific consequences of the anemia. Thus, a classification for developmental toxicity is not warranted.

A detailed discussion can be found in chapter M-CA 5.6 of this dossier.

The effects leading to the current classification with Repr. Cat. 2 (H361d) (which is not warranted supported by new data) are clearly not related to endocrine disruption.

Exclusion of other ED related endpoints

There is no indication for an endocrine disruption potential of dimoxystrobin from pivotal animal studies. Organ weight changes of male and female sexual organs and endocrine glands observed in subchronic and chronic studies at higher dose levels were secondary to the observed decrease of terminal body weights, or incidental findings in single animals only and typically not accompanied by histopathological findings.

The decrease of absolute and relative adrenal weights in high dose female mice in the subchronic toxicity study was accompanied by a decrease of lipid deposition in the x-zone, which is specific to mice and of unknown function. The decreased lipid content is considered secondary to the impaired body weight development associated with reduced triglycerides. Moreover, this effect was not seen in the long-term study in mice.
A slight increase in focal fatty change of adrenals of mid and high dose female rats in the rat carcinogenicity study was an incidental finding as no dose-response was observed. Furthermore, focal fatty change occurred to an even larger extent in control animals of another carcinogenicity study conducted in the same lab during the same time.

Likewise, long-term administration of dimoxystrobin did not indicate treatment-related effects on neoplastic or non-neoplastic findings in sexual organs or endocrine tissues.
A slight increase in c-cell adenoma in the thyroid of high dose female rat in the chronic and carcinogenicity study was well within the historical control data and without a dose-response. In males, the number of animals with c-cell adenomas was even slightly decreased in the top dose group compared to controls. Therefore, this effects is regarded as incidental. No histological effects on the thyroid were seen in mice and dogs.

No treatment-related adverse effects on fertility or reproductive performance could be detected in the 2-generation toxicity study and in the modified 1-generaion study up to the highest dose tested. No teratogenic effects could be detected in the teratogenicity studies in rats and rabbits.
The slight delay of vaginal opening and preputial separation observed in the 2-generation reproduction toxicity study is considered to be secondary to the reduced body weights at the high dose and therefore no endocrine mediated effect.

As a conclusion, there is no evidence for endocrine active properties of dimoxystrobin.

Impact assessment in literature

Impact assessments by different organisations were cited in the EC roadmap:

- PSD, United Kingdom (*Revised assessment of the impact on crop protection in the UK of the 'cut-off criteria'…*)
- KEMI, Sweden (*Interpretation in Sweden of the impact of the "cut-off" criteria…*)
- European Parliament (*The benefits of strict cut-off criteria on human health in relation to the proposal for a Regulation concerning plant protection products*)
- HSE (*Extended impact assessment study of the human health and environmental criteria for endocrine disrupting substances proposed by HSE, CRD*)

All of these assessments did not consider dimoxystrobin to be an endocrine disruptor.

HSE, CRD came to the conclusion that dimoxystrobin is **not** considered to be an endocrine disruptor. This was based on an *Extended impact assessment study of the human health and environmental criteria for endocrine disrupting substances* conducted by the Water Research Council (Ewence et al., 2013; WRc Ref: Defra9088.02) on behalf of the HSE, CRD.

Furthermore, in *An approach to the identification and regulation of endocrine disrupting pesticides* Ewence et al. (2015; BASF DocID 2015/1174503) did not consider dimoxystrobin to be an endocrine disruptor.

Marx-Stoelting et al. (2014; BASF DocID 2014/1326032) published an *Assessment of three approaches for regulatory decision making on pesticides with endocrine disrupting properties.* Dimoxystrobin was not considered an endocrine disruptor, based either purely on hazard identification (adversity, mode of action, and the plausibility that both are related), or additional elements of hazard characterisation (severity and potency); dimoxystrobin was only considered an endocrine disruptor based on the interim criteria described above.

Conclusion

The mechanisms of action leading to duodenum tumors in mice, and with this the classification with Carc. Cat. 2, as well as the effects on offspring animals in the multi-generation study leading to the current legal classification with Repr. Cat. 2 (not warranted supported by new data available) can be clearly related to effects of dimoxystrobin on iron absorption in the duodenum on a receptor level.

An endocrine mode of action can therefore clearly be excluded. Thus, the categorization of dimoxystrobin as an endocrine active substance based on the interim criteria is scientifically inappropriate and draws a wrong picture on the toxicological properties of the active substance.

References

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CA 5.9 Medical Data

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of dimoxystrobin. Thus, the medical monitoring programme is designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.
The surveillance program includes a general physical examination including neurological status, red and white blood cell counts, liver enzymes. Adverse health effects suspected to be related to dimoxystrobin exposure have not been observed.

CA 5.9.2 Data collected on humans

No human cases of intoxication or poisoning deriving from dimoxystrobin are known to BASF SE.

CA 5.9.3 Direct observations

Neither data on exposure of the general public nor epidemiologic studies on dimoxystrobin are available for BASF SE, nor is BASF SE aware on any epidemiologic studies performed by third parties.

CA 5.9.4 Epidemiological studies

Report: CA 5.9.4/1
Nasterlack M., 2010a
Relevance of iron deficit for the development of duodenal cancer (small bowel adenocarcinoma, SBA) in humans?
2010/1228652
Guidelines: none
GLP: no

Executive Summary

I. INTRODUCTION

The fungicide dimoxystrobin has been assessed by the European authority EFSA (EFSA 2005). lt was found to be carcinogenic in mice but not in rats, causing an increased incidence of adenoma and adenocarcinoma in the duodenum at the top dose. There was also focal hyperplasia in the duodenum at the top dose, interpreted as a pre-neoplastic lesion. lt has been proposed that increased duodenal thickening is related to decreased levels of iron in serum. Mechanistic studies indicated that duodenal tumors in mice were caused by a persistent cell proliferation related to increased functional demand on the duodenum to compensate for decreased iron levels in serum. Based on these results, the risk phrase and classification Xn; R40 "Limited evidence of a carcinogenic effect" has been proposed.
, BASF decided to perform an assessment from a medical and epidemiological point of view to answer the question: ls the finding of duodenal cancer in mice relevant to humans? Or, more specifically: ls there an indication for a causal role in humans of iron deficit and/or anemia in the development of duodenal cancer?

II. RESULTS AND DISCUSSION

Epidemiology

Small bowel adenocarcinoma (SBA) is extremely rare in humans, with an estimated incidence of 0.9-1.4/100,000 in Western countries (Hoffmann et al. 2010). Although the small intestine makes up for some 75% of the total length of the intestine, and more than 90% of its mucosal surface area, small intestine malignant tumors of all cell types account for only 1-3% of all malignant intestinal tumors, and less than half of them are adenocarcinomas. Roughly 50% of SBA arise in the duodenum, 30% in the jejunum, and 20% in the ileum.

lron deficiency (ID) and anemia are comparatively common findings in many populations, with the highest rates in regions where chronic malnutrition is prevalent. According to a World Health Organization (WHO) estimate ID affects 47% of children below age 5 world-wide, and 25% of children between age 5-14.

Females are more affected than males, with 30% of females considered iron deficient, rising to 42% in pregnancy. The prevalence of ID in males is estimated around 13% (WHO 2001). However, also in affluent Western countries ID is not uncommon. The respective figures for the United Kingdom are 21 % of children aged 11-18, and 18% of females between age 16-64. In the USA 9-11 % of nonpregnant females aged 16-64 are considered iron deficient, and 2-5% are anemic due to ID (Andersson et al. 2010).

lt may thus be tentatively and preliminarily concluded that the extreme rarity of SBA in humans on the one hand, and the relatively high frequency of iron deficits and anemia on the other hand do not provoke an a *priori* hypothesis of a causal link between these two conditions.

Causes and risk factors for SBA and ID

The causes for SBA in humans are largely unknown. Both sexes are affected almost to the same extent, with a slight preponderance of males (American Cancer Society 2010, Hoffmann et al. 2010). Medical conditions and hereditary diseases such as Crohn's disease, familial adenomatous polyposis, Gardner's syndrome, Lynch syndrome (HNPCC) and a few others have been identified as going with an increased risk for the development of SBA. Dietary factors like high fat intake and consumption of red, cured or smoked meat have been proposed as external risk factors. The role of smoking and alcohol consumption for the development of colon cancer is considered clear, but remains inconclusive for SBA. Occupational risk factors for SBA have rarely been studied. Female dry cleaners; textile workers; dockers and freight handlers; general farm laborers; and male welders have been suggested to be at a higher risk for SBA in one case-control study (Kaerlev et al. 2000).

lron absorption normally occurs in the duodenum and upper part of the jejunum. lt is regulated according to the body's needs, increasing in ID and decreasing in iron overload (Morgan and Oates 2002). The underlying causes for ID can apparently be found either in nutritional deficits, e.g. chronic malnutrition or extreme dietary habits (vegans) or in increased iron loss or turnover, like in acute or chronic blood loss, chronic inflammation, etc. This latter circumstance provides the only established connection between intestinal cancer on the one side and ID on the other side: the latter being a sequel to the former.

Plausibility of a link between SBA and ID

As a matter of fact, an intestinal tumor with occult bleeding is comparatively frequently found during the diagnostic work-up of an ID of unknown origin (Ho et al. 2008). This finding is then rightfully interpreted as the underlying cause for the ID (Prutki et al. 2006, Raje et al. 2007). There exist no published findings that would indicate a reverse causality. On the contrary, in a large prospective study employing colonoscopy and biopsy in 1,000 consecutive patients, hemoglobin concentration was not predictive for any pathological finding in the duodenal region (Tischendorf et al. 2008). In another study, albeit targeted at colon cancer, the prevalence of colorectal carcinoma was not different between anemic and non-anemic patients, although those with a proximal colorectal carcinoma had a lower hemoglobin and ferritin level and a higher prevalence of iron-deficiency anemia compared with patients with a distal colorectal carcinoma (Joosten et al. 2008). In any case, many cancers are accompanied by significant ID and/or anemia (Prutki et al. 2006).

lt has been pointed out that vegetarians and vegans are at increased risk for developing ID because iron derived from heme, and thus from animal sources, is more readily absorbed in the intestine than from any other source (Morgan and Oates 2002). These groups of persons have, however, a decreased risk for developing cancers of the upper gastrointestinal tract, probably also including SBA (Key et al. 2009). On the other hand, as mentioned above, heavy consumption of red meat (as an excellent source of iron) has been found to be a risk factor for the development of such cancers (Fraser et al. 2009). Further, while for obvious reasons ID and anemia are more common in females than in males, there is a preponderance of SBA cases in males (American Cancer Society 2010, Hoffmann et al. 2010). These circumstances also argue against a causation of SBA through ID.

lt may finally be mentioned that there is no medical experience, let alone published evidence, indicating that iron substitution should decrease the risk for SBA or any other intestinal cancer in humans.

III. CONCLUSION

- There is sufficient evidence that cancer in general and intestinal cancers specifically, can be a cause for ID and/or anemia in humans.
- There is no evidence and no biological plausibility for the causation of intestinal cancers, and more specifically SBA, through ID.
- The finding of increased incidence of adenocarcinoma in the duodenum of mice following dimoxystrobin-induced ID is, thus considered irrelevant for humans.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Not known

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

See safety data sheet / precautions; symptomatic and supportive treatment, no specific antidote known

CA 5.9.7 Expected effects of poisoning

Expected effects were derived for acute and subacute studies in animals

Dimoxystrobin

Document M-CA, Section 6

RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

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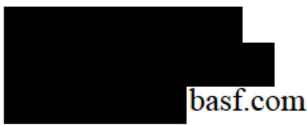

Version history[1]

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

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

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CA 6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED AND PLANT METABOLISM

Dimoxystrobin (BAS 505 F), a fungicide for use in oilseed rape and sunflower, is registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive No 2006/75/EC. Inclusion entered into force on 11 September 2006. The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011. Approval extension was granted until 31 January 2018 by Implementing Regulation (EU) No 1136/2012.

All relevant information on the first Annex I review and the endpoints used in consumer dietary assessments can be found in the monograph of dimoxystrobin, in the EFSA conclusion on dimoxystrobin (2005) and in SANCO/10531/05-Final document (EU Review Report of April 2006).

For the current renewal of approval under Regulation (EC) 1107/2009, a data gap analysis according to new guidelines and new guidance documents was performed and new studies or evaluations were initiated where considered necessary. All new data are provided in this section or in the respective sections of the dossier for the new representative formulation.

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. Although title and abstract of several publications indicated a potential connection to respective consumer safety chapters of this dossier, the detailed evaluation of these publications showed no endpoint of sufficient reliability which could be used for the required risk assessments. Consequently, for consumer safety, no summaries of public literature data on dimoxystrobin are provided in this section. Further information on the literature assessment and respective justifications can be found in M-CA 9.

An overview of metabolites identified during consumer safety studies is given below. The list of metabolites occurring in rats is not complete; the complete list can be found in M-CA 5.1. The information in the table allows a comparison between the pathways in different test systems.

The metabolite overview below is including the different code numbers that are available for each metabolite. Due to historic reasons (e.g. use of different metabolite codes in different study reports), it is unfortunately not possible to use always only one and the same metabolite code for a certain metabolite. In the following chapters and study summaries synonym metabolite codes are given in brackets where deemed to be helpful.

Code Numbers			CAS-No	Compound found in	Structure
Substance Code	Reg. No.	Synonyms			
BAS 505 F	285028	Dimoxystrobin parent M505F000	149961-52-4	rat, goat, hen, wheat, rape, rot. crop, soil/water	
505M01	358104	BF 505-4 M505F001	133409-73-1	rat, goat, hen, rot. crop, soil, water (aqueous photolysis + irrad. Water/sediment)	
505M02	356310	BF 505-5 M505F002	not assigned	rat, goat, hen, rot. crop	
505M04	4035807	BF 505-12 M505F004	not assigned	goat, soil, water (aqueous photolysis)	
505M06	not assigned	M505F006	not assigned	rot. crop	
505M09	354563	BF 505-8 M505F009	1418095-11-0	rat, goat, hen, rot. crop, soil, water (irrad. water/sediment)	
505M33	not assigned	M505F033	not assigned	rat, rot. crop	
505M47	not assigned	M505F047	not assigned	hen	
505M48	not assigned	M505F048	not assigned	hen	

Code Numbers			CAS-No	Compound found in	Structure
Substance Code	Reg. No.	Synonyms			
505M49	not assigned	M505F049	not assigned	goat	
505M50	not assigned	M505F050	not assigned	rat, goat	
505M51	not assigned	M505F051	not assigned	goat	
505M63	not assigned	M505F063	not assigned	rat, rot. crop	
505M76	4040914	BF 505-11 M505F076	not assigned	goat, hen	
505M78	not assigned	M505F078	not assigned	goat, hen	
505M79	not assigned	M505F079	not assigned	goat	
505M80	not assigned	M505F080	not assigned	rot. crop	
505M81	not assigned	M505F081	not assigned	rat, goat	

Code Numbers			CAS-No	Compound found in	Structure
Substance Code	Reg. No.	Synonyms			
505M82	not assigned	M505F082	not assigned	hen	
505M84	not assigned	M505F084	not assigned	goat	
505M86	not assigned	M505F086	not assigned	goat	
505M88	not assigned	M505F088	not assigned	rot. crop	
505M89	not assigned	M505F089	not assigned	rot. crop	
505M91	not assigned	M505F091	not assigned	rot. crop	
505M93	not assigned	M505F093	not assigned	rot. crop	
505M94	not assigned	M505F094	not assigned	rot. crop	

Code Numbers			CAS-No	Compound found in	Structure
Substance Code	Reg. No.	Synonyms			
505M95	not assigned	M505F095	not assigned	rot. crop	
505M98*	360056	M505F098 Parent Z-isomer	159023-37-7	goat, rape, soil/water	
505M105	not assigned	M505F105	not assigned	goat	
505M107	not assigned	M505F107	not assigned	rape	

* 505M98 is not a metabolite. 505M98 is the M-code for the Z-isomer of dimoxystrobin which is present in application solutions (3-10%) used in the studies therefore it is detectable in all treated matrices.

CA 6.1 Storage stability of residues

Storage stability data in different crop commodities is provided for dimoxystrobin (BAS 505 F) and its Z-Isomer 505M98, in order to support new residue trials.
Storage stability for animal matrices is not provided as samples of the feeding study were analyzed within 30 days.

Stability of dimoxystrobin was studied for different crop commodities, results demonstrated that dimoxystrobin is stable up to 2 years in plant matrices. An ongoing storage stability study is being conducted, preliminary data demonstrated, that the Z-isomer 505M98 is stable up to 5 months (interim report). Taking into account structure similitudes and data from the storage stability study of 505M98 in soil matrices (M-CA 7.1.2.2.1/4, DocID 2014/1286541), it is also expected that 505M98 is stable up to two years in the different crop commodities.

Report:	CA 6.1/1 Lehmann A.,Mackenroth C., 2002a Investigation of the stability of residues of BAS 505 F in plant matrices under normal storage conditions 2002/1004102
Guidelines:	IVA Guideline Residue Chemistry Part II Storage Stability 1992, EPA 171-4(e)
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The stability of BAS 505 F in different plant matrices stored deep frozen such as wheat (green plant without roots, grain and straw), oil rape seed, sugar beet (roots), white cabbage (head), peach (fruit) and peas was investigated over a period of two years. The plant material was spiked with the test substance at a concentration level of 0.5 mg/kg. The spiked samples were then stored under the usual storage conditions for field samples (polyethylene bottles, -20°C). At different intervals samples were analyzed with BASF method No. 445/0. The method allows quantitation of residues of BAS 505 F to a limit of 0.05 mg/kg in plant material. The analytical results used for the stability calculation were corrected for recoveries and were evaluated by means of an exponential function. Over the period tested of about 2 years, BAS 505 F was demonstrated to be stable in wheat green plant, grain and straw as well as in oil rape seed, sugar beet, white cabbage, peach and pea.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** BAS 505 F
   Description: Dimoxystrobin
   Lot/Batch #: 01171-55
   Purity: 99.7%
   CAS#: 145451-07-6
   Development code: not relevant
   Spiking levels: 0.5 mg/kg

2. **Test Commodity:**
   Crop: wheat, oil seed rape, sugar beet, cabbage, peach and peas
   Type: not reported
   Variety: not reported
   Botanical name: *Triticum aestivum, Brassica oleracea, Beta vulgaris, Prunus persica, Pisum sativum*
   Crop parts(s) or processed commodity: wheat (green plant without roots, grain and straw), oilrape seed, sugar beet (roots), white cabbage (head), peach (fruit) and peas
   Sample size: 5 g

B. STUDY DESIGN

1. Test procedure

The storage stability of dimoxystrobin in frozen raw agricultural commodities (RAC; namely wheat, oil seed rape, sugar beet, cabbage, peach and peas) was tested for up to 2 years. Approximately 3 kg of the sample material was processed in the same way as routine residue samples: Green plant material was ground in a grinder. Seed samples were sieved and thoroughly mixed. Straw was milled. Sugar beets, cabbage heads and fruits were cut. Aliquots of 5 g of each sample material were weighed into polyethylene containers. For every sampling date, a set of 5 containers were filled with sample material. Two of these were fortified with BAS 505 F at a level of concentration equivalent to 0.5 mg/kg. One was used as control, the other two containers were used for procedural recoveries. Samples were stored deep frozen and analyzed after day 0, month 1, 3, 6, 12 18 and 24. Each sample was measured in duplicate.

2. Description of analytical procedures

All samples were analysed according to BASF Method 445/0. BAS 505 F was extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane. The final determination of BAS 505 F was performed by HPLC-MS/MS.
All samples were analyzed in duplicate. The mean procedural recovery was reported to be 94.0% with a CV of 9.6%. The limit of quantitation of the method was 0.05 mg/kg.

II. RESULTS AND DISCUSSION

The summarized storage stability data of BAS 505 F are shown in Table 6.1-1 and Table 6.1-2.

Table 6.1-1: Summarized storage stability of BAS 505 F in various RACs

	BAS 505 F found [1] (mg/kg)															
Day	**Wheat plant**		**Wheat grain**		**Wheat straw**		**Oilrape seed**		**Sugar beet**		**White cabbage**		**Peach**		**Pea**	
0	0.53	0.51	0.47	0.50	0.51	0.54	0.48	0.46	0.48	0.51	0.54	0.48	0.53	0.51	0.50	0.50
33	0.47	0.48	0.46	0.46	0.51	0.53	0.54	0.49	0.49	0.45	0.48	0.46	0.48	0.50	0.52	0.48
96	0.37	0.37	0.47	-	0.46	0.47	0.44	0.47	0.48	0.47	0.49	0.47	0.52	0.48	0.48	0.48
182	0.45	0.46	0.45	0.43	0.47	0.45	0.40	0.42	0.53	0.53	0.44	0.43	0.50	-	0.47	0.49
356	0.56	0.58	0.47	0.46	0.46	0.48	0.46	0.48	0.51	0.53	0.45	0.42	0.52	0.49	0.51	0.52
566	0.43	0.44	0.49	0.49	0.43	0.44	0.50	0.49	0.46	0.47	0.43	0.43	0.51	0.49	0.44	0.47
720	0.45	0.45	0.48	0.50	0.45	0.48	0.43	0.41	0.53	0.52	0.41	0.46	0.48	0.47	0.49	0.51
	Degradation after 720 days (%)															
	7.0		stable [2]		15.7		7.8		stable [2]		13.9		4.3		1.1	

1) Corrected for individual procedural recovery
2) Negative degradation = apparent increase

Table 6.1-2: Summarized procedural recoveries of BAS 505 F in various RACs

	Recoveries (%)															
Day	**Wheat plant**		**Wheat grain**		**Wheat straw**		**Oilrape seed**		**Sugar beet**		**White cabbage**		**Peach**		**Pea**	
0	68.3	70.1	97.4	96.5	72.9	75.7	82.8	79.3	90.6	94.4	91.5	110.1	98.0	89.4	94.4	90.1
33	86.5	79.4	99.5	99.3	71.8	93.2	94.6	99.5	104.3	109.8	99.1	95.0	94.3	111.2	106.7	94.6
96	77.4	81.5	95.0	94.4	77.1	76.4	93.9	89.7	89.3	98.9	84.9	103.2	96.4	89.5	93.5	90.9
182	104.4	99.9	101.7	102.5	105.5	104.6	100.1	103.3	97.5	72.4	96.6	93.3	98.5	94.2	98.7	-
356	78.4	77.6	100.0	99.7	97.5	98.2	93.7	94.6	97.5	96.3	96.5	97.2	97.1	93.8	99.5	97.2
566	96.0	98.1	98.1	84.5	99.6	101.9	90.9	93.5	100.2	98.4	102.1	92.1	83.2	94.0	92.2	92.5
720	94.7	100.3	100.8	101.8	100.8	96.9	99.9	106.0	85.5	92.1	-	92.1	105.6	97.4	82.0	106.9
Mean	86.6%		97.9%		90.9%		94.4%		94.8%		96.4%		95.9%		95.3%	
SD	+/- 11.7		+/- 2.7		+/- 13.7		+/- 7.5		+/- 10.0		+/- 6.7		+/- 6.1		+/- 5.1	
CV	13.5%		2.8%		15.1%		8.0%		10.5%		7.0%		6.4%		5.3%	
Overall mean: 94.0% - SD: +/- 9.1 - CV: 9.6%																

The data in Table 6.1-1 and Table 6.1-2 show that there is no significant decrease of the concentration of BAS 505 F in the spiked samples of this study. BAS 505 F has been demonstrated to be stable in wheat (plant, grain, straw), oilseed rape (seeds) white cabbage and peas for up to two years of frozen storage on all RACs tested.

III. CONCLUSION

The data showed that BAS 505 F was stable in frozen wheat (plant, grain, straw), oilseed rape (seeds) white cabbage and peas over a period of approximately 2 years. This indicates that specimens of these commodities containing BAS 505 F can be stored deep frozen for at least 2 years prior analytical measurements.

Report:	CA 6.1/2 Shen X.,Guo D., 2015a Freezer storage stability of 505M98 (Reg. No. 360056) in plant matrices 2015/7000746
Guidelines:	EPA 860.1380, OECD 506, EPA 860.1500, EEC 7032/VI/95 rev. 5
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

The purpose of this study was to determine the storage stability of 505M98 (Z-isomer of dimoxystrobin, Reg. No. 360056) in plant matrices stored frozen for up to 2 years. In this report, the stored samples have been tested for stability up to 5 months (151 days). The study is still ongoing. Five plant matrices were studied including lettuce, sunflower seeds, beans, barley grain and orange.

Samples of homogenized untreated plant matrix (5 g each) were treated with a standard solution of 505M98 at a level of 0.1 mg kg^{-1} (equivalent to 20 times the LOQ), and were stored frozen at approximately -20ºC for 5 months (151 days). They were analysed after 0, 30, 90 and 151 days after fortification and storage using validated BASF Method No.L0076/08 (CA 4.1.2/6, BASF DocID 2014/1233896). The limit of quantitation (LOQ) of the method is 0.005 mg kg^{-1} and the method limit of detection (LOD) is 0.001 mg kg^{-1} for analyte 505M98.

The results of the study show that 505M98 is stable in plant matrices while stored frozen for up to 5 months (151 days), with the average corrected recovery above 70% in all samples. Acceptable procedural recoveries for each plant matrix were also obtained for 505M98.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Z-isomer of dimoxystrobin
   Description: 505M98
   Lot/Batch #: 1520-181
   Purity: 99.5%
   CAS#: 149923-06-8
   Development code: BASF Reg. No. 360056
   Spiking levels: 0.1 mg kg^{-1}

2. **Test Commodity:** lettuce, sunflower seed, white navy bean, barley grain, orange
   Crop: leafy vegetables, pulses and oilseeds, cereals, fruits and fruiting vegetables
   Type: n/a
   Variety: n/a
   Botanical name: *Lactuca sativa, Helianthus annuus, Phaseolus vulgaris, Hordeum vulgare, citrus sinesis.*

   Crop parts(s) or processed commodity: lettuce, sunflower seed, white navy bean, barley grain, orange
   Sample size: 5 g

B. STUDY DESIGN AND METHODS

1. Test procedure

The untreated control plant samples obtained from five different plant matrices including lettuce, sunflower seed, white navy bean, barley grain and orange were received intact and frozen, and were immediately placed in frozen storage.
Approximately 5 g of each homogenized untreated plant matrix were fortified with a standard solution of 505M98 at a level of 0.1 mg kg-1 equivalent to 20 times the LOQ of the analytical method. The stored-fortified samples were prepared by adding 250 µL of a 2 µg mL-1 standard solution (dissolved in methanol), using a calibrated mechanical pipette. The other samples remained unfortified to be used as control and procedural fortifications at time of analysis.

All fortified samples and controls were stored in a freezer at approximately -20ºC and analyzed after different intervals (0, 1, 3 and 5 months). On the day of analysis, for each plant matrix, two stored-fortified samples corresponding to the designated time period, together with three control samples, were removed from the freezer. Two of the control samples were fortified with 505M98 at 20 times the LOQ to generate procedural (concurrent) recovery data. Thus, each sample set consisted of calibration standards and blanks, and one untreated control sample, two freshly-fortified procedural fortification samples, and two stored-fortified samples for each plant matrix.

2. Description of analytical procedures

The storage stability samples were analyzed using validated BASF Analytical Method Number L0076/08 entitled: “Method for the determination of BAS 505F (Reg. No. 285028) and 505M98 (Reg. No. 360056) in plant matrices by LC-MS/MS", dated November 17, 2014, successfully tested during method validation on tomato, oilseed rape (seeds), beans, barley grain and orange for both analytes. The limit of quantitation (LOQ) of the method is 0.005 mg kg-1 and the method limit of detection (LOD) is 0.001 mg kg-1 for analyte 505M98.

The plant samples are extracted with a mixture of methanol, water and hydrochloric acid (70:25:5). The extracts are diluted 1:1 with water, partitioned against cyclohexane, evaporated to dryness and reconstituted with acetonitrile/water (50:50). The residues are determined using LC-MS/MS.

Quantitation of residues in all samples is achieved using an external calibration curve. The performance of the instrument is evaluated during each injection set. The method-detector response was linear (r > 0.99) over the tested calibration concentration range of about 0.2 - 7.5 ng mL-1 for all analytical sets.

II. RESULTS AND DISCUSSION

The performance of the analytical method was evaluated during each sample set by fortifying controls with standards containing the reference substance 505M98 at 20 times the LOQ level. Acceptable concurrent recovery data for each plant matrix were obtained (see Table 6.1-3). Individual recoveries were generally within the range of 70 – 120 %, and overall mean concurrent recoveries for 505M98 from all plant matrices ranged from 71 - 112% (%RSD ≤20%).

Table 6.1-3: Procedural recoveries from plant matrices

Matrix	Fortification Level (ppm)	Storage Interval (Months)	Replicates (n)	Concurrent Recovery (%)	Concurrent Mean Recovery (%)
Lettuce	0.1	0	2	84, 88	86
		1	2	87, 85	86
		3	2	90, 93	91
		5	2	65, 81	73
Overall Recovery (%) ± SD (CV*)			**8**	**Range = 65 - 93**	**84 ± 8 (9)**
Sunflower Seeds	0.1	0	2	98, 101	99
		1	2	115, 109	112
		3	2	85, 84	85
		5	2	86, 66	76
Overall Recovery (%) ± SD (CV*)			**8**	**Range = 66 - 115**	**93 ± 16 (17)**
Beans	0.1	0	2	94, 89	91
		1	2	105, 91	98
		3	2	84, 82	83
		5	2	89, 79	84
Overall Recovery (%) ± SD (CV*)			**8**	**Range = 79 - 105**	**89 ± 7 (8)**
Barley Grain	0.1	0	2	112, 110	111
		1	2	96, 93	95
		3	2	86, 90	88
		5	2	93, 74	83
Overall Recovery (%) ± SD (CV*)			**8**	**Range = 74 - 112**	**94 ± 12 (13)**
Orange	0.1	0	2	87, 83	85
		1	2	94, 99	97
		3	2	89, 92	91
		5	2	75, 67	71
Overall Recovery (%) ± SD (CV*)			**8**	**Range = 67 - 99**	**86 ± 11 (13)**

* = Coefficient of Variation (CV or RSD) = (Standard Deviation (SD)/mean recoveries) times 100

Recoveries from the stored-fortified samples, including recoveries corrected for the mean of the duplicate concurrent recoveries, are presented in Table 6.1-4 together with the procedural recovery data. The results show that 505M98 is stable in plant matrices while stored frozen for up to 5 months (151 days), with the average corrected recovery above 70% in all samples.
The average corrected recoveries for 505M98 after a storage period of 5 months (151 days) are 95% in lettuce, 115% in sunflower seeds, 101% in beans, 108% in barley grain and 111% in orange.

Table 6.1-4: Storage stability of 505M98 residues in five plant matrices

Matrix	Analyte	Fortification Level (mg kg^{-1})	Storage Interval (Months)	Stored Sample recovery (mg kg^{-1})	Stored Sample Recovery (%) Uncorrected [mean] †	Concurrent Recovery (%) [mean] †	Stored Sample Recovery (%) Corrected*
Lettuce	505M98	0.1	0	0.0825, 0.0805	83, 81 [82]	84, 88 [86]	95
			1	0.0821, 0.0819	82, 82 [82]	87, 85 [86]	96
			3	0.0815, 0.0923	82, 92 [87]	90, 93 [91]	95
			5	0.0603, 0.0771	60, 77 [69]	65, 81 [73]	95
Sunflower Seeds	505M98	0.1	0	0.0980, 0.1065	98, 107 [102]	98, 101 [99]	103
			1	0.1030, 0.1030	103, 103 [103]	115, 109 [112]	92
			3	0.0878, 0.0914	88, 91 [90]	85, 84 [85]	106
			5	0.0859, 0.0886	86, 89 [87]	86, 66 [76]	115
Beans	505M98	0.1	0	0.0860, 0.0880	86, 88 [87]	94, 89 [91]	96
			1	0.1015, 0.1005	102, 101 [101]	105, 91 [98]	103
			3	0.0964, 0.0842	96, 84 [90]	84, 82 [83]	109
			5	0.0826, 0.0862	83, 86 [84]	89, 79 [84]	101
Barley Grain	505M98	0.1	0	0.1075, 0.1030	108, 103 [105]	112, 110 [111]	95
			1	0.0926, 0.0967	93, 97 [95]	96, 93 [95]	100
			3	0.1010, 0.1020	101, 102 [102]	86, 90 [88]	115
			5	0.0935, 0.0861	94, 86 [90]	93, 74 [83]	108
Orange	505M98	0.1	0	0.0830, 0.0840	83, 84 [84]	87, 83 [85]	96
			1	0.0956, 0.0904	96, 90 [93]	94, 99 [97]	96
			3	0.0921, 0.0950	92, 95 [94]	89, 92 [91]	103
			5	0.0880, 0.0698	88, 70 [79]	75, 67 [71]	111

* = Corrected for concurrent recoveries

$$\text{Corrected Recovery} = \frac{\%\,Recovery\ of\ Stored\ Sample}{\%\,Concurrent\ Recovery} \times 100\%$$

† = Mean % recovery for this time interval is shown in brackets

III. CONCLUSION

Samples of different plant matrices fortified with 505M98 (Z-isomer of dimoxystrobin, Reg. No. 360056) at 0.1 mg kg^{-1} were stored frozen for duration of up to approximately 5 months (151 days). The analysis results show that 505M98 is stable in plant matrices for this time period under frozen conditions (-20°C).

CA 6.2 Metabolism, distribution and expression of residues

During the previous EU Review of the active substance dimoxystrobin, the metabolism of dimoxystrobin has been studied in wheat, goat and hens. All of these studies have been part of the previous evaluation, are still scientifically valid and are therefore not submitted again in this supplementary dossier. For reasons of convenience, a short overview of the main conclusions is given below.

Study type	Title	Test system	Results	Reference (BASF DocID)
Study according to OECD 501	Metabolism of BAS 505 F in wheat	Wheat	The investigation of the metabolism of ^{14}C-BAS 505 F in wheat leads to the conclusion that the relevant residue in wheat forage, straw and grain only consists of the parent compound BAS 505 F.	2002/1007095
Study according to OECD 503	Nature of the residue of 14C BAS 505F in lactating goats	Goat	^{14}C-BAS 505 was rapidly absorbed and almost completely excreted. There was no indication of accumulation of ^{14}C-BAS 505 F in goat tissues and milk. Milk and tissues of goats contained residues of 8 identified metabolites at levels of 0.05 mg/kg or less.	2001/5002332
Radiovalidation study	14C-Validation of the extractability of methods 478/0 and D0006 for the determination of BAS 505 F (Reg. No. 354563) and its metabolites in matrices of animal origin	Goat	The study demonstrates that BASF Methods 478/0 and D0006 are capable of extracting and quantifying aged residues of BAS 505 F in livestock commodities.	2001/5002312
Study according to OECD 503	The metabolism of 14C-BAS 505 F in laying hens	Hen	The investigation of the metabolism of ^{14}C-BAS 505 F in hens leads to the conclusion that eggs and tissues of hens contained residues at low levels consisting of four identifiable metabolites.	2000/1013139
Study according to OECD 503	14C-BAS 505 F - Absorption, distribution and excretion after repeated oral administration in laying hens	Hen	^{14}C-BAS 505 F 505 was rapidly absorbed and almost completely excreted. There was no indication of accumulation of ^{14}C-BAS 505 F in hen tissues, organs and eggs	1998/10589 1998/10986 (amendment)

CA 6.2.1 Metabolism, distribution and expression of residues in plants

In context of the previous submission of dimoxystrobin for EU review, a plant metabolism in wheat (foliar application) was submitted and evaluated. According to the results of this study, dimoxystrobin is metabolized in wheat only to a small degree:

A metabolism study was conducted to determine the amount and nature of residual radioactivity in wheat grain, straw and forage following two post-emergence applications of ^{14}C labeled dimoxystrobin at a rate of 300 g a.s./ha. The first application was performed when stem elongation started (BBCH growth stage 31), the second followed when flowering began (growth stage 61). The interval between the applications was 56 days. Samples were taken 0 days after the first application, before and after the second application and at harvest. The samples collected at harvest were separated into straw, chaff and grains. The level and the nature of the radioactivity (TRR) in the RAC (forage, straw and grain) was investigated. The highest TRRs were found in straw (8.6 - 11.3 mg/kg) and the lowest in grain (0.09 - 0.10 mg/kg), the TRRs in forage were in between (0.7 - 0.8 mg/kg). The extractability of ^{14}C residues (ERR) with methanol was good. The highest value (about 94% TRR) was found in straw and the lowest in grain (about 58% TRR). In all matrices, the metabolic patterns of the methanol extracts were almost identical. By means of HPLC and MS investigations it could be shown that the parent molecule was the only prominent peak. Its structure and the structure of the Z-isomer were confirmed by LC-MS/MS analysis. From the RRRs, the residues bound to cellulose, lignin and protein could be released, but only in smaller quantities.

This assessment was recently confirmed by EFSA during the re-evaluation of the established MRLs according to Regulation (EC) No 396/2005, Art. 12. The following conclusion is directly copied from the Reasoned Opinion (see: EFSA Journal 2013;11(11):3464):

Dimoxystrobin is the most important compound in both, cereals and oilseeds. In immature plants shortly after application, parent dimoxystrobin is by far the main residue. With longer PHI intervals the amounts of dimoxystrobin decrease and the proportion of non-extractable residues increases, but parent dimoxystrobin remains the main residue in all analysed matrices at harvest.

For covering the use in oilseed rape and sunflower two new crop metabolism studies in rape have been conducted. The older oilseed rape study was already evaluated during the process according to Regulation 396/2005, Art. 12, but has not been peer-reviewed. In the study conducted in 2002 both, a benzyl and a phenyl label, were applied. Parent and two metabolites in traces were found in hulls but no indication of cleavage of the molecule was observed. To investigate the bound residues in more detail a second study in oilseed rape was conducted with only one (phenyl) label. Both studies are summarized below.

Report: CA 6.2.1/1
Veit P.,Eisert R., 2002a
Metabolism of 14C-BAS 505 F in rape
2002/1007938
Guidelines: EPA 860.1300, EPA 860.1000, BBA IV 3-2
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Dimoxystrobin
   Description:
   Lot/Batch #: 596-1106 (benzyl-U-14C): 98%, specific activity 5.48 MBq/mg; 597-1102 (phenyl-U-14C): 98.0%, specific activity 4.74 MBq/mg; 596-2010: > 97%
   Purity: >98%
   CAS#: Not reported
   Development code: Not reported
   Spiking levels:

3. **Test Commodity:**
   Crop: Oilseed rape
   Type: Oilseeds
   Variety: Jumbo
   Botanical name: *Brassica napus*
   Crop parts(s) or processed commodity: Forage, hulls, seed, straw
   Sample size: Not reported

B. STUDY DESIGN AND METHODS

1. Test procedure
In total, two post-emergence applications at an intended use rate of 100 g a.s./ha were performed. The first application was performed when flower buds were visible (BBCH growth stage 18/19), the second followed at flowering (growth stage 63/65).

The plants were grown in pots filled with sandy loam under Limburgerhof natural conditions. Specimens were taken immediately after the first application, prior and after the second application and at harvest. The samples collected at harvest were separated into straw, hulls and seeds. An overview of the plant-uptake study design is given in Table 6.2.1-1:

Table 6.2.1-1: Design of the plant-uptake part - rape

Position of label	Benzyl label	Phenyl label
Intended use rate [g a.s./ha]	100	100
Number of applications	2	2
Interval between applications [days]	28	28
Comparison to the maximum recommended use rate	1 x	1 x
Sampling of unripe material [days after treatment]	1 [a)], 14 [a)], 1 [b)]	1 [a)], 15 [a)], 1 [b)]
PHI [days after last application]	64	65

[a)] first application
[b)] last application

2.Description of analytical procedures

Forage, seed, hull and straw samples of both labels were extracted with methanol. Prior to methanol extraction, the seeds were first extracted using cyclohexane. The cyclohexane extracts were further purified by SPE. The extractable radioactivity was characterised and quantified by radio-HPLC. Identification of the residue is based on HPLC comparison with authentic reference standards and LC/MS/MS experiments. The residual radioactivity was determined by combustion analysis. The non-extractable radioactivity in hulls and straw was further investigated by treatment with ammonia and enzymes (macerozyme and a mixture consisting of pectinase, cellulase and ß-glucosidase). The enzyme treatment was performed simultaneously from two aliquots.

During the metabolism study, the extractability according to the residue analytical methods 445/0 (data generation method) and S19 (proposed enforcement method) was additionally examined. Forage and seed were extracted with methanol/water and acetone/water mixtures.

II. RESULTS AND DISCUSSION

The total radioactive residues (based on calculated TRR = ERR + RRR) are summarized in Table 6.2.1-2. The extraction behavior is described in Table 6.2.1-3.

Table 6.2.1-2: Total radioactive residues after treatment of spring rape with 14C-BAS 505 F

Total radioactive residues [mg/kg]		
Sample material	**Benzyl label**	**Phenyl label**
Forage (1 DAT, 1. Appl.)	1.480	3.666
Forage (14-15 DAT)	0.517	0.346
Forage (1 DAT, 2. Appl.)	0.947	1.717
Seed	0.049	0.042
Straw	0.301	0.305
Hull	0.459	0.198

Table 6.2.1-3: Extraction of radioactivity after treatment of spring rape with 14C-BAS 505 F

Extraction of radioactivity [mg/kg] (% TRR)			
Sample material	**Methanol**	**Cyclohexane**	**Residue**
Benzyl label			
Forage (1 DAT, 1.Appl.)	1.466 (99.1)	n.a.	0.014 (0.9)
Forage (14 DAT)	0.504 (97.5)	n.a.	0.013 (2.5)
Forage (1 DAT, 2. Appl.)	0.920 (97.1)	n.a.	0.027 (2.9)
Seed	0.010 (19.5)	0.03 (61.6)	0.009 (18.9)
Straw	0.180 (59.9)	n.a.	0.121 (40.1)
Hull	0.334 (72.7)	n.a.	0.125 (27.3)
Phenyl label			
Forage (1 DAT)	3.648 (99.5)	n.a.	0.018 (0.5)
Forage (15 DAT)	0.338 (97.7)	n.a.	0.008 (2.3)
Forage (1 DAT)	1.659 (96.6)	n.a.	0.058 (3.4)
Seed	0.008 (19.3)	0.03 (61.9)	0.008 (19.3)
Hull	0.168 (55.2)	n.a.	0.137 (44.8)
Straw	0.134 (67.6)	n.a.	0.064 (32.4)

n.a.: not applicable

The extraction behaviour was comparable for both labels. In forage the solvent extractability was high (>95 % of the TRR). In hull and straw, it was slightly lower, 55 to 73 % of the TRR were extractable with methanol. In seeds, about 19 % of the TRR were extractable with methanol. Most of the radioactivity present in seeds could be extracted with cyclohexane (<61% TRR). The extractability investigations according to the residue methods 445/0 and S19 resulted in comparable values. By extraction with methanol/water, 89% (seed) and 98% (forage) of the TRR were extractable. The mixture acetone/water released more than 97% of the TRR in forage.

In all matrices, the metabolite patterns looked almost identical with unchanged BAS 505 F being the most prominent compound of the residue. In addition to BAS 505 F several minor metabolites were detected; most of them were significantly below 5 % TRR. The Z-isomer could not be detected or only traces are visible in the HPLC profiles. The quantification of the extractable radioactivity in the different plant matrices under investigation is summarized in Table 6.2.1-4 to Table 6.2.1-7. There is no significant difference between the both labels. No cleavage between both ring systems has been observed.

Table 6.2.1-4: Summary of identified components in spring rape samples after treatment with 14C-[benzyl]-BAS 505 F (forage)

Metabolite code (Reg.-No. of reference item)	Metabolite identity	Benzyl label		
		Forage (1 DAT)	Forage (14 DAT)	Forage (1 DAT)
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
BAS 505 F (285028, E-isomer)		1.466 (99.1)	0.504 (97.5)	0.920 (97.1)

Table 6.2.1-5: Summary of identified components in spring rape samples after treatment with 14C-[benzyl]-BAS 505 F (harvest)

Metabolite code (Reg.-No. of reference item)	Metabolite identity	Benzyl label		
		Seed	Straw	Hulls
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
BAS 505 F (285028, E-isomer)		0.026 (53.1)	0.182 (60.6)	0.231 (50.5)

Table 6.2.1-6: Summary of identified components in spring rape samples after treatment with 14C-[phenyl]-BAS 505 F (forage)

Metabolite code (Reg.-No. of reference item)	Metabolite identity	Phenyl label		
		Forage (1 DAT)	Forage (15 DAT)	Forage (1 DAT)
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
BAS 505 F (285028, E-isomer)	[structure: CH3, O, H N, H3C, N, O, CH3, CH3, O]	3.648 (99.5)	0.338 (97.7)	1.659 (96.6)

Table 6.2.1-7: Summary of identified components in spring rape samples after treatment with 14C-[phenyl]-BAS 505 F (harvest)

Metabolite code (Reg.-No. of reference item)	Metabolite identity	Phenyl label		
		Seed	Straw	Hulls
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
BAS 505 F (285028, E-isomer)	[structure: CH3, O, H N, H3C, N, O, CH3, CH3, O]	0.024 (57.5)	0.139 (45.7)	0.116 (58.4)

The storage stability investigations carried out clearly demonstrate that no changes in the metabolic pattern could be observed. Under the chosen conditions, the radioactive residue in rape matrices was stable over the period of investigation (ca. 7 months).

The non-extractable residues present in straw and hulls (see Table 6.2.1-8) ranged from 27.3 to 44.8 % TRR. In straw and hulls, extraction with ammonia as initial step released about 20 % of the TRR. The ammonia soluble fractions were further characterised by HPLC. According to the HPLC patterns obtained the radioactivity was distributed among several compounds (up to five). The enzyme incubation clearly indicated that only minor amounts of radioactivity were bound to carbohydrates. The characterization of the non-released radioactivity is summarized Table 6.2.1-8.

Table 6.2.1-8: Summary of released non-extractable radioactivity from rape straw and hulls

Fraction name	Benzyl label		Phenyl label	
	Straw	Hulls	Straw	Hulls
	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
Residue (after MeOH extraction)	0.121 (40.1)	0.125 (27.3)	0.137 (44.8)	0.064 (32.4)
Ammonia extraction	0.046 (15.2)	0.086 (18.8)	0.050 (16.4)	0.039 (19.5)
Maceroenzyme	0.007 (2.2)	0.011 (2.3)	0.007 (2.4)	0.008 (3.9)
Residue after Maceroenzyme	0.069 (22.8)	0.024 (5.2)	0.076 (24.9)	0.023 (11.4)
P/C/G*	0.008 (2.6)	0.011 (2.4)	0.007 (2.5)	0.007 (3.6)
Residue after P/C/G*	0.064 (21.3)	0.023 (5.1)	0.066 (21.6)	0.021 (10.8)

*P/C/G: Pectinase/Cellulase/Glucosidase treatment

III. CONCLUSION

The investigation of the metabolism of BAS 505 F in rape using material labelled either in the benzyl or the phenyl ring system leads to the conclusion that the relevant residue in forage, seed and straw only consists of the parent compound BAS 505 F.

All other metabolites were only present in trace amounts. The low non-extractable residues in forage and seed demonstrate that BAS 505 F and its metabolites are not firmly associated with cell wall polymers. Somewhat higher amounts of non-extractable residues were found in straw and hulls; minor portions of the radioactivity were associated with or incorporated into carbohydrate fractions.

Report: CA 6.2.1/2
Funk D., 2015a
Metabolism of 14C-Dimoxystrobin in rape
2015/1017698

Guidelines: EPA 860.1300: EPA Residue Chemistry Test Guidelines, EPA 860.1300: Nature of the Residue in Plants Livestock, EPA 860.1000: Background - PMRA Section 97.2 (Canada): Residue Chemistry Guidelines: Plants and Lifestock (June 1997), EPA 860.1000: EPA Residue Chemistry Test Guidelines, BBA IV 3-2, Lundehn III: 7028/VI/95 rev. 3 Appendix A (EU) Metabolism and distribution in plants (draft), OECD 501, JMAFF 59 NohSan No 4200

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

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: ^{14}C-dimoxystrobin (phenyl-U-^{14}C label)
^{13}C-dimoxystrobin (acetamide-2-^{13}C)
^{12}C-dimoxystrobin (unlabeled)

Lot/Batch #: ^{14}C-dimoxystrobin: 597-1301
^{13}C-dimoxystrobin: 596-2010
^{12}C-dimoxystrobin: 00956-117

Purity: Radiochemical purity:
^{14}C-dimoxystrobin: 99.1%%
Chemical purity:
^{14}C-dimoxystrobin: 96.7%
^{13}C-dimoxystrobin: >97%
^{12}C-dimoxystrobin: 99.9%
Specific activity:
^{14}C-dimoxystrobin: 4.6 MBq/mg

CAS#: 149961-52-4

2. Test Commodity:

Crop: Oilseed rape
Type: Oilseeds
Variety: Salsa CL (summer rape)
Botanical name: *Brassica napus*
Crop parts(s) or processed commodity: Seeds, hulls, rest of plants
Sample size: Not relevant

3. Soil: A sandy loam soil (Bruch West, USDA) was used. The soil physicochemical properties are described below

Table 6.2.1-9: Soil physicochemical properties

Soil series	Soil type	pH	OM %	Sand %	Silt %	Clay %	Maximal water holding capacity[1]	CEC[2] cmol/kg
Bruch West	*Sandy Loam	**7.3	***2.6	*70.4	*19.9	*9.7	28.4	12.4

* USDA scheme ** ($CaCl_2$) ***Organic matter calculated as 1.72 x percent organic carbon
[1] In g/100 g dry soil
[2] Cation exchange capacity

B. STUDY DESIGN

The study was carried out at the Agricultural Research Centre of BASF in Limburgerhof, Germany. The plant uptake part of the study was conducted in plastic containers located in climatic chambers.

1. Test procedure

The maintenance of the crop was performed in accordance with normal agricultural practice; fertilizers and additional pesticides were applied to achieve an adequate plant growth. According to the study protocol, the crop was treated twice with radiolabeled dimoxystrobin (BAS 505 F) at a nominal rate of 100 g a.s./ha (0.089 lb/A) at each application. The first application was carried out at BBCH 35 and the second 49 days before harvest.
The in-life part was performed with ^{14}C-labeled test substance. For each preparation of the application solutions, defined amounts of ^{14}C-labeled, ^{13}C-labeled and unlabeled forms of dimoxystrobin (ratio 71:14:14, ^{14}C:^{13}C:^{12}C) were dissolved in acetone. The acetone was evaporated with nitrogen and a weighed amount of the BAS 540 01 F blank SC formulation was added. The mixture was ground, homogenized and transferred, adjusted to a defined weight with water. Aliquots of the application solutions were diluted with acetonitrile:water (1:1). The radioactivity of the solutions was determined by liquid scintillation counting and the purity and isotope pattern was determined using HPLC/LC-MS. The purities after the preparation of the two application formulations were 86.7% and 89.3%, the specific activities were 197205 dpm/µg and 198841 dpm/µg, respectively. The radiochemical purities of the original test items were above 95%. Although the active concentration in the preparations was reduced, presumably due to the grinding process the purity was considered as sufficient. Additionally, co-chromatographies using HPLC were performed to demonstrate that the test item already contained the *Z*-isomer 505M98 due to the manufacturing process.
For the first and second application 100.18 g a.s./ha and 102.203 g a.s./ha of spray solution was applied to the rape plants using an automatic spray track system, respectively. Hulls, seeds and rest of plants samples were taken from mature rape plants at BBCH growth stage 89 (49 DALA).

2. Description of analytical procedures

Combustion: Homogenized solid plant samples were weighed, dried and combusted by means of an automatic sample oxidizer. The $^{14}CO_2$ was trapped by an absorption and scintillation liquid, and the collected radioactivity was measured by liquid scintillation counting. All measurements of radioactivity were corrected for oxidizer efficiency and LSC quench curves. ^{14}C standards were combusted to determine the recovered radioactivity and the measurements were corrected accordingly. In order to determine the background radioactivity, aliquots of untreated rest of plant samples and rape seed samples were combusted under the same conditions. The limits of quantitation in terms of mg/kg were calculated from the twofold background radioactivity levels (dpm per gram matrix) divided by the corresponding specific radioactivity.

Homogenization / solvent extraction: Samples were homogenized with dry ice and aliquots of homogenized samples were combusted. Aliquots of homogenized rape hull and rest of plant samples were extracted 3 times with methanol and 2 times with water. Homogenized seeds were extracted three times with cyclohexane and the extracts pooled. The residual sample was extracted four times with methanol, followed by two water extractions. The pooled samples for each extraction method were radioassayed by LSC for determination of extractable radioactive residues (ERR).

The residual samples after the respective solvent extractions were dried, homogenized and combusted for determination of residual radioactive residues (RRR). To elucidate the RRR further solubilization steps were performed.

Solubilization: The residue after solvent extraction was solubilized with ammonia and the residue after ammonia solubilization was successively incubated with macerozyme / cellulase, tyrosinase / laccase, amylase / amyloglucosidase, pepsin and pancreatin. All extracts were radioassayed by LSC.

Isolation of metabolites for HPLC-MS investigations:

Hulls: were extracted 3 times with methanol and partitioned 3 times with water and ethyl acetate. For the isolation of the metabolites for the HPLC-MS investigations, the ethyl acetate phase was purified by SPE and HPLC fractionation. The separated fractions were analysed by HPLC-MS. The water phase was concentrated and fractionated using HPLC.

Rest of plants: were extracted 3 times with methanol and 2 times with water. Samples for HPLC analysis were prepared from the pooled methanol and water extracts.

Seeds: To the oil phase of the cyclohexane extract (see solvent extraction for seeds above) iso-hexane was added. The iso-hexane phase was partitioned twice with a water:acetonitrile mixture (70:30). Subsequently, the iso-hexane phase was partitioned four times with a water:methanol mixture (1:1). The different aqueous and the iso-hexane phases were pooled, concentrated, brought to volume with acetonitrile:methanol and iso-hexane, respectively, and analysed by HPLC.

Identification of metabolites: The structure elucidation of metabolites was based on HPLC-MS investigations of extracts and isolated fractions. Peak assignment of the HPLC analyses was based on HPLC-MS and co-chromatography experiments.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

In the present study, the TRR was calculated by summarizing the ERR and the RRR after solvent extraction. Additionally, the TRR was measured by direct combustion followed by LSC analysis. The combusted TRR of all three matrices showed no major differences when compared to the calculated TRR values (see Table 6.2.1-10). The calculated TRR of rape seeds was 0.157 mg/kg. The TRR in rape hulls and rape rest of plants were significantly higher and accounted for 4.658 mg/kg and 2.373 mg/kg, respectively. The calculated TRR values were set to 100% TRR for all matrices.

Table 6.2.1-10: Total radioactive residues (TRRs) in oilseed rape matrices

Matrix[1]	TRR determined by direct combustion [mg/kg]	TRR calculated [mg/kg]*
	Phenyl label	
Seeds	0.127	0.157
Hulls	4.729	4.658
Rest of plant	2.158	2.373

1 Harvested at plant maturity (49 DALA)
* Sum of ERR (extraction with methanol, cyclohexane (seeds) and water) and RRR (extraction residue)

B. EXTRACTION, CHARACTERIZATION AND IDENTIFICATION OF RESIDUES

The extractability of the oilseed rape matrices with cyclohexane, methanol and water is summarized in Table 6.2.1-11.

Table 6.2.1-11: Extraction efficiency of radioactive residues in oilseed rape samples

Matrix[1]	TRR calc.*	Distribution of radioactive residues						ERR[2]		RRR[3]	
		Cyclohexane extract		Methanol extract		Water extract					
	[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Oilseed rape	Phenyl label										
Seeds	0.157	0.098	62.3	0.023	14.8	0.007	4.4	0.128	81.5	0.029	18.5
Hulls	4.658	N/A	N/A	3.421	73.4	0.560	12.0	3.981	85.5	0.677	14.5
Rest of plants	2.373	N/A	N/A	2.003	84.4	0.175	7.4	2.179	91.8	0.194	8.2

1 Harvested at plant maturity (49 DALA)
* TRR was calculated as the sum of ERR + RRR
2 ERR = extractable radioactive residue, calculated as the sum of cyclohexane, methanol and water extract
3 RRR = residual radioactive residue
N/A Not applicable

1. Extraction and characterization of residues in oilseed rape

The extractabilities of rape rest of plants and rape hulls with methanol and water were high and accounted for 91.8% and 85.5% of the TRR, respectively (see Table 6.2.1-11). For rape rest of plants, the major part of radioactivity was extracted with methanol (84.4% TRR), and smaller amounts were extracted with water (7.4% TRR). For rape hulls, comparable amounts were extracted with methanol (73.4% TRR) and water (12.0% TRR). The extractability of rape seeds with cyclohexane followed by methanol and water was high and accounted for 81.5% TRR. The major part was extracted with cyclohexane (62.3% TRR) and smaller amounts were extracted with methanol (14.8% TRR) and water (4.4% TRR).

Table 6.2.1-12: Summary of identified and characterized residues in matrices of oilseed rape

Matrix	Seed		Hulls		Rest of plant	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
BAS 505 F	0.084	53.8	3.483	74.8	1.990	83.9
505M98	0.002	1.1	0.129	2.8	0.050	2.1
505M107	ND	ND	0.035	0.8	0.006	0.3
Total identified	**0.086**	**54.9**	**3.647**	**78.3**	**2.046**	**86.2**
Total characterized	**0.052**	**33.0**	**0.639**	**13.7**	**0.235**	**9.9**
Total identified and characterized	**0.138**	**87.8**	**4.287**	**92.0**	**2.282**	**96.2**
Final residue	**0.012**	**7.8**	**0.260**	**5.6**	**0.068**	**2.8**
Grand total	**0.150**	**95.7**	**4.547**	**97.6**	**2.349**	**99.0**

ND Not detected

2. Identification and quantitation of extractable residues in oilseed rape

The parent compound BAS 505 F, its isomer 505M98 and the metabolite 505M107 were identified and quantified by analysis of the fractionated methanol extracts of rape hull with 62.9% of TRR, 2.8% of TRR and 0.8% of TRR, respectively. Similarly, compound BAS 505 F, its isomer 505M98 and the metabolite 505M107 were identified by analysis of the fractionated methanol extracts of rape rest of plant with 76.3% of TRR, 2.1% of TRR and 0.3% of TRR, respectively. The parent compound BAS 505 F and its isomer 505M98 were identified and quantified with comparable efficiency by analysis of the acetonitrile/methanol phase (24.8% and 1.1%) and iso-hexane extracts of rape seeds (20.2% and 0%), respectively.

In rape seed, 0.138 mg/kg or 87.8% TRR were identified and characterized. The parent compound BAS 505 F (0.084 mg/kg or 53.8% TRR) and its *Z*-isomer (0.002 mg/kg or 1.1% TRR) were identified only in the ERR and not in the RRR. Further 0.052 mg/kg or 33.0% TRR were characterized in the ERR and the RRR of rape seed.

In rape hull, 4.287 mg/kg or 92.0% TRR were identified and characterized. The parent compound was identified with 3.295 mg/kg or 70.7 %TRR in the ERR and with 0.189 mg/kg or 4.0 %TRR in the RRR. In total, BAS 505 F accounted for 3.483 mg/kg or 74.8% TRR.

The *Z*-isomer 505M98 was identified only in the ERR with 0.129 mg/kg or 2.8% TRR. In minor amounts (0.035 mg/kg or 0.8% TRR), the metabolite 505M107 was identified in the ERR. Further 0.470 mg/kg or 10.1% TRR were characterized in the ERR and 0.169 mg/kg or 3.6% TRR in the RRR. In total, 0.639 mg/kg or 13.7% TRR were characterized in the ERR and the RRR of rape hull.
In rape rest of plant, 2.282 mg/kg or 96.2 % TRR were identified and characterized. The parent compound accounted for 1.948 mg/kg or 82.1 % TRR in the ERR and 0.043 mg/kg or 1.8% TRR in the RRR. In total, BAS 505 F accounted for 1.990 mg/kg or 83.9% TRR.
The *Z*-Isomer 505M98 was identified only in the ERR with 0.050 mg/kg or 2.1% TRR. In minor amounts (0.006 mg/kg or 0.3% TRR), the metabolite 505M107 was identified in the ERR. Further 0.190 mg/kg or 8.0% TRR were characterized in the ERR and 0.045 mg/kg or 1.9% TRR in the RRR by HPLC. In total, 0.235 mg/kg or 9.9% TRR were characterized in the RRR.
In order to analyse if either BAS 505 F or its *Z*-isomer 505M98 were preferably metabolized in rape, the ratio between the parent compound and its *Z*-isomer were compared. The relative amounts of the isomers were approximately 30:1 in the application formulation as well as in rape seed, hull and rest of plant. The relative amounts ranged from 87.5 to 95.9% ROI for BAS 505 F and from 2.3 to 4.1 % ROI for its *Z*-isomer 505M98.

3. Metabolic pathway

The proposed metabolic pathway of BAS 505 F in rape is shown in Figure 6.2.1-1. The main residue in rape is dimoxystrobin; the compound is metabolized only to a small extent. Hydroxylation of the dimethylphenyl moiety of the parent compound (either at the methyl groups or at the phenyl group) results in a postulated intermediate metabolite, which is conjugated to glucose to form metabolite 505M107. No cleavage between both ring systems has been observed. The compound 505M98 represents the *Z*-isomer of the parent compound, which is present in the application formulation due to the manufacturing process. The E/*Z*-ratio remained constant over the course of the study.

5. Storage stability

Storage stability investigations (initial analysis, re-analysis of stored extract and re-extraction of stored samples and analysis) were performed exemplarily for all rape matrices. Generally, metabolite patterns observed were comparable before and after storage. Residues in extracts and samples were shown to be stable. The longest storage period between the initial analysis and the re-analysis of extracts was 579 days. Re-extraction of samples was carried out after 1064 days of storage of samples. The maximal difference between the two extractions was 768 days. In the iso-hexane phase of rape seed after re-extraction the partitioning of the re-extracted cyclohexane extract resulted in a different distribution of the parent compound between the phases. However, the sum of BAS 505 F over the different phases was comparable to the initial extraction.
Concerning the time period until initial analysis, a previously conducted rape metabolism study (see report 6.2.1/1) confirms that parent is the major compound detected, and indicates stability during storage until initial analysis. Additionally the storage stability of BAS 505 F in rape was investigated before in a variety of crops, including rape. The study indicated that BAS 505 F was stable in all matrices under investigation; in rape for over 24 months.

III. CONCLUSIONS

After foliar application of dimoxystrobin (BAS 505 F) in summer rape the highest amounts of total radioactive residues were detected in rape hulls (4.658 mg/kg), followed by rest of plant samples (2.373 mg/kg); and the lowest concentration was observed in seeds (0.157 mg/kg).

The soluble radioactive residues were most efficiently extracted with cyclohexane from seeds, (62.3% TRR) and with methanol from rape hulls and rest of plants (73.4% TRR and 84.4% TRR).

BAS 505 F was identified as the main compound of the radioactive residues in all three matrices with 53.8% TRR, 74.8% TRR and 83.9% TRR in rape seeds, hulls and rest of plants, respectively.

Dimoxystrobin translocation into seeds is low and the parent compound is metabolized only to a small extent. The main pathway is characterized by hydroxylation of the dimethylphenyl ether moiety of the parent compound (either at the methyl groups or at the phenyl group) resulting in a postulated intermediate metabolite, which is conjugated with glucose to form metabolite 505M107. This metabolite was identified in the methanol extracts of rape hulls and rest of plants only in minor amounts (0.3-0.8%TRR).

In agreement with the low metabolization rates enzymatic treatments simulating gastrointestinal conditions resulted in a very minor release of BAS 505 F and hence, no other degradation product would be expected to be released during digestion. In addition, residues in extracts and samples were stable for periods of 579-768 days in oilseed rape extracts and matrix. The presence of the *Z*-isomer (505M98) of dimoxystrobin in the applied formulation is due to the manufacturing process. The parent to 505M98 ratio remained similar (about 30:1) throughout analyses procedures in all rape matrices.

Overall conclusions of plant metabolism studies in oilseed rape

The two plant metabolism studies conducted with oilseed rape yielded comparable results; the extraction behaviour and metabolite patterns were comparable. Unchanged dimoxystrobin constituted the predominant residue in the metabolism studies, identified minor metabolites were in general formed by oxidation of the dimethylphenyl moiety. There was no indication of cleavage of the dimoxystrobin molecule and the isomer ratio of parent to its Z-isomer (505M98) remained constant throughout the new study.

Figure 6.2.1-1: Proposed metabolic pathway of dimoxystrobin (BAS 505 F) in oilseed rape

BAS 505 F
Reg.No. 285028

Hydroxylation

or isomer

or isomer

505M107

Report: CA 6.2.1/3
Funk D., 2015b
Investigation of the extractability of BAS 505 F and its Z-isomer M505F098 in samples from plant metabolism studies
2015/1020186

Guidelines: OECD 501, SANCO/825/00 rev. 7 (17 March 2004), SANCO/3029/99 rev. 4 (11 July 2000), OECD Guidance Document on Pesticide Residue Analytical Methods (ENV/JM/MONO(2007)17 - 13-Aug-07, EPA 860.1340: Residue Chemistry Test Guidelines - Residue Analytical Method

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

The information in this report fulfils the portion of the OECD 501, SANCO/825/00 rev. 7 17/03/04 and the OECD ENV/JM/MONO (2007) 17 guideline requirements that demand a check of analytical methods (for both enforcement and data collection) to efficiently extract the observed residues from the crop metabolism studies.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: ^{14}C-dimoxystrobin (phenyl-U-^{14}C label)
^{13}C-dimoxystrobin (acetamide-2-^{13}C)
^{12}C-dimoxystrobin (unlabeled)

Lot/Batch #: ^{14}C-dimoxystrobin: 597-1301
^{13}C-dimoxystrobin: 596-2010
^{12}C-dimoxystrobin: 00956-117

Purity: Radiochemical purity:
^{14}C-dimoxystrobin: 99.1%%
Chemical purity:
^{14}C-dimoxystrobin: 96.7%
^{13}C-dimoxystrobin: >97%
^{12}C-dimoxystrobin: 99.9%
Specific activity:
^{14}C-dimoxystrobin: 4.6 MBq/mg

CAS#: 149961-52-4

Development code: BAS 505 F

Spiking levels: Not applicable

2. Test Commodity:

Crop: Oilseed rape
Type: Oilseeds
Variety: not reported
Botanical name: *Brassica napus* L.
Crop parts(s) or processed Commodity: Rest of plants, seed
Sample size: 5 g of rape seeds, 10 g of rest of plants (method 1)
5 g of plant samples (method 2)
50 g of rape seeds, 25 g of rest of plants (method 3)
10 g of plant samples (method 4)

B. STUDY DESIGN AND METHODS

1. Test procedure

During this study, selected samples from a metabolism study in oilseed rape were extracted (BASF DocID 2015/1017698). The extraction efficiency (by LSC) and the extracted amount of dimoxystrobin (BAS 505 F) and its *Z*-isomer 505M98 were determined by radio-HPLC for four extraction protocols. The study allows two types of comparisons: 1) the overall extractability of the radiolabelled material and 2) the quantitation of the parent compound dimoxystrobin (BAS 505 F) and its *Z*-isomer 505M98 in the different extracts. The results of each extraction analysis were compared to the extraction performed in the metabolism study as a reference method. In the present study, the calculated TRR of the rape metabolism study was taken as reference.

2. Description of analytical procedures

Homogenization
The oilseed rape matrices were obtained from the metabolism study as homogenized material.

Extraction Methods

Extraction Method 1 (extraction procedure based on the QuEChERS multi-residue method)

Rape Seeds:
10 g water were added to 5 g of homogenized rape seeds followed by addition of 10 mL acetonitrile. The extraction was performed using a homogenizer for 15 min (10000 rpm, Polytron). Subsequently, the mixture was centrifuged for 10 min. The supernatants were adjusted to a defined volume and measured by LSC. After a concentration step, the samples were again measured by LSC and subjected to HPLC analysis.

Rest of plants:
50 g water were added to 10 g of homogenized plant material. Then, 50 mL acetonitrile were added. The extraction was performed using a homogenizer for 10 min (5000 rpm, Polytron). Subsequently, the mixture was centrifuged for 15 min. The supernatants were adjusted to a defined volume and measured by LSC. After a concentration step, the samples were again measured by LSC and subjected to HPLC analysis.

Extraction Method 2 (extraction procedure based on BASF methods Numbers L0076/01 and L0076/09)
5 g of the homogenized plant samples were extracted once with 100 mL of methanol / water / 2 N HCl in a ratio of 70/25/5 (v/v/v) using a homogenizer for 2 min (5000 rpm, Polytron). After centrifugation for approximately 15 min, the supernatant was measured by LSC. After a concentration step, the samples were again measured by LSC and subjected to HPLC analysis.

Extraction Method 3 (extraction procedure based on DFG S19 multi-residue method)
To an aliquot of the homogenized rape seeds (50 g) 95 g water (40°C) were added. The suspension was mixed and incubated for 20 min. For extractions of rest of plants, 10 g water were added to 25 g of the homogenized plant material.

After adding 200 mL acetone to the sample, the extraction was performed using a homogenizer for 4 min (10000 rpm, Polytron). The samples were subsequently centrifuged and measured by LSC. After concentration, the aliquot was measured again by LSC and subjected to HPLC analysis.

Extraction Method 4 (extraction procedure based on the multi-residue method SweEt)
To 10 g of homogenized plant material 3 g of $NaHCO_3/Na_2SO_4$ in a 1/3 (m/m) ratio were added. The initially intended amount of 20 mL ethyl acetate was increased to 100 mL, since the original amount was not sufficient for the extraction. After the addition of ethyl acetate, the sample was homogenized for 4 min (10000 rpm, Polytron). Samples were centrifuged for 15 min. The supernatants were adjusted to a defined volume and measured by LSC. After concentration, the aliquot was again measured by LSC and subjected to HPLC analysis.

LSC and HPLC Measurements
For the quantitation of radioactivity in liquid samples a liquid scintillation counter (LSC) was used (Tri-Carb 2910 TR, Perkin Elmer). Aliquots of liquid samples were mixed with a sufficient volume of a suitable scintillator prior to measurement. All data were corrected using appropriate quench curves and are expressed in decays per minute (dpm).
For the investigation of the extracted amount, HPLC method LC07 (with the solid scintillator cell YG 600 S5D) was used.

II. RESULTS AND DISCUSSION

The extractability of radioactive residues from oilseed rape using four different extraction protocols and HPLC results for extracts are summarized in Table 6.2.1-13.

Table 6.2.1-13: Extractability and HPLC results for extracts of plant matrices

Extraction method		ERR			BAS 505 F			505M98		
		[mg/kg]	[% TRR]	[% Met][1]	[mg/kg]	[% TRR]	[% Met][1]	[mg/kg]	[% TRR]	[% Met][1]
Rape Seeds										
Rape Metabolism Study (389879)		0.128	81.5	100	0.084	53.8	100	0.002	1.1	100
1	QuEChERS	0.040	25.4	31.2	0.040	25.8	48.2	n.d.	n.d.	n.d.
2	BASF Method L0076	0.098	62.3	76.4	0.099	63.4	118	n.d.	n.d.	n.d.
3	DFG S19	0.093	59.4	72.8	0.091	57.8	108	n.d.	n.d.	n.d.
4	SweEt Method	0.073	46.3	56.8	0.072	46.2	86.2	n.d.	n.d.	n.d.
Rape Rest of Plant										
Rape Metabolism Study (389879)		2.179	91.8	100	1.948	82.1	100	0.050	2.1	100
1	QuEChERS	1.505	63.4	69.1	1.263	53.2	64.9	0.047	2.0	93.8
2	BASF Method L0076	1.997	84.2	91.7	1.992	83.9	102	0.054	2.3	108
3	DFG S19	1.393	58.7	63.9	1.335	56.3	68.5	0.040	1.7	80.5
4	SweEt Method	1.365	57.5	62.7	1.291	54.4	66.3	0.045	1.9	89.9

n.d. Not detected
[1] Extraction efficiency as percentage of mg/kg values related to the extractions performed in the rape metabolism study

The results of each extraction analysis were compared to the extraction performed in the rape metabolism study as a reference method. For the matrix rape seeds, a sequential extraction with cyclohexane methanol and water was performed in the metabolism study. The matrix rape rest of plant was extracted with methanol and water.

For both matrices, the extractability with method 2 (based on BASF method L0076) was highest with 76.4% (seeds) and 91.7% (rest of plant) of the ERR obtained in the metabolism study. Methods 3 (according to DFG S19 method) and 4 (according to SweEt method) showed slightly lower extractabilities with 72.8% (seeds, method 3), 56.8% (seeds, method 4), 63.9% (rest of plant, method 3) and 62.7% (rest of plant, method 4) of the ERR obtained in the metabolism study, respectively. With method 1 (adapted from QuEChERS multi-residue method, BASF method L0248/01) only small amounts of extractable radioactive residues were extracted from seeds, accounting for 31.2% of the ERR obtained in the metabolism study. Higher amounts of radioactive residues were extracted from rest of plant with method 1, amounting to 69.1% compared with the metabolism study extractions.

In the extracts obtained from rape seeds by using the four extraction methods, only dimoxystrobin (BAS 505 F) was identified. 505M98 was not detected in seeds due to the overall low abundance of the isomer in this matrix (0.002 mg/kg in the seeds extract in the metabolism study). The extraction methods 2 and 3 yielded the highest amounts of BAS 505 F of 0.099 mg/kg (63.4% TRR) and 0.091 mg/kg (57.8% TRR). In the extracts obtained by extraction methods 1 and 4, less BAS 505 F was extracted. Here, BAS 505 F accounted for 0.040 and 0.072 mg/kg (25.8 and 46.2% TRR).

In comparison to the metabolism study extractions, the methods 1 to 4 yielded 48.2-118% BAS 505 F.

Similar to the results in rape seeds, extraction method 2 yielded the highest extracted amount of BAS 505 F from rape rest of plant (1.992 mg/kg or 83.9% TRR). The *Z*-isomer 505M98 was identified with 0.054 mg/kg (2.3% TRR). In the extracts that were obtained by extraction methods 1, 3 and 4, the amount of the parent compound BAS 505 F ranged from 1.263 mg/kg to 1.335 mg/kg (53.2-56.3% TRR). Additionally, in all three extracts 505M98 was quantified with 0.040-0.047 mg/kg (1.7-2.0% TRR).

In comparison to the metabolism study extractions, the methods 1 to 4 yielded 64.9-102% BAS 505 F and 80.5-108% 505M98.

III. CONCLUSION

Overall, the extractability of both plant matrices was highest with extraction method 2 (based on BASF Method L0076). The extractability of radioactive residues from rape seeds and rape rest of plant was in the range of 76.4-91.7% compared to the extractions in the rape metabolism study. Extraction method 3 (adapted from DFG S19 multi-residue method) and 4 (according to SweEt multi-residue method) demonstrated 63.9-72.8% and 56.8-62.7% extraction efficiency compared with the rape metabolism study. Extraction procedure 1 (based on QuEChERS multi-residue method, BASF Method L0248/01) yielded more variability with a range of 31.2-69.1% of the metabolism study extractions.

The extractable radioactive residue (ERR) in the metabolism study is comparable to the ERR obtained with extraction method 2, while extraction methods 3 and 4 yielded slightly lower extraction efficiencies.

HPLC analyses were performed to quantify the amount of dimoxystrobin (BAS 505 F) and 505M98 in the extracts and to determine their relative amounts compared to the reference metabolism study.
In rape seeds, only the parent compound BAS 505 F was identified. 505M98 accounts only for very low amounts in this matrix (0.002 mg/kg in the metabolism study), hence, the focus to investigate the extractability of this compound should be on the rest of plants.

Similar to the extractability of radioactive residues, the highest amounts of BAS 505 F in seeds were identified in the extracts obtained by extraction method 2 (118% of the amount in the metabolism study). In the extracts of rape seeds obtained by extraction methods 3 and 4, slightly lower amounts of BAS 505 F were extracted (86.2-108% of the amount in the metabolism study). Extraction method 1 yielded relatively low amounts of BAS 505 F (48.2% of the amount in the metabolism study).

For the matrix rest of plant, extraction method 2 yielded the highest amounts of BAS 505 F and the *Z*-isomer 505M98 (102% and 108% of the amount in the metabolism study, respectively). Extracts obtained by extraction methods 1, 3 and 4 were comparable regarding their extraction efficiencies. For the parent compound BAS 505 F, 64.9-68.5% of the amounts in the metabolism study were extracted, while the extraction efficiency for the *Z*-isomer ranged from 80.5% to 93.8% of the metabolism study extractions.

For the parent compound BAS 505 F, method 2 yielded the highest extraction efficiencies in both matrices. Extracts obtained by methods 3 and 4 demonstrated slightly lower efficiencies, while extracts obtained by method 1 showed lower amounts of BAS 505 F in the matrix rape seeds.

The *Z*-isomer 505M98 was not detected in the extracts of rape seeds due to the overall relatively low abundance of 505M98 in this matrix. In the extracts of rest of plant, the extraction efficiency was highest for method 2, while methods 1, 3 and 4 yielded slightly lower extraction efficiencies.

CA 6.2.2 Poultry

Two studies in laying hens using [^{14}C]-benzyl or [^{14}C]-phenyl dimoxystrobin had been conducted and the summaries were submitted for the EU review of dimoxystrobin. The data have been found to be adequate and no further metabolism studies in poultry are required.

For the sake of completeness the results of these studies are summarized briefly in the following.

After 7 consecutive daily oral administrations of ^{14}C-BAS 505 F there was a rapid absorption and almost complete excretion of radioactivity. There was no indication of accumulation of ^{14}C-BAS 505 F in hen tissues and eggs (see Table 6.2.2-1 and Table 6.2.2-2). Because of the very low concentration of radioactivity in muscle tissue no further investigations were performed. Eggs and tissues of hens that had received doses of 12.7 mg/kg feed of [benzyl-^{14}C] BAS 505 F and 13.3 mg/kg feed of [phenyl-^{14}C] BAS 505 F (which has to be considered a very exaggerated dose level compared to expected residue levels in ruminant feed under normal agricultural practice) contained residues at low levels consisting of four identifiable metabolites. The parent compound was found in eggs, fat, and liver, but, except for fat, in very low proportions. It can be excluded that any of the identified components can be found in quantifiable amounts when hens are fed with feed containing residues from normal agricultural practice. Therefore a residue transfer study in poultry is not necessary. For enforcement purposes, a method is considered appropriate, which analyses for metabolite 505M09.

Table 6.2.2-1: Summary of metabolite identities and quantities in edible matrices of laying hens after dosing with 14C-BAS 505 F, benzyl label

Metabolite Code	Structure	Eggs [mg/kg] (%TRR)	Fat [mg/kg] (%TRR)	Liver* [mg/kg] (%TRR)
BAS 505 F		0.001 (2.7)	0.002 (15.1)	0.003 (0.9)
505M01		- -	- -	0.030 (8.3)
505M02		- -	0.001 (13.2)	0.028 (7.8)
505M09 (BF 505-8)		- -	0.001 (7.6)	0.008 (2.3)
505M78		- -	- -	0.083 (23.4)

* includes pronase digest

Table 6.2.2-2: Summary of metabolite identities and quantities in edible matrices of laying hens after dosing with 14C-BAS 505 F, phenyl label

Metabolite Code	Structure	Eggs [mg/kg] (%TRR)	Fat [mg/kg] (%TRR)	Liver* [mg/kg] (%TRR)
BAS 505 F		0.001 (4.1)	0.001 (7.2)	0.002 (0.6)
505M02		- -	0.006 (33.2)	0.033 (11.1)
505M09 (BF 505-8)		- -	0.001 (5.4)	0.005 (1.5)
505M78		- -	- -	0.054 (17.8)

* includes pronase digest

The proposed metabolic pathway for BAS 505 F in laying hen is given in Figure 6.2.2-1. Four main routes of biotransformation were observed. First, the predominant transformation was the hydroxylation of the phenyl ring in para position in relation to the connecting ether bridge (505M02). Second, the hydroxylated compound was conjugated with glucuronic acid to form 505M78. Third, oxidation reactions of the methyl groups at the phenyl ring took place to form the carboxylic acid 505M09 as well as the bis-hydroxylic compounds 505M47 and 505M48 (starting from 505M02) and 505M82, that can be defined either as the glucuronide of 505M48 or the alcohol of 505M78. Another oxidation product is metabolite 505M76 that can be formed from the carboxylic acid 505M09 by hydroxylation of the phenyl ring as well as from the hydroxylated compound 505M02 by oxidation of the related methyl group to a carboxylic acid. Finally, the parent compound was cleaved at the connecting ether bridge, eliminating the substituted phenyl ring to form a benzyl alcohol 505M01 of the entirely unchanged benzyl part of the molecule.

Figure 6.2.2-1: Metabolic pathway of ^{14}C BAS 505 F in laying hens

BAS 505 F

505M01

505M47

505M09

505M02

505M78

505M76

505M48

505M82

CA 6.2.3 Lactating ruminants

The nature of dimoxystrobin residues in commodities of animal origin was investigated in the context of the previous submission of dimoxystrobin for EU review. Reported metabolism studies include two studies in lactating goats using [^{14}C]-phenyl labeled dimoxystrobin; one nature of the residue study and one study on the extractability of the analytical methods used. The studies were found adequate, however, the goat metabolism study was conducted with only a ^{14}C-label in the phenyl moiety. As metabolism studies in hens and rats indicate cleavage of the molecule, an additional metabolism study in goat was conducted with a ^{14}C-label in the benzyl moiety (see report 6.2.3/1) to provide a comprehensive view on the metabolism in ruminants. Furthermore, the extraction efficiency of representative analytical methods used for animal matrices was investigated during this study.

For the sake of completeness a brief summary of the former metabolism study is given here. After 5 consecutive daily oral administrations ^{14}C-BAS 505 (phenyl label) was rapidly absorbed and almost completely excreted. There was no indication of accumulation of ^{14}C-BAS 505 F in goat tissues and milk. Milk and tissues of goats that had received a dose of 12.6 mg/kg feed (which has to be considered an exaggerated dose level compared to expected residue levels in ruminant feed under normal agricultural practice) contained residues of 8 identified metabolites at levels of 0.05 mg/kg or less. The parent compound was detected in milk, liver and kidney at levels of 0.02 mg/kg or less. Carbon atoms on the phenyl ring and the methyl side chains of the phenyl ring were oxidised to form hydroxy groups (505M04 and intermediates). Further oxidation of the benzyl groups formed the corresponding carboxylic acids (505M09, 505M76) which are the major constituents in all matrices. Conjugation of the OH-groups with glucuronic acid (505M86, 505M49, 505M78, and 505M79) resulted in the formation of numerous polar metabolites. From these findings it is rather unlikely that the parent compound or any of the identified metabolites would reach quantifiable concentrations in milk or tissues from ruminants under normal agricultural practice. However, for the analysis of samples generated in the course of a cow feeding study which was performed to confirm this extrapolation, unchanged BAS 505 F and the metabolites 505M09 (BF 505-8) and 505M76 (BF 505-11) were considered suitable marker analytes.

Table 6.2.3-1: Summary of identified metabolites and quantities in edible matrices of lactating goats after dosing with ^{14}C-BAS 505 F

Metabolite Code	Structure	Liver* mg/kg (% TRR)	Kidney* mg/kg (% TRR)	Milk mg/kg (% TRR)
BAS 505 F		0.018 (7.5)	0.001 (1.7)	<0.001 (9.2)
505M09 (BF 505-8)		0.052 (21.8)	0.013 (17.1)	<0.001 (14.4)
505M76 (BF 505-11)		0.046 (19.2)	0.005 (7.5)	<0.001 (10.3)
505M49		0.008 (3.8)	0.009 (13.6)	----- -----
505M78		0.029 (12.1)	0.008 (12.0)	----- -----
505M79		0.024 (10.1)	0.017 (22.8)	----- -----
505M84		0.008 (3.4)	0.005 (6.9)	----- -----
505M86		----- -----	----- -----	<0.001 (4.9)

Table 6.2.3-1: Summary of identified metabolites and quantities in edible matrices of lactating goats after dosing with ^{14}C-BAS 505 F

Metabolite Code	Structure	Liver*	Kidney*	Milk
		mg/kg (% TRR)	mg/kg (% TRR)	mg/kg (% TRR)
505M04		----- -----	----- -----	<0.001 (3.9)

* includes protease digest

Report:	CA 6.2.3/1 [redacted] 2015a The metabolism of 14C-Reg. No. 285028 (BAS 505 F) in the lactating goat 2015/1001730
Guidelines:	EPA 860.1300: Nature of the Residue in Plants Livestock, EEC 91/414 (7030(VI/95 Rev. 3), OECD Test Guideline 503 - Metabolism in livestock
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	CA 6.2.3/2 [redacted] 2015b Report amendment 1 - The metabolism of 14C-Reg. No. 285028 (BAS 505 F) in the lactating goat 2015/1125782
Guidelines:	EPA 860.1300: Nature of the Residue in Plants Livestock, EEC 91/414 (7030(VI/95 Rev. 3), OECD Test Guideline 503 - Metabolism in livestock
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Description:	^{14}C-dimoxystrobin (benzyl-U-^{14}C-label) ^{13}C-dimoxystrobin (acetamide-2-^{13}C-label) ^{12}C-dimoxystrobin (unlabeled)
Lot/Batch #:	^{14}C-dimoxystrobin: 596-3013 ^{13}C-dimoxystrobin: 596-2010 ^{12}C-dimoxystrobin: 00956-117
Purity:	Radiochemical purity: ^{14}C-dimoxystrobin: 98.2% Chemical purity: ^{14}C-dimoxystrobin: 96.1% ^{13}C-dimoxystrobin: > 97% ^{12}C-dimoxystrobin: 99.9% Specific activity: ^{14}C-dimoxystrobin: 7.6 MBq/mg
CAS#:	149961-52-4
Development code:	Not reported
Stability of test compound:	The test item was stable for the test period

2. Test Animals

Species:	Goat
Variety:	"Saanen cross Toggenburg"
Gender:	Female
Age:	Approximately 2 years
Weight at dosing:	63 kg
Number of animals:	1
Acclimation period:	14 days
Diet:	2 x 0.5 kg non-medicated commercially available concentrate (Dodson and Horrell Goat Mix supplied by Dodson and Horrell Limited, Kettering Road) + hay *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Metabolic cage
Environmental conditions	
Temperature:	15-18°C
Humidity:	21-47%
Photoperiod:	16 h light / 8 h dark

B. STUDY DESIGN AND METHODS

1. Dosing regime

Oral:	**Amount of dose:**	11 mg/kg feed dry matter/day (nominal), 11.8 mg/kg feed dry matter/day (mean actual)
	Food consumption:	1.0 kg concentrate fresh matter/animal/day 0.86 kg concentrate dry matter/animal/day
	Vehicle:	Gelatin capsule by dosing gun
	Timing:	Once daily
	Duration:	7 consecutive days

2. Sample collection

Milk collection:	Twice daily
Urine and feces collection:	Daily
Interval from last dose to sacrifice:	6 h
Tissues harvested & analyzed:	Blood, urine, feces, milk, bile, liver, kidney, muscle, fat, GI tract

3. Test system

The metabolism and distribution of dimoxystrobin was investigated in one lactating goat following a repeated oral administration of benzyl-U-^{14}C-BAS 505 F at a mean dose level of 11.8 mg/kg feed for seven consecutive days. The test item was prepared in gelatin capsules and administered orally by dosing gun. Details of the study outline are summarized in Table 6.2.3-2.

Table 6.2.3-2: Dosing of lactating goat with ^{14}C-dimoxystrobin

Animal	Treatment days	Nominal daily dose	Actual daily dose		Sacrifice time after last dose (hours)
		mg/kg feed intake	mg/kg feed intake	mg/kg bw**	
Benzyl-U-^{14}C label					
1	7	11	9.1-16.3* (mean 11.8)	0.15-0.27* (mean 0.19)	6

* Since the goat was sacrificed 6 h after the seventh dose administration (not a full day) day seven was not taken into account
** Body weight mean calculated based on weight on 1 day before dosing and day 7 of dosing = 60.75 kg

4. Sampling and storage

Blood samples were taken prior and at 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post first dose. Urine and faeces samples were collected for the 24 h period prior to first dose and for each 24 h period until sacrifice. The goat was milked twice daily and immediately prior to sacrifice. At approximately 6 h post final dose the goat was humanely killed and edible tissues (liver, kidney, muscle and fat), bile, blood and the GI tract were removed *post mortem*. All samples were stored at ca. –20°C.

5. Description of analytical methods

The radioactivity of homogenized extracts of the liquid samples (milk, bile, urine, blood) was determined by LSC (liquid scintillation counting) analysis. Solid debris (faeces, tissues) were analysed for radioactivity content by combustion analysis followed by LSC.
Faeces, liver and kidney were generally homogenized and extracted using 3 extraction solutions with different water:methanol ratios. Further investigation of non-solvent extractable residues in liver and kidney debris (RRR) was conducted using protease enzyme. In addition larger subsamples of liver and kidney were extracted to aid metabolite elucidation and these extracts were also used for the enzyme deconjugation experiments and chiral analysis of BAS 505 F. Extracts containing significant amounts of radioactivity were proportionately combined and concentrated. Additionally, subsamples of liver and kidney were extracted using 2 different extraction methods [BASF method D0006 (methanol based) and BASF method L0232/01 (acetone and ethyl acetate:cyclohexane based] in order to measure the components of the residue definition (BAS 505 F and 505M09).

Samples were analysed using two HPLC methods; on-line radiodetection for quantification and fraction collection and TopCount analysis to confirm the assignment and quantification. A mix of the reference standards was prepared and each sample/extract analysed by HPLC was fortified with the standard mix. The identity of radiolabeled components was then based on co-chromatography with the authentic reference items. Two HPLC methods were used to quantify individual residues and to confirm the assignment and quantification. To identify components which did not correspond to a reference standard and to confirm the assignments made using co-chromatography HPLC-MS/MS was used.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The total radioactive residues (TRR) in all matrices are summarized in Table 6.2.3-3. Rapid excretion was observed. Until sacrifice, the radioactive residues excreted via urine and feces amounted to 17.6% and 62.6% of the total radioactivity administered, respectively. Of approximately 94% of the total dose that was recovered 11.3% was found in the GI tract and contents (bile). Radioactivity associated with edible portions (milk and tissues) accounted for <1% of the administered dose. Radioactive residues in milk were low and accounted for a plateau concentration of approximately 0.002 mg equiv/kg within 2 days for the test goat. The radioactive residues in muscle and fat were low and ranged from 0.005-0.009 mg/kg and from 0.005-0.006 mg/kg, respectively. The residues in the liver were 0.365 mg/kg and in kidney 0.110 mg/kg.

Table 6.2.3-3: Total radioactive residues in edible matrices and excreta after dosing of lactating goat with benzyl-U-^{14}C-BAS 505 F

Matrix	TRR [mg/kg]	
	measured	calculated
Benzyl-U-^{14}C label		
Milk[1]	0.002	-
Bile	9.180	-
Liver	0.365	0.385
Kidney	0.110	0.113
Fat (omental/renal/subcutaneous)	0.005/0.006/0.005	-
Muscle (loin/flank)	0.005/0.009	-

TRR: Total radioactive residue (sum of ERR + RRR)
1 Mean of six pooled milk samples (PM and AM milk collection)
2 Pool samples day 4-6

B. EXTRACTION OF RESIDUES

The extractability of the edible tissues was high, ranging from 78.0% (liver) to 85.0% (kidney) of the TRR (see Table 6.2.3-4). The residue after solvent extraction of liver and kidney was 21.9% TRR and 15.1% TRR, respectively and was further investigated with protease enzyme. An additional 23% and 16.1% of the TRR was released for liver and kidney, respectively.

Table 6.2.3-4: Residues after [^{14}C]-BAS 505 F treatment in goat matrices extracted with methanol and methanol/water (4:1 and 3:7, v/v)

Matrix	TRR		ERR[1]		RRR[2]		Total[3]
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	%
Liver	0.385	100.0	0.300	78.0	0.084	21.9	99.9
Kidney	0.113	100.0	0.096	85.0	0.017	15.1	100.1

[1] ERR = Extractable radioactive residue
[2] RRR = Residual radioactive residues
[3] Sum of ERR + RRR

C. IDENTIFICATION AND CHARACTERIZATION OF RESIDUES

Summaries of identified and characterized residues are shown in Table 6.2.3-5 and Table 6.2.3-6. A summary of all identified metabolites and their distribution in excreta, bile, milk, muscle, fat, liver, and kidney is given in Table 6.2.3-7. Identification was accomplished by HPLC co-chromatography with reference standards. To confirm the thus identified metabolic pattern and to identify additional components HPLC-MS/MS was utilized.

For quantification of labeled components, liver concentrated extract was analyzed directly by HPLC pre- and post treatment with glucuronidase enzyme (see Table 6.2.3-5 and Table 6.2.3-7). Analysis of pre-enzyme hydrolysis liver extract showed 17 peaks; two of which were identified as unchanged BAS 505 F (0.014 mg/kg; 3.8% TRR) and 505M09 (0.051 mg/kg; 13.4 % TRR). Of the other peaks five were identified as glucuronide conjugates and two of them were identified as isomers of 505M50 and individually accounted for 0.037 mg/kg (9.7% TRR) and 0.022 mg/kg (5.6% TRR). Three other conjugates were assigned as 505M105 (0.033 mg/kg; 8.6% TRR), 505M51 (0.022 mg/kg; 5.8% TRR) and 505M81 (0.044 mg/kg; 11.5% TRR). The other peaks were not identified and individually accounted for less than 0.006 mg/kg (1.6% TRR). Deconjugation experiments with glucuronidase enzyme confirmed the formation of aglycons form the glycon identified glucuronide conjugates

For quantification of labeled components, kidney concentrated extract was analyzed directly by HPLC pre- and post treatment with glucuronidase enzyme (see Table 6.2.3-5 and Table 6.2.3-7), without further purification. Analysis of pre-enzyme hydrolysis kidney extract showed 19 peaks; two of which were identified as unchanged BAS 505 F (0.005 mg/kg; 4.6% TRR) and 505M09 (0.009 mg/kg; 7.5% TRR). Of the other peaks four were identified as glucuronide conjugates and two of them were assigned as isomers 505M50 and individually accounted for 0.009 mg/kg (7.8% TRR) and 0.008 mg/kg (6.8%TRR). Two other conjugates were assigned as 505M51 (0.014 mg/kg; 12.3% TRR) and 505M81 (0.022 mg/kg; 19.5% TRR). The other peaks were not identified and individually accounted for less than 0.003 mg/kg (2.7% TRR).

Deconjugation experiments with liver and kindney samples with glucuronidase enzyme confirmed the formation of aglycons from the identified glucuronide conjugates.

Table 6.2.3-5: Summary of identified and characterized residues in liver of lactating goat dosed with [benzyl-U-^{14}C]-BAS 505 F

Components	Liver	
	[mg/kg]	[% TRR]
BAS 505 F (Reg No.285028) [1]	0.014	3.8
505M105 (M505F105)[2]	0.033	8.6
505M50 (M505F050)[2,3]	0.037	9.7
505M51 (M505F051)[2]	0.022	5.8
505M81 (M505F081)[2]	0.044	11.5
505M50 (M505F050)[2,3]	0.022	5.6
505M09 (Reg. No. 354563)[1]	0.051	13.4
Total identified in extractable radioactivity (ERR)	**0.223**	**58.4**
Total characterized in extractable radioactivity (ERR)	**0.033**	**8.6**
Total identified/characterized radioactivity (RRR[4])	**0.089**	**23.0**
Sum identified/characterized (from ERR+RRR)	**0.345**	**90.0**
Final Residue	**0.008**	**2.2**
Grand Total	**0.353**	**92.2**

1 Identified metabolites were resolved and quantified by co-chromatography using HPLC. Qualitative confirmation was achieved by co-chromatography using HPLC-MS/MS
2 Identified metabolites were resolved and quantified using HPLC and identification was performed by mass spectral analysis
3 Two isomers of 505M50 have been detected
4 Further investigated using protease enzyme

Table 6.2.3-6: Summary of identified and characterized residues in kidney of lactating goat dosed with [benzyl-U-^{14}C]-BAS 505 F

Components	Kidney	
	[mg/kg]	[% TRR]
BAS 505 F (Reg No.285028)[1]	0.005	4.6
505M50 (M505F050)[2,3]	0.009	7.8
505M51 (M505F051)[2]	0.014	12.3
505M81 (M505F081)[2]	0.022	19.5
505M50 (M505F050)[2,3]	0.008	6.8
505M09 (M505F009, Reg No.354563)[1]	0.009	7.5
Total identified in extractable radioactivity (ERR)	**0.067**	**58.5**
Total characterized in extractable radioactivity (ERR)	**0.020**	**17.4**
Total identified/characterized radioactivity (RRR[4])	**0.018**	**16.1**
Sum identified/characterized (from ERR+RRR)	**0.105**	**92.0**
Final Residue	**0.001**	**1.1**
Grand Total	**0.106**	**93.1**

1 Identified metabolites were resolved and quantified by co-chromatography using HPLC. Qualitative confirmation was achieved by co-chromatography using HPLC-MS/MS

2 Identified metabolites were resolved and quantified using HPLC and identification was performed by mass spectral analysis

3 Two isomers of 505M50 have been detected

4 Further investigated using protease enzyme

Table 6.2.3-7: Summary of identified residues in goat after dosing with [benzyl-U-^{14}C]-BAS 505 F

Metabolite	Urine		Faeces		Bile		Liver		Kidney	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
BAS 505 F (Reg No 285028)	ND	ND	3.181	62.5	ND	ND	0.014	3.8	0.005	4.6
505M105 (M505F105)	ND	ND	ND	ND	ND	ND	0.033	8.6	ND	ND
505M109 (M505F109)	0.246	7.5	ND	ND	ND	ND	ND	ND	ND	ND
505M50[1] (M505F050)	0.823	24.9	ND	ND	2.087	22.7	0.037	9.7	0.009	7.8
505M51 (M505F051)	0.698	21.2	ND	ND	1.050	11.4	0.022	5.8	0.014	12.3
505M81 (M505F081)	ND	ND	ND	ND	3.858	42.0	0.044	11.5	0.022	19.5
505M50[1] (M505F050)	0.234	7.1	ND	ND	1.272	13.9	0.022	5.6	0.008	6.8
505M02 (Reg. No 356310)	ND	ND	0.509	10.0	ND	ND	ND	ND	ND	ND
505M04 (Reg. No 035807)	ND	ND	0.259	5.1	ND	ND	ND	ND	ND	ND
505M09 (Reg. No 354563)	0.882	26.7	1.586	31.2	0.329	3.6	0.051	13.4	0.009	7.5

[1] Two isomers of 505M50 have been detected
ND Not detected

Comparability of residue extraction methods used for liver and kidney samples

Table 6.2.3-8: Comparison of residues in liver and kidney (BAS 505 F and 505M09) following extraction using three different extraction methods

Extraction	ERR			BAS 505 F			505M09		
	[mg/kg]	[% TRR]	[% Met][1]	[mg/kg]	[% TRR]	[% Met][1]	[mg/kg]	[% TRR]	[% Met][1]
Liver									
Residue Method D0006 (Methanol)	0.243	64.7	88.1	0.017	4.5	118.4	0.076	20.1	150.0
Residue Method L0232/01 (Acetone and ethyl acetate:cyclohexane)	0.146	39.9	54.4	0.005	1.3	34.2	0.058	15.8	117.9
Goat Metabolism Study (Methanol and Water)	0.283	73.4	N/A	0.014	3.8	N/A	0.051	13.4	N/A
Kidney									
Residue Method D0006 (Methanol)	0.087	78.0	96.2	0.004	4.0	89.0	0.009	8.5	88.2
Residue Method L0232/01 (Acetone and ethyl acetate:cyclohexane)	0.013	11.6	14.3	0.005	4.4	95.7	0.009	8.5	88.2
Goat Metabolism Study (Methanol and Water)	0.092	81.1	N/A	0.005	4.6	N/A	0.009	7.5	N/A

ERR Extractable radioactive residue (solvents: acetonitrile, isohexane, water)
[1] % Met = Extraction efficiency compared to the extraction method used in the metabolism study
N/A not applicable

Subsamples of liver and kidney were extracted by residue methods BASF Method D0006 and BASF Method L0232/01 in order to measure the components of the residue definition (BAS 505 F and 505M09) and to identify these components by HPLC using co-chromatography with authentic reference standards. A comparison summary table detailing the residues observed between the profiling and two residue extraction methods is presented in Table 6.2.3-8. The levels of 505M09 and BAS 505 F observed in liver and kidney extracts generated by the D0006 and L0232/01 residue methods are generally comparable with the levels following analysis of the extracts obtained by the metabolite profiling extraction method (used in the already evaluated goat metabolism study with phenyl label). Slightly less 505M09 (0.051 mg/kg; 13.4% TRR) and BAS 505 F (0.014 mg/kg; 3.8% TRR) was observed in the liver extract following the metabolite profiling extraction method when compared to the D0006 and L0232/01 residue methods, respectively.

2. Storage stability

Initial analyses of the tissue and excreta extracts were carried out within 6 months of sacrifice and storage at -20°C for liver, kidney, urine, faeces and bile. Liver and kidney samples were extracted and profiled by HPLC-MS/MS twelve months after the original extraction and analysis was carried out. The original concentrated and reconstituted extracts from liver and kidney were analysed fifteen months after their initial analysis. The profiles were comparable showing stability over the course of the study in both the tissues and extracts.

3. Metabolic pathway

The proposed metabolic pathway of BAS 505 F (Reg. No. 285028) in the lactating goat is provided in Figure 6.2.3-1. The unchanged parent BAS 505 F (Reg. No. 285028) was extensively metabolized in the lactating goat. Metabolic transformations occurred mainly *via* two routes i) hydroxylation followed by glucuronide conjugation and ii) carboxylation followed by glucuronide conjugation. Main components of the former category in extracts of liver and kidney were 505M50 (as two isomers) and 505M51. The main component of the latter category in extracts of liver and kidney was 505M81, a glucuronide conjugate of 505M09. A glucuronide conjugate of the hydroxy carboxylic acid of BAS 505 F was observed in liver and designated as 505M105. 505M01, a cleavage product also observed in rat and laying hens was identified indirectly in the new goat study. The aglycon 505M01 was detected upon glucuronidase treatment of urine, bile and liver.

4. Stereoisomer analysis

The E/Z ratio of BAS 505 F was investigated in the dose solution and in extracts of liver and kidney. The ratio of the E/Z isomers of BAS 505 F was *ca* 9:1 in the dose solution and in the liver extract. Due to the low overall amount of radioactivity in the sample only the E-isomer (BAS 505 F) was detected in the extract of kidney extract.

Conclusion

Parent BAS 505 F (Reg. No. 285028) was extensively metabolized in the lactating goat. Among all the metabolites identified from the tissues and urine, all structural modifications occurred at the phenyl ring and the basic structural backbone of the parent compound remained intact. To a small extent the parent compound was cleaved at the connecting ether bridge eliminating the substituted phenyl ring to form a benzyl alcohol 505M01 of the entirely unchanged benzyl part of the molecule. This could only be shown indirectly by treating urine, bile and liver with glucuronidase enzyme and is in alignment with the results of rat and hen metabolism.

Metabolic transformations occurred mainly *via* two routes i) hydroxylation followed by glucuronide conjugation and ii) carboxylation followed by glucuronide conjugation. Main components of the former category in extracts of liver and kidney were two isomers of 505M50 and 505M51. The main component of category ii) in extracts of liver and kidney of the study was 505M81, a glucuronide conjugate of 505M09. A glucuronide conjugate of the hydroxy carboxylic acid of BAS 505 F was observed in liver and designated as 505M105. Deconjugation experiments with glucuronidase enzyme confirmed the formation of aglycons from the identified glucuronide conjugates. By treatment with glucoronidase 505M01, a cleavage product observed in rat and laying hens was also identified indirectly.

Overall conclusion on goat metabolism studies

BAS 505 F is extensively metabolized in the lactating goat. In terms of encountered metabolization pathways the two goat studies are largely comparable. The following general conclusions can be drawn:

- In all identified metabolites, structural modification occurred at the phenyl ring while the basic structural backbone of the parent compound remained unchanged.
- 505M01, a cleavage product observed in rat and laying hens was also identified indirectly in the new goat study. The aglycon 505M01 was detected upon glucuronidase enzyme treatment of goat urine, bile and kidney. This underlines the similarity of dimoxystrobin metabolism in different animal species.
- As observed in the older goat study metabolic transformations occurred mainly *via* two routes i) hydroxylation followed by glucuronide conjugation and ii) carboxylation followed by glucuronide conjugation. Main components of category i) were 505M50 and 505M51 (new study) and 505M49, 505M78 and 505M79 (old study). The main components of category ii) were 505M09 and its glucuronide conjugate 505M81 (new study) and 505M09 and 505M76 in the old study.
- The observed metabolic pathways are similar to the ones observed in rat and laying hens. Main routes are based on hydroxylation and carboxylation of the aromatic ring system, cleavage of the two ring system was observed to a lesser extent.
- The ratio of the E and Z stereoisomers of BAS 505 F was investigated in the new study in the dose solution and extracts of liver and kidney. The ratio of the E/Z-isomers of BAS 505 F was approximately 9:1 in the dose solution and in the liver extract. Due to the low overall amount of radioactivity only the E-isomer (BAS 505 F) was detected in the kidney extract. The isomer ratio of parent to its Z-isomer (505M98) remained constant throughout the study and no change of the ratio is to be expected.

Summarizing the above, it can be noted that the metabolic processing in the two lactating goat studies aligns very well and provides a comprehensive picture of dimoxystrobin metabolism in lactating ruminants. Additionally, it can be concluded that the metabolic pathways in the different animal species (rat, hen, goat) are quite similar. Main routes are based on hydroxylation and carboxylation of the aromatic ring system, cleavage of the two ring system was observed to a lesser extent.

Figure 6.2.3-1: Proposed Biotransformation Pathway for [^{14}C]-BAS 505 F (Reg. No. 285028) in the Lactating Goat

CA 6.2.4 Pigs

No separate metabolism study for pigs is required since the metabolic pathways in rodents (rats) and ruminants (goats) are comparable. Metabolism data for pigs can be extrapolated from ruminants.

CA 6.2.5 Fish

A metabolism study in fish is required if the Log Po/w is higher than 3 and residues in fish feed are ≥0.1 mg/kg feed DM.

Although the Log Po/w of dimoxystrobin is higher than 3 (DAR, 2003) no significant residue is expected in fatty tissues and milk as the relevant metabolites formed in the animal, including hydroxylated metabolites and sugar conjugates, are supposed to be more hydrophilic than dimoxystrobin.

Due to the expected low feed burden (0.03 mg/kg feed DM for carp, 0.02 mg/kg feed DM for trout; see also M-CA chapter 6.7) of plant commodities fed to fish and the fact that a suitable EU guidance document for the conduct of fish metabolism studies is lacking a metabolism study in fish was not performed.

Additional information on the nature of residues found in fish is available from the BCF study peer-reviewed during the last Annex I-process (M-II, point 8.2.3). It was concluded that despite the relatively high lipophilicity of BAS 505 F there is no risk of accumulation in fish or other aquatic organisms because of its metabolic lability and the rapid excretion of the compound and its metabolites.

CA 6.3 Magnitude of residues trials in plants

Dimoxystrobin is registered in cereals, oilseed rape, mustard seed, radish and sunflower belonging to different EU crop groups. Within this dossier residue data are provided only for the representative uses in sunflower and oilseed rape supporting the renewal of approval. Several magnitude of residue studies for oilseed rape and sunflower have not been peer-reviewed during the Annex I process of the active substance dimoxystrobin but have been submitted for Article 12 evaluation and are presented in this dossier for the sake of completeness (see supportive information in the table below). However, only the residues reported in the most recent studies representing the critical GAP and using a lower LOQ of 0.005 mg/kg were used for MRL calculation (see below and chapter M-CA 6.7). In these studies the SC formulation BAS 540 01 F (containing dimoxystrobin and boscalid) has been used as representative formulation. In order to increase the readability of the dossier, the data for mixing partners are not shown in this dossier.

Table 6.3-1: Overview of residue trials included in the dossier

Crop	Formulation	Applied dose (kg a.s./ha)	Application timing	PHI* (days)	Residues** (mg/kg)	Reference
Studies included as supportive information						
OSR	BAS 510 01 F (WG) BAS 505 02 F (SC)	2 x 0.100 2 x 0.100	1st appl: 51-55 BBCH 2nd appl: 65 BBCH	Maturity	<0.05	M-CA 6.3.1/1 2001/1006138
	BAS 540 KA F (SC)	2 x 0.100	1st appl: 51-55 BBCH 2nd appl: 65 BBCH	Maturity	<0.05	M-CA 6.3.1/2 2002/1007081
	BAS 540 00 F (SC)	2 x 0.100	1st appl: 51-55 BBCH 2nd appl: 65 BBCH	Maturity	<0.05	M-CA 6.3.1/3 2004/1015921
Sunflower	BAS 540 01 F (SC)	3 x 0.100	1st appl: 10-20 BBCH 2nd appl: 61-81 BBCH 3rd appl: 81-87 BBCH	28	<0.05	M-CA 6.3.2/1 2005/1025862
	BAS 540 01 F (SC)	2 x 0.100	1st appl: 42 DBH 2nd appl: 28 DBH	28	<0.01-0.01	M-CA 6.3.2/2 2007/1006111
	BAS 540 01 F (SC)	2 x 0.100	1st appl: 6-12 BBCH 2nd appl: after flowering/30 DBH	28	<0.05	M-CA 6.3.2/3 2005/1035114
	BAS 540 01 F (SC)	2 x 0.100	28±1 DBH 42±1 DBH	28	<0.05-0.163	M-CA 6.3.2/4 2006/1018116
Studies supporting the cGAP used for MRL derivation						
OSR	BAS 540 01 F (SC)	2 x 0.100	28±1 days before 2ndappl./BBCH 75	***	<0.005-0.063	M-CA 6.3.1/4 2013/1003732
	BAS 540 01 F (SC)	2 x 0.100	28±1 days before 2ndappl./BBCH 75	***	<0.005-0.093	M-CA 6.3.1/5 2014/1010806
Sunflower	BAS 540 01 F (SC)	1 x 0.100	BBCH 75	30	<0.005-0.04	M-CA 6.3.2/5 2013/1003723
	BAS 540 01 F (SC)	1 x 0.100	BBCH 75	30	<0.005-0.018	M-CA 6.3.2/6 2014/1010807

* Intended pre-harvest interval
** Residues at PHI. If higher residues were found at later harvest times these are indicated.
*** Defined by growth stage at last application
DBH Days before harvest

CA 6.3.1 Oilseed rape

The use in oilseed rape was not part of the previous Annex I inclusion process. In this dossier sufficient data supporting the representative GAP are submitted for evaluation on the EU level. The critical GAP is shown below for differentiation between studies used for MRL calculation and studies included as supporting information (see Table 6.3.1-1).

Table 6.3.1-1: Representative GAP for the use of dimoxystrobin (BAS 505 F) in/on oilseed rape

Crop	Maximum applied dose (kg a.s./ha)	Water volume (L/ha)	PHI (days)	Application method	Application timing
Oilseed rape	2 x 0.1	100-400	N/A	Foliar spray	BBCH 20-75

PHI Pre-harvest interval
N/A Not applicable. Defined by growth stage at latest application.

Report: CA 6.3.1/1
Raunft E.,Lehmann A., 2001a
Study on the residue behavior of BAS 505 F and BAS 510 F in winter rape after treatment with BAS 505 02 F and BAS 510 01 F under field conditions in Belgium, Denmark, Germany and Great Britain, 2000
2001/1006138

Guidelines: UK Guidance on Crop Residue Data Requirements PSD October 1992, BBA IV 3-3, FAO Guidelines Rome 1990, IVA Guidelines for Residue Studies Sections IA and IB 2nd edition 1992

GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** Dimoxystrobin (BAS 505 F), boscalid (BAS 510 F),
Description: BAS 510 01 F (WG), BAS 505 02 F (SC)
Lot/batch #: BAS 505 02 F : 99-1 (500 g/L dimoxystrobin, nominal)
BAS 510 01 F : 99-10 (50% boscalid, nominal)
Purity: Not relevant
CAS#: 149961-52-4 (dimoxystrobin)
Development code:
Spiking levels: 0.05-5.0 mg/kg

3. **Test commodity:**
Crop: Oilseed rape
Type: Oilseeds
Variety: Colosse, Capitol, Artus, Express, Apex
Botanical name: *Brassica napus L.*
Crop parts(s) or processed
Commodity: Whole plant without roots, seed
Sample size: Not reported

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2000 growing season, 5 field trials were conducted in winter oil seed in Belgium, Denmark, Germany and the United Kingdom in order to determine the residue level of dimoxystrobin (BAS 505 F) after application of BAS 505 02 F and BAS 510 01 F. The test substances BAS 510 01 F (50% BAS 510 F, WG) and BAS 505 02 F (500 g/L BAS 505 F, SC) were applied twice as tank mix at an application rate equivalent to 0.1 kg/ha BAS 510 F and BAS 505 F. The first application was made at BBCH stage 53-55 and the second at BBCH 65. The spray volumes used were approximately 300 L/ha. Oilseed rape plants were sampled directly after the last application (0 DALA). Seeds were sampled at maturity 62-83 DALA (BBCH 87-89).

Table 6.3.1-2: Target application rates and timings for oilseed rape

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date / timing
2000	5	2	F	Tank mix: BAS 510 01 F (WG) BAS 505 02 F (SC)	Boscalid Dimoxystrobin	0.100 0.100	300	1st appl: 51-55 BBCH 2nd appl: 65 BBCH

2. Description of analytical procedures

The specimens were analyzed for dimoxystrobin (BAS 505 F) using BASF method No 445/0. Residues of dimoxystrobin were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method is 0.05 mg/kg for all sample matrices.

Table 6.3.1-3: Summary of procedural recoveries for dimoxystrobin

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 445/0		Dimoxystrobin (BAS 505 F)		
Plants*	0.05/5.0	2	93.4	N/A
Seed	0.05/5.0	2	86.8	N/A
Overall Recovery	0.05/5.0	4	90.0	14.7

N/A Not applicable
* Without roots

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-3, detailed residue levels are shown in Table 6.3.1-4.

After two tank mix applications of BAS 510 01 F and BAS 505 02 F, the residue levels of BAS 505 F ranged between 0.28 and 1.48 mg/kg at 0 DALA. No residues of BAS 505 F, at or above the limit of quantitation (LOQ, 0.05 mg/kg), were detected in any of the mature oilseed rape seed specimens.

In the control samples no residues of dimoxystrobin at or above the LOQ were found.

Table 6.3.1-4: Summary of dimoxystrobin residues in oilseed rape

Region	Year	Application	DALA[1]	Growth stage (BBCH)[2]	Range of residues (mg/kg)	
					Matrix	BAS 505 F
EU North	2000	Tank mix: BAS 505 02 F (SC) BAS 510 01 F (WG)	0 62-83	65 87-89	Plants* Seed	0.28-1.48 <0.05

1 Days after last application
2 At last application
* Without roots

III. CONCLUSION

No residues at or above the LOQ of dimoxystrobin were found in the mature oilseed rape seed specimens after two tank mix applications of BAS 510 01 F and BAS 505 02 F.

Table 6.3.1-5: Residues dimoxystrobin in oilseed rape after two tank mix applications of BAS 510 01 F and BAS 505 02 F in the EU North

Study Details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	NEU/FR/02/00 2001/1006138 AGR/02/00 Yes 2000	Oilseed rape	Belgium	Tank mix: BAS 510 01 F BAS 505 02 F BAS 505 F: 2 x 0.10	65	0 71	Plants* Seed	0.59 <0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	NEU/FR/02/00 2001/1006138 ALB/01/00 Yes 2000	Oilseed rape	Denmark	Tank mix: BAS 510 01 F BAS 505 02 F BAS 505 F: 2 x 0.10	65	0 70	Plants* Seed	1.52 <0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	NEU/FR/02/00 2001/1006138 D05/02/00 Yes 2000	Oilseed rape	Germany	Tank mix: BAS 510 01 F BAS 505 02 F BAS 505 F: 2 x 0.10	65	0 81	Plants* Seed	0.99 <0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	NEU/FR/02/00 2001/1006138 DU2/11/00 Yes 2000	Oilseed rape	Germany	Tank mix: BAS 510 01 F BAS 505 02 F BAS 505 F: 2 x 0.10	65	0 62	Plants* Seed	0.91 <0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	NEU/FR/02/00 2001/1006138 OAT/02/00 Yes 2000	Oilseed rape	United Kingdom	Tank mix: BAS 510 01 F BAS 505 02 F BAS 505 F: 2 x 0.10	65	0 83	Plants* Seed	1.04 <0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

Report: CA 6.3.1/2
Trewhitt J. et al., 2002a
Residue behaviour of BAS 505 F and BAS 510 F in winter oil seed rape after application of BAS 505 02 F / BAS 510 01 F and BAS 540 KA F under field conditions in Denmark, France (N and S), The United Kingdom and The Netherlands, 2001
2002/1007081

Guidelines: EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 2, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999

GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** Dimoxystrobin (BAS 505 F), boscalid (BAS 510 F),
   Description: BAS 540 KA F (SC), BAS 510 01 F (WG), BAS 505 02 F (SC)
   Lot/batch #: BAS 540 KA F: 20002 (200 g/L dimoxystrobin, 200 g/L boscalid nominal)
   BAS 505 02 F : 99-1 (500 g/L dimoxystrobin, nominal)
   BAS 510 01 F : 2001-1 (50% boscalid, nominal)
   Purity: Not relevant
   CAS#: 149961-52-4 (dimoxystrobin)
   Development code:
   Spiking levels: 0.05-5.0 mg/kg

3. **Test commodity:**
   Crop: Oilseed rape
   Type: Oilseeds
   Variety: Lisabeth, Cymbal, Elite, Madrigal, Escort
   Botanical name: *Brassica napus L.*
   Crop parts(s) or processed
   Commodity: Shoots, seed
   Sample size: 12 plants (> 1 kg)

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2001 growing season, 5 field trials were conducted in winter oil seed rape in Denmark, France, the United Kingdom and the Netherlands in order to determine the residue level of dimoxystrobin (BAS 505 F) in or on raw agricultural commodities after application of BAS 540 KA F. The test items BAS 505 02 F (500 g/L BAS 505 F, SC) / BAS 510 01 F (500 g/kg BAS 510 F, WG) as a tank mix, and BAS 540 KA F (200 g/L BAS 505 F and 200 g/L BAS 510 F, SC) were applied twice to oil seed rape plants at a dose rate of 100 g a.s./ha. The first application was made at BBCH stage 55-57 and the second at BBCH 65. The spray volumes used were approximately 300 L/ha. Oilseed rape plants were sampled directly after the last application (0 DALA). Seeds were sampled at crop BBCH stage 87 (68-76 DALA). The maximum storage interval from harvest until analysis was 329 days.

Table 6.3.1-6: Target application rates and timings for oilseed rape

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date / timing
2001	5	2	F	BAS 540 KA F (SC)	Dimoxystrobin Boscalid	0.100 0.100	300	1st appl: 51-55 BBCH 2nd appl: 65 BBCH
2001	5	2	F	Tank mix: BAS 505 02 F (SC) BAS 510 01 F (WG)	Dimoxystrobin Boscalid	0.100 0.100	300	1st appl: 51-55 BBCH 2nd appl: 65 BBCH

2. Description of analytical procedures

The specimens were analyzed for dimoxystrobin (BAS 505 F) using BASF method No 445/0. Residues of dimoxystrobin were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method is 0.05 mg/kg for all samples.

Table 6.3.1-7: Summary of recoveries for dimoxystrobin

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 445/0		**Dimoxystrobin (BAS 505 F)**		
Shoots	0.05/5.0	2	84.8	N/A
Seed	0.05/5.0	2	86.6	N/A
Overall recovery	0.05/5.0	4	85.7	1.4

N/A Not applicable

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-8, detailed residue levels are shown in Table 6.3.1-9 through Table 6.3.1-12.

After application of BAS 540 KA F, the residue levels of BAS 505 F in oilseed rape plants without roots ranged between 0.68 and 1.78 mg/kg. No residues of BAS 505 F, at or above the limit of quantitation (LOQ: 0.05 mg/kg), were detected in any of the oilseed rape seed specimens. The results were similar to the results obtained after the application of a tank mix containing BAS 505 02 F and BAS 510 01 F. The residue levels of BAS 505 F ranged between 0.43 and 1.68 mg/kg. No residues at or above the limit of quantitation (LOQ: 0.05 mg/kg) were detected in oilseed rape seed specimens.

In the control samples no residues of dimoxystrobin at or above the LOQ were found.

Table 6.3.1-8: Summary of dimoxystrobin residues in oilseed rape

Region	Year	Application	DALA[1]	Growth stage (BBCH)[2]	Range of residues (mg/kg)	
					Matrix	BAS 505 F
EU North	2001	BAS 540 KA F (SC)	0	65	Plants*	0.68-1.78
			69-76		Seed	<0.05
		Tank mix: BAS 510 01 F (WG) BAS 505 02 F (SC)	0	65	Plants*	0.43-1.68
			69-76		Seed	<0.05
EU South	2001	BAS 540 KA F (SC)	0	65	Plants*	1.53
			68		Seed	<0.05
		Tank mix: BAS 510 01 F (WG) BAS 505 02 F (SC)	0	65	Plants*	0.99
			68		Seed	<0.05

1 Days after last application
2 At last application
* Without roots

III. CONCLUSION

After application of BAS 540 KA F or the tank mix containing BAS 505 02 F and BAS 510 01 F no residues at or above the LOQ (0.05 mg/kg) of dimoxystrobin were detected in the oilseed rape seed specimens at the intended harvest.

Table 6.3.1-9: Residues of dimoxystrobin in oilseed rape after two applications of BAS 540 KA F in the EU North

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS [2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	72551 2002/1007081 AGR/17/01 Yes 2001	Oilseed rape	The Netherlands	BAS 540 KA F BAS 505 F: 2 x 0.10	65	0 69	Plants* Seeds	0.68 <0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	72551 2002/1007081 ALB/04/01 Yes 2001	Oilseed rape	Denmark	BAS 540 KA F BAS 505 F: 2 x 0.10	65	0 73	Plants* Seeds	1.23 <0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	72551 2002/1007081 FBM/03/01 Yes 2001	Oilseed rape	France	BAS 540 KA F BAS 505 F: 2 x 0.10	65	0 76	Plants* Seeds	0.91 <0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	72551 2002/1007081 OAT/03/01 Yes 2001	Oilseed rape	United Kingdom	BAS 540 KA F BAS 505 F: 2 x 0.10	65	0 70	Plants* Seeds	1.78 <0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

Table 6.3.1-10: Residues of dimoxystrobin in oilseed rape after two applications of BAS 540 AK F in the EU South

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	72551 2002/1007081 FBD/02/01 Yes 2001	Oilseed rape	France	BAS 540 KA F BAS 505 F: 2 x 0.10	65	0 68	Plants* Seeds	1.53 <0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

Table 6.3.1-11: Residues of dimoxystrobin in oilseed rape after two applications of a tank mix of BAS 505 02 F with BAS 510 01 Fin the EU North

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS [2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code: Doc ID: Trial No: GLP: Year:	72551 2002/1007081 AGR/17/01 Yes 2001	Oilseed rape	The Netherlands	Tank mix: BAS 510 01 F BAS 505 02 F BAS 505 F: 2 x 0.10	65	0 69	Plants* Seeds	0.43 <0.05
Study code: Doc ID: Trial No: GLP: Year:	72551 2002/1007081 ALB/04/01 Yes 2001	Oilseed rape	Denmark	Tank mix: BAS 510 01 F BAS 505 02 F BAS 505 F: 2 x 0.10	65	0 73	Plants* Seeds	1.51 <0.05
Study code: Doc ID: Trial No: GLP: Year:	72551 2002/1007081 FBM/03/01 Yes 2001	Oilseed rape	France	Tank mix: BAS 510 01 F BAS 505 02 F BAS 505 F: 2 x 0.10	65	0 76	Plants* Seeds	0.79 <0.05
Study code: Doc ID: Trial No: GLP: Year:	72551 2002/1007081 OAT/03/01 Yes 2001	Oilseed rape	United Kingdom	Tank mix: BAS 510 01 F BAS 505 02 F BAS 505 F: 2 x 0.10	65	0 70	Plants* Seeds	1.68 <0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

Table 6.3.1-12: Residues of dimoxystrobin in oilseed rape after two applications of a tank mix of BAS 505 02 F with BAS 510 01 Fin the EU South

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code: Doc ID: Trial No: GLP: Year:	72551 2002/1007081 FBD/02/01 Yes 2001	Oilseed rape	France	Tank mix: BAS 510 01 F BAS 505 02 F BAS 505 F: 2 x 0.10	65	0 68	Plants* Seeds	0.99 <0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

Report: CA 6.3.1/3
Schulz H., 2004a
Study on the residue behaviour of BAS 510 F and BAS 505 F in oilseed rape after application of BAS 510 01 F and BAS 540 00 F under field conditions in Denmark, France (North and South), Germany, the Netherlands and the United Kingdom, 2003
2004/1015921

Guidelines: EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 7, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** Dimoxystrobin (BAS 505 F), boscalid (BAS 510 F),
   Description: BAS 540 00 F (SC)
   Lot/batch #: BAS 540 00 F: 2000-1 (200 g/L dimoxystrobin, 200 g/L boscalid nominal)
   Purity: Not relevant
   CAS#: 149961-52-4 (dimoxystrobin)
   Development code:
   Spiking levels: 0.05-0.5 mg/kg

3. **Test commodity:**
   Crop: Oilseed rape
   Type: Oilseeds
   Variety: Lion, Canberra, Expreß, Pollen, Parabole, Banjo, Borneo
   Botanical name: Brassica napus L.
   Crop parts(s) or processed commodity: Whole plant without root, seed, pods with seeds, rest of plant without roots
   Sample size: 0.5-1 kg (nominal)

B. STUDY DESIGN AND METHODS

1. Test procedure

During the 2003 growing season, 7 trials in oilseed rape were conducted in Denmark, France, Germany, the Netherlands and the United Kingdom in order to determine the residue level of dimoxystrobin (BAS 505 F) after application of BAS 540 00 F. Plots were treated two times with BAS 540 00 F (200 g/L BAS 510 F and 200 g/L BAS 505 F, SC) at an application rate equivalent to 0.1 kg/ha BAS 510 F and BAS 505 F. The target applications took place at BBCH growth stages 51-55 and 75 in spray volumes of about 300 L/ha. Specimens were collected 21± 1, 28± 1, 35± 1 and 42 ± 1 days after the last application. The maximum storage interval from harvest until analysis was 280 days.

Table 6.3.1-13: Target application rates and timings for oilseed rape

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date / timing
2003	7	2	F	BAS 540 00 F (SC)	Boscalid Dimoxystrobin	0.100 0.100	300	1st appl: 51-55 BBCH 2nd appl: 75 BBCH

2. Description of analytical procedures

The specimens were analyzed for dimoxystrobin (BAS 505 F) using BASF method No 445/0. Residues of dimoxystrobin were extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method is 0.05 mg/kg for all samples.

Table 6.3.1-14: Summary of recoveries for dimoxystrobin

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 445/0		**Dimoxystrobin (BAS 505 F)**		
Whole plants*	0.05/0.5	2	85.2	N/A
Pods with seed	0.05/0.5	2	81.8	N/A
Seed	0.05/0.5	4	91.1	10.4
Overall Recovery	0.05/0.5	8	87.3	9.0

N/A Not applicable
* Without roots

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-15, detailed residue levels are shown in Table 6.3.1-16 and Table 6.3.1-17.

After application of BAS 540 00 F the BAS 505 F residues in oilseed rape at 0 DALA were between 0.82-1.69 mg/kg. They decreased to <0.05 mg/kg in the specimens taken at the subsequent sampling events including the intended harvest of mature seeds.
In the control samples of all matrices no residues of dimoxystrobin at or above the limit of quantitation (LOQ) were found.

Table 6.3.1-15: Summary of dimoxystrobin residues in oilseed rape

Region	Year	Application	DALA[1]	Growth stage (BBCH)[2]	Range of residues (mg/kg)	
					Matrix	BAS 505 F
EU North	2003	BAS 540 00 F (SC)	0	75	Whole plants*	0.82-1.41
			20-21		Whole plant*	<0.05
			28		Pods with seed	<0.05
			28		Rest of plants*	<0.05
			21		Seed	<0.05
			27-29		Seed	<0.05
			34-35		Seed	<0.05
			41-43		Seed	<0.05
EU South	2003	BAS 540 00 F (SC)	0	75	Whole plants*	0.84-1.69
			21-22		Seed	<0.05
			27-29		Seed	<0.05
			34-35		Seed	<0.05
			42		Seed	<0.05

1 Days after last application
2 At sampling
* Without roots

III. CONCLUSION

The study showed that after application of BAS 540 00 F, the BAS 505 F residues in oilseed rape seed specimens were <0.05 mg/kg at the intended harvest (BBCH 87-89).

Table 6.3.1-16: Residues of dimoxystrobin in oilseed rape after two applications of BAS 540 00 F in the EU North

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code:	150304	Oilseed rape	Germany	BAS 540 00 F BAS 505 F: 2 x 0.10	75	0	Whole plants*	0.87
Doc ID:	2004/1015921					21	Whole plants*	<0.05
Trial No:	AGR/05/03					28	Seed	<0.05
GLP:	Yes					35	Seed	<0.05
Year:	2003					42	Seed	<0.05
Study code:	150304	Oilseed rape	Denmark	BAS 540 00 F BAS 505 F: 2 x 0.10	75	0	Whole plant*	0.87
Doc ID:	2004/1015921					21	Whole plant*	<0.05
Trial No:	ALB/02/03					29	Seed	<0.05
GLP:	Yes					34	Seed	<0.05
Year:	2003					42	Seed	<0.05
Study code:	150304	Oilseed rape	Germany	BAS 540 00 F BAS 505 F: 2 x 0.10	75	0	Whole plant*	1.12
Doc ID:	2004/1015921					21	Seed	<0.05
Trial No:	DU4/09/03					27	Seed	<0.05
GLP:	Yes					35	Seed	<0.05
Year:	2003					43	Seed	<0.05
Study code:	150304	Oilseed rape	France	BAS 540 00 F BAS 505 F: 2 x 0.10	75	0	Whole plant*	0.82
Doc ID:	2004/1015921					20	Whole plant*	<0.05
Trial No:	FAN/07/03					27	Seed	<0.05
GLP:	Yes					35	Seed	<0.05
Year:	2003					41	Seed	<0.05
Study code:	150304	Oilseed rape	United Kingdom	BAS 540 00 F BAS 505 F: 2 x 0.10	75	0	Whole plant*	1.41
Doc ID:	2004/1015921					21	Whole plant*	<0.05
Trial No:	OAT/05/03					28	Rest of plants*	<0.05
GLP:	Yes					28	Pods with seed	<0.05
Year:	2003					34	Seed	<0.05
						41	Seed	<0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

Table 6.3.1-17: Residues of dimoxystrobin in oilseed rape after two applications of BAS 540 00 F in the EU South

Study details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code:	150304	Oilseed rape	France	BAS 540 00 F	75	0	Whole plants*	0.84
Doc ID:	2004/1015921			BAS 505 F:		21	Seed	<0.05
Trial No:	FBD/03/03			2 x 0.10		27	Seed	<0.05
GLP:	Yes					34	Seed	<0.05
Year:	2003					42	Seed	<0.05
Study code:	150304	Oilseed rape	France	BAS 540 00 F	75	0	Whole plants*	1.69
Doc ID:	2004/1015921			BAS 505 F:		22	Seed	<0.05
Trial No:	FTL/04/03			2 x 0.10		29	Seed	<0.05
GLP:	Yes					35	Seed	<0.05
Year:	2003					42	Seed	<0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

Report:	CA 6.3.1/4 Erdmann H.-P., 2015a Study on the residue behaviour of BAS 505 F and BAS 510 F in oilseed rape after application of BAS 540 01 F under field condition in United Kingdom, Northern and Southern France, The Netherlands, Italy, Spain, Greece and Germany, 2012 2013/1003732
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 9 (March 2011), EEC 79/117
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test material:** Dimoxystrobin (BAS 505 F), boscalid (BAS 510 F),
   Description: BAS 540 01 F (SC)
   Lot/batch #: BAS 540 01 F: 2000-1 (200 g/L dimoxystrobin, 200 g/L boscalid nominal)
   Purity: Not reported
   CAS#: 149961-52-4 (dimoxystrobin)

   Development code: Not reported
   Spiking levels: 0.005-2.0 mg/kg (dimoxystrobin)

2. **Test Commodity:**
   Crop: Oilseed rape
   Type: Oilseeds
   Variety: RNX3922, Castille, Dynastie, Billy, Albatros, PR46W31, Avenir, Kabel
   Botanical name: *Brassica napus*
   Crop parts(s) or processed commodity: Whole plant without roots, rest of plant without roots, seeds
   Sample size: 12 units, 0.5 kg nominal

B. STUDY DESIGN

1. Test procedure

During the 2012 growing season 8 residue field trials in oilseed rape were conducted in the EU, 4 in the northern (Germany, France, The Netherlands, United Kingdom) and 4 in the southern part (France, Italy, Spain, Greece) to determine the residue levels of dimoxystrobin. BAS 540 01 F (SC) containing 200 g/L dimoxystrobin and 200 g/L boscalid was applied twice at individual rates equivalent to 0.100 kg dimoxystrobin/ha in spray volumes of about 200 L/ha. The applications were performed targeting 28 days before the second application and BBCH 75 for the second application. Whole plant specimens without roots were harvested immediately after the last application (0 DALA). Rest of plant specimens without roots and seed specimens were collected at maturity (BBCH 89), from 25 to 50 DALA. Samples were frozen within 6 hours and stored below -18°C until analysis. In trial L120380 temperature raised shortly to -12°C during sample shipment. The maximum storage interval (-12 to -18°C) from sampling until extraction was 708 days.

Table 6.3.1-18: Target application rates and timings for oilseed rape

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ timing
2012	8	2	F	BAS 540 01 F (SC)	BAS 505 F	0.100	200	28±1 DB2ndA
					BAS 510 F	0.100		BBCH 75

DB2ndA Days before second application, which was intended to be made on BBCH 75

2. Description of analytical procedures

The specimens were analyzed for dimoxystrobin and its *Z*-isomer 505M98 using BASF method No L0076/08 quantifying the analytes with a limit of quantitation (LOQ) of 0.005 mg/kg, each. Residues of dimoxystrobin and its *Z*-isomer 505M98 were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane before final determination by LC-MS/MS.

Table 6.3.1-19: Summary of procedural recovery data for dimoxystrobin

Matrix	Fortification level (mg/kg)	Summary recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0076/08		Dimoxystrobin (BAS 505 F)			*Z*-isomer (505M98)		
Whole plants*	0.005-2.0	5	103	9.4	3	86	9.1
Rest of plants*	0.005-2.0	5	87	7.4	3	84	7.2
Seeds	0.005-0.5	3	95	11	3	86	6.9

* Without roots

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-20, detailed residue levels are shown in Table 6.3.1-21 and Table 6.3.1-22.

Residues of dimoxystrobin parent and the *Z*-isomer in whole plant specimens without roots collected immediately after the last application ranged from 0.41 mg/kg to 1.6 mg/kg and from <0.005 to 0.0065 mg/kg, respectively. Dimoxystrobin residues in rest plant specimen without roots ranged from <0.0058 mg/kg to 1.3 mg/kg and the *Z*-isomer residues ranged from <0.005 to 0.087 mg/kg. Residues of dimoxystrobin parent and *Z*-isomer were between <0.005 and 0.063 mg/kg and <0.005 mg/kg in seeds.

Table 6.3.1-20: Summary of dimoxystrobin residues in oilseed rape

Region	Year	Application	DALA[1]	Growth stage (BBCH)[2]	Range of residues (mg/kg)		
					Matrix	BAS 505 F	*Z*-isomer (505M98)
EU North	2012	BAS 540 01 F (SC)	0	75-77	Whole plants*	0.41-1.5	<0.005
			30-50		Rest of plants*	0.0058-0.048	<0.005
			30-50		Seed	<0.005	<0.005
EU South	2012	BAS 540 01 F (SC)	0	57-77	Whole plants*	0.84-1.6	<0.005-0.0065
			25-48		Rest of plants*	0.0077-1.3	<0.005-0.087
			25-48		Seed	<0.005-0.063	<0.005

1 Days after last application
2 At last application
* Without roots

III. CONCLUSION

Residues of dimoxystrobin parent and *Z*-isomer were between <0.005 and 0.063 mg/kg and <0.005 mg/kg in mature seeds, respectively.

Table 6.3.1-21: Residues of dimoxystrobin in oilseed rape after two applications of BAS 540 01 F in the EU North

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	Growth stage [2] BBCH	DALA[1]	Residues found (mg/kg)		
							Matrix	BAS 505 F	Z-isomer (505M98)
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390013 2013/1003732 L120374 Yes 2012	Oilseed rape	Germany	BAS 540 01 F BAS 505 F: 2 x 0.10	75	0 50 50	Whole plants* Rest of plants* Seed	0.41 0.048 <u><0.005</u>	<0.005 <0.005 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390013 2013/1003732 L120375 Yes 2012	Oilseed rape	United Kingdom	BAS 540 01 F BAS 505 F: 2 x 0.10	75	0 30 30	Whole plant* Rest of plant* Seed	1.1 0.026 <u><0.005</u>	<0.005 <0.005 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390013 2013/1003732 L120376 Yes 2012	Oilseed rape	France	BAS 540 01 F BAS 505 F: 1 x 0.10 1 x 0.113[#]	77	0 50 50	Whole plant* Rest of plant* Seed	1.5 0.0058 <u><0.005</u>	<0.005 <0.005 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390013 2013/1003732 L120377 Yes 2012	Oilseed rape	The Nether-lands	BAS 540 01 F BAS 505 F: 2 x 0.10	75	0 48 48	Whole plant* Rest of plant* Seed	1.1 0.019 <u><0.005</u>	<0.005 <0.005 <0.005

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots
# Second application was performed with 225 L water/ha and exceeded 10% deviation
_ Underlined values were taken for MRL calculation (see chapter 6.7)

Table 6.3.1-22: Residues of dimoxystrobin in oilseed rape after two applications of BAS 540 01 F in the EU South

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	Growth stage [2] BBCH	DALA[1]	Residues found (mg/kg)		
							Matrix	BAS 505 F	*Z*-isomer (505M98)
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390013 2013/1003732 L120378 Yes 2012	Oilseed rape	France	BAS 540 01 F BAS 505 F: 2 x 0.10	77	0 48 48	Whole plants* Rest of plants* Seed	1.2 0.0077 <u><0.005</u>	<0.005 <0.005 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390013 2013/1003732 L120379 Yes 2012	Oilseed rape	Greece	BAS 540 01 F BAS 505 F: 2 x 0.10	75	0 32 32	Whole plants* Rest of plants* Seed	1.6 0.15 <u>0.0092</u>	0.0065 <0.005 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390013 2013/1003732 L120380 Yes 2012	Oilseed rape	Italy	BAS 540 01 F BAS 505 F: 2 x 0.10	75	0 25 25	Whole plants* Rest of plants* Seed	1.2 0.31 <u>0.063</u>	<0.005 0.016 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390013 2013/1003732 L120381 Yes 2012	Oilseed rape	Spain	BAS 540 01 F BAS 505 F: 2 x 0.10	75	0 36 36	Whole plants* Rest of plants* Seed	0.84 1.3 <u>0.053</u>	<0.005 0.087 <0.005

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots
_ Underlined values were taken for MRL calculation (see chapter 6.7)

Report: CA 6.3.1/5
Erdmann H.-P., 2015a
Residue behaviour of BAS 505 F (Dimoxystrobin) and BAS 510 F (Boscalid) in oilseed rape after application of BAS 540 01 F under field condition in Germany, Belgium, The Netherlands, Northern and Southern France, Greece, Italy, Spain 2013
2014/1010806
Guidelines: EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)
GLP: yes
(certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

Report: CA 6.3.1/6
Erdmann H.-P., 2015b
Amendment No. 1 - Residue behaviour of BAS 505 F (Dimoxystrobin) and BAS 510 F (Boscalid) in oilseed rape after application of BAS 540 01 F under field condition in Germany, Belgium, The Netherlands, France, Greece, Italy, Spain 2013
2015/1071837
Guidelines: EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)
GLP: yes
(certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

1. **Test Material:**
   Description: BAS 540 01 F (SC)
   Lot/Batch #: 0003354823 (200 g/L dimoxystrobin, 200 g/L boscalid, nominal)
   Purity: Not reported
   CAS#: 149961-52-4 (dimoxystrobin)
   Development code: Not reported
   Spiking levels: 0.005-50 mg/kg (dimoxystrobin)

2. **Test Commodity:**
   Crop: Oilseed rape
   Type: Oilseeds
   Variety: Sherpa, Arsenal, Deca Expertise, Visby, Extorm, Nelson, Excalibur, Salsa CL
   Botanical name: *Brassica napus*
   Crop parts(s) or processed commodity: Whole plant without roots, rest of plant without roots, seeds
   Sample size: 12 units, 0.5 kg nominal

B. STUDY DESIGN

1. Test procedure

During the 2013 growing season 8 residue field trials in oilseed rape were conducted the EU, 4 in the northern (Germany, France, The Netherlands, Belgium) and 4 in the southern part (France, Italy, Spain, Greece) to determine the residue levels of dimoxystrobin. BAS 540 01 F (SC) containing 200 g/L dimoxystrobin and 200 g/L boscalid was applied twice at individual rates equivalent to 0.100 kg dimoxystrobin/ha and 0.100 kg boscalid/ha in spray volumes of about 200 L/ha. The first applications were targeted to 28 days before the second application and the second application to BBCH stage 75. Whole plant specimens without roots were harvested immediately after the last application (0 DALA). Rest of plant specimens without roots and seed specimens were collected at plant maturity (BBCH 89), at 30 to 64 days after the last application (DALA). The maximum storage interval (<-18°C) from sampling until extraction was 476 days.

Table 6.3.1-23: Target application rates and timings for oilseed rape

Year	No. of trials	No. of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ timing
2013	8	2	F	BAS 540 01 F (SC)	BAS 505 F	0.100	200	28±1 DB2ndA
					BAS 510 F	0.100	200	BBCH 75

DB2ndA Days before second application, which was intended to be made on BBCH 75

2. Description of analytical procedures

The specimens were analyzed for dimoxystrobin and its *Z*-isomer 505M98 using BASF method No L0076/08 quantifying the analytes with a limit of quantitation (LOQ) of 0.005 mg/kg, each. Residues of dimoxystrobin and its *Z*-isomer 505M98 were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane before final determination by LC-MS/MS.

Table 6.3.1-24: Summary of procedural recovery data for dimoxystrobin

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0076/08		Dimoxystrobin (BAS 505 F)			*Z*-isomer (505M98)		
Whole plants*	0.005-50	6	96	10.2	6	99	5.5
Rest of plants*	0.005-5.0	6	95	10.2	6	97	9.5
Seeds	0.005-0.5	3	93	1.7	3	99	4.2

* Without roots

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.1-25, detailed residue levels are shown in Table 6.3.1-26 and Table 6.3.1-27.

Residues of dimoxystrobin parent and the *Z*-isomer in whole plant specimens without roots collected immediately after the last application ranged from 0.43 mg/kg to 1.6 mg/kg and from <0.005-0.014 mg/kg, respectively. At maturity residues of parent and the Z-isomer ranged from <0.005 mg/kg to 2.9 mg/kg and were between <0.005 mg/kg and 0.11 mg/kg in in rest plant specimens without roots. In mature seeds residues of parent ranged from <0.005 mg/kg to 0.093 mg/kg and were below LOQ for the *Z*-isomer.

No residues of dimoxystrobin parent and the *Z*-isomer has been found in the control samples, except for trial L130073 (The Netherlands), where 0.46 mg/kg of dimoxystrobin and 0.016 mg/kg of 505M98 were found in whole plant at 0 DALA and 0.034 mg/kg of dimoxystrobin in the rest of plant specimen at 51 DALA.

Table 6.3.1-25: Summary dimoxystrobin residues in oilseed rape

Region	Year	Application	DALA[1]	Growth stage (BBCH)[2]	Range of residues (mg/kg)		
					Matrix	BAS 505 F	*Z*-isomer (505M98)
EU North	2013	BAS 540 01 F (SC)	0	75	Whole plants*	0.94-1.5	<0.005-0.014
			46-61		Rest of plants*	<0.005-0.20	<0.005-0.0079
			46-61		Seed	<0.005-0.013	<0.005
EU South	2013	BAS 540 01 F (SC)	0	75	Whole plants*	0.43-1.6	<0.005
			30-64		Rest of plants*	<0.005-2.9	<0.005-0.11
			30-64		Seed	<0.005-0.093	<0.005

1 Days after last application
2 At last application
* Without roots

III. CONCLUSION

Residues of dimoxystrobin parent and Z-isomer were between <0.005 and 0.093 mg/kg and <0.005 mg/kg in mature seeds, respectively.

Table 6.3.1-26: Residues of dimoxystrobin in oilseed rape after two applications of BAS 540 01 F in the EU North

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	Growth stage [2] BBCH	DALA[1]	Residues found (mg/kg)		
							Matrix	BAS 505 F	Z-isomer (505M98)
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390014 2014/1010806 L130070 Yes 2013	Oilseed rape	Germany	BAS 540 01 F BAS 505 F: 1 x 0.111 1 x 0.10	75	0 46 46	Whole plants* Rest of plants* Seed	0.95 0.0066 <0.005	<0.005 <0.005 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390014 2014/1010806 L130071 Yes 2013	Oilseed rape	Belgium	BAS 540 01 F BAS 505 F: 2 x 0.10	75	0 53 53	Whole plants* Rest of plants* Seed	1.3 0.0035 0.0093	<0.005 <0.005 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390014 2014/1010806 L130072 Yes 2013	Oilseed rape	France	BAS 540 01 F BAS 505 F: 2 x 0.10	75	0 61 61	Whole plants* Rest of plants* Seed	0.94 <0.005 <0.005	<0.005 <0.005 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390014 2014/1010806 L130073 Yes 2013	Oilseed rape	The Nether-lands	BAS 540 01 F BAS 505 F: 2 x 0.10	75	0 51 51	Whole plants* Rest of plants* Seed	1.5 0.20 0.013	0.014 0.0079 <0.005

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots
# First application was performed with 222 L water/ha and exceeded 10% deviation
_ Underlined values were taken for MRL calculation (see chapter M-CA 6.7)

Table 6.3.1-27: Residues of dimoxystrobin in oilseed rape after two applications of BAS 540 01 F in Southern Europe

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	Growth stage [2] BBCH	DALA[1]	Residues found (mg/kg)		
							Matrix	BAS 505 F	Z-isomer (505M98)
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390014 2014/1010806 L130074 Yes 2013	Oilseed rape	France	BAS 540 01 F 2 x 0.10	75	0 64 64	Whole plant* Rest of plant* Seed	0.43 <0.005 <0.005	<0.005 <0.005 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390014 2014/1010806 L130075 Yes 2013	Oilseed rape	Greece	BAS 540 01 F 2 x 0.10	75	0 30 30	Whole plant* Rest of plant* Seed	0.68 0.11 0.034	<0.005 0.012 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390014 2014/1010806 L130076 Yes 2013	Oilseed rape	Italy	BAS 540 01 F 2 x 0.10	75	0 30 30	Whole plant* Rest of plant* Seed	1.6 0.83 0.093	<0.005 0.028 <0.005
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	390014 2014/1010806 L130077 Yes 2013	Oilseed rape	Spain	BAS 540 01 F 2 x 0.10	75	0 47 47	Whole plant* Rest of plant* Seed	1.0 2.9 0.092	<0.005 0.11 <0.005

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots
_ Underlined values were taken for MRL calculation (see chapter M-CA 6.7)

CA 6.3.2 Sunflower

The use in sunflower was not part of the previous active substance inclusion process. In this dossier sufficient data supporting the representative GAP are submitted for evaluation on the EU level. The critical GAP is shown below for differentiation between studies used for MRL calculation and studies included as supporting information (see Table 6.3.2-1).

Table 6.3.2-1: Representative GAP for the use of dimoxystrobin (BAS 505 F) in/on sunflower

Crop	Maximum applied dose (kg a.s./ha)	Water volume (L/ha)	PHI (days)	Application method	Application timing
Sunflower	1 x 0.1	100-400	30	Foliar spray	BBCH 51-75

PHI Pre-harvest interval

Report: CA 6.3.2/1
Toth F., 2005a
Determination of the residues of active ingredients of Pictor SC (Boscalid/BAS 510 F Dimoxystrobin/BAS 505 F) in sunflower
2005/1025862
Guidelines: none
GLP: yes
(certified by National Institute of Pharmacy, Budapest, Hungary)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Boscalid, dimoxystrobin BAS 510 F, BAS 505 F
   Description: BAS 540 01 F (SC)
   Lot/Batch #: 1007, Dimoxystrobin: 200 g/L; Boscalid: 200 g/L
   Purity: Not reported
   CAS#: BAS 505 F: 149961-52-4
   Development code: Not reported
   Spiking levels: 0.10-0.20 mg/kg

3. **Test Commodity:**
   Crop: Sunflower
   Type: Oilseeds
   Variety: Matis, Arena, Alexandra, Pioneer/310448/YF-3312
   Botanical name: *Helianthus annuus*
   Crop parts(s) or processed Commodity: Sunflower seeds
   Sample size: > 1 kg (from >14 heads)

B. STUDY DESIGN AND METHODS

1. Test procedure

In 2004 four trials were conducted in sunflower in Hungary in order to determine the residue levels of BAS 505 F. The test item BAS 540 01 F (200 g/L BAS 505 F and 200 g/L BAS 510 F, SC) was applied three times to sunflower at a rate of 100 g a.s./ha. The first application was made at BBCH stage 11 to 18, the second at BBCH stage 61 to 81 and the third at BBCH 81 to 87. The spray volume used was ranging from 60 to 300 L/ha. Samples were stored deep frozen (-18°C) for less than 4 months.

Table 6.3.2-2: Target application rates and timings for sunflower

Year	No. of trials	No. of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ timing
2004	4	3	F	BAS 540 01 F (SC)	Boscalid Dimoxystrobin	0.100 0.100	300	1st appl: 10-20 BBCH 2nd appl: 61-81 BBCH 3rd appl: 81-87 BBCH

2. Description of analytical procedures

The specimens were analysed for BAS 505 F according to BASF method no. 445/0. BAS 505 F was extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane. The final determination of the analyte was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method was 0.05 mg/kg for all samples.

Table 6.3.2-3: Summary of procedural recoveries for dimoxystrobin

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 445/0			**Dimoxystrobin (BAS 505 F)**	
Seed	0.10, 0.20	8	78	15.8

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-4, detailed residue levels are shown in Table 6.3.2-5.

Sunflower seeds obtained from heads collected 29 to 30 DALA did not show residues of BAS 505 F at or above the LOQ of the analytical method (0.05 mg/kg).

In the control samples no residues of dimoxystrobin at or above the limit of quantitation (LOQ) were found.

Table 6.3.2-4: Summarized results of the residue trials with BAS 505 F in sunflower

Sampling no.	Portion analyzed	DALA1	Range of residues BAS 505 F (mg/kg)
1	Sunflower seeds	28	<0.05

1 Days after last application

Table 6.3.2-5: Residues of dimoxystrobin in sunflower after three applications of BAS 540 01 F in the EU North

Study details		Crop	Country	Formulation, Application rate[0)] (kg a.s./ha)	GS[2] BBCH	DA LA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	04 BASF 01 01 2005/1025862 04 BASF 01 01/1 Yes 2004	Sunflower	Hungary	BAS 540 01 F BAS 505 F: 3 x 0.10	85-87	28	Seed	<0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	04 BASF 01 01 2005/1025862 04 BASF 01 01/2 Yes 2004	Sunflower	Hungary	BAS 540 01 F BAS 505 F: 3 x 0.10	81-83	28	Seed	<0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	04 BASF 01 01 2005/1025862 04 BASF 01 01/3 Yes 2004	Sunflower	Hungary	BAS 540 01 F BAS 505 F: 3 x 0.10	85	28	Seed	<0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	04 BASF 01 01 2005/1025862 04 BASF 01 01/4 Yes 2004	Sunflower	Hungary	BAS 540 01 F BAS 505 F: 3 x 0.10	87	28	Seed	<0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

III. CONCLUSION

At the proposed PHI of 30±2 days, no residues above the limit of quantitation of BAS 505 F were detected.

Report: CA 6.3.2/2
Wicks R., 2007a
Residue study (decline) with BAS 540 01 F applied to sunflower in Belgium, The Netherlands, Northern France, Germany and Southern France in 2006
2007/1006111

Guidelines: EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Boscalid, dimoxystrobin BAS 510 F, BAS 505 F
Description: BAS 540 01 F (SC)
Lot/Batch #: 1011, Boscalid: 200.0 g/L; dimoxystrobin: 200.0 g/L
Purity: Not reported
CAS#: 149961-52-4 (dimoxystrobin)
Development code:
Spiking levels: 0.01-1.0 mg/kg

3. **Test Commodity:**
Crop: Sunflower
Type: Oilseeds
Variety: LG 54.50 HO, Sunrich orange, Aria, Aurasol, Heliaroc, LG 5435, Prodisol
Botanical name: *Helianthus annuus*
Crop parts(s) or processed Commodity: Whole plant without roots, seed
Sample size: > 1 kg (from > 12 sunflowers)

B. STUDY DESIGN AND METHODS

1. Test procedure

The purpose of this study was to determine the magnitude and decline of the residue of BAS 505 F (dimoxystrobin) in sunflowers after application of 2 times 0.1 kg a.s./ha of formulation BAS 540 01 F and a pre-harvest interval (PHI) of 30 days. Seven residue trials were conducted in Belgium, the Netherlands, northern France, Germany and southern France. BAS 540 01 F (containing 200 g/L boscalid and 200 g/L dimoxystrobin) was applied at a 14 day interval in spray volumes of 300 L/ha. Sunflower crop specimens were collected at 0 DALA (plants without roots) and at 21±1, 28±1 and 35±1 DALA (sunflower seeds). The samples were stored for a maximum of 178 days until analysis.

Table 6.3.2-6: Target application rates and timings for sunflower

Year	No. of trials	No. of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ timing
2006	7	2	F	BAS 540 01 F (SC)	Boscalid Dimoxystrobin	0.100 0.100	300	1st appl: 42 DBH 2nd appl: 28 DBH

2. Description of analytical procedures

The specimens were analysed for BAS 505 F according to BASF method No 535/2. BAS 505 F was extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane under alkaline conditions. The final determination of the analyte was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method was 0.01 mg/kg for all samples.

Table 6.3.2-7: Summary of procedural recoveries for dimoxystrobin

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 535/2		**Dimoxystrobin (BAS 505 F)**		
Plants without roots	0.01/0.10	2	84.95	N/A
Seed	0.01/0.10	2	89.15	N/A
Overall Recovery		4	87	6.4

N/A Not applicable

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-8, detailed residue levels are shown in Table 6.3.2-9 and Table 6.3.2-10.

Directly after the last application dimoxystrobin residues ranged between 0.28 and 1.17 mg/kg. At the intended PHI of 28±1 days residues ranged from <0.01-0.01 mg/kg in sunflower seeds.

In the control samples no residues of either boscalid or dimoxystrobin at or above the limit of quantitation (LOQ) were found.

Table 6.3.2-8: Summary of dimoxystrobin residues in sunflower

Region	Year	Application	DALA[1] (days)	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
					Matrix	BAS 505 F
EU North	2006	BAS 540 01 F	0 21-22 28-29 35-36	68-73	Plants* Seed Seed Seed	0.28-1.17 <0.01 <0.01-0.01 <0.01-0.02

Table 6.3.2-8: Summary of dimoxystrobin residues in sunflower

Region	Year	Application	DALA[1] (days)	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
					Matrix	BAS 505 F
EU South	2006	BAS 540 01 F	0	69-71	Plants*	0.80-1.00
			21		Seed	<0.01-0.01
			28		Seed	<0.01
			35-36		Seed	<0.01-0.01

1 Days after last application
2 At last application
* Without roots

III. CONCLUSION

At the intended PHI of 28±1 days residues ranged from <0.01-0.01 mg/kg in sunflower seeds.

Table 6.3.2-9: Residues of dimoxystrobin in sunflower after two applications of BAS 540 01 F in the EU North

Study details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	GS[2] BBCH	DA LA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF 0648/072364 2007/1006111 BSF/648-01 Yes 2005	Sunflower	Belgium	BAS 540 01 F BAS 505 F: 2 x 0.10	73	0 21 28 35	Plants* Seed Seed Seed	0.69 <0.01 <0.01 <0.01
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF 0648/072364 2007/1006111 BSF/648-02 Yes 2005	Sunflower	The Netherlands	BAS 540 01 F BAS 505 F: 2 x 0.10	68-69	0 22 29 35	Plants* Seed Seed Seed	0.91 <0.01 <0.01 <0.01
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF 0648/072364 2007/1006111 BSF/648-03 Yes 2005	Sunflower	France North	BAS 540 01 F BAS 505 F: 2 x 0.10	71	0 21 28 35	Plants* Seed Seed Seed	0.28 <0.01 0.01 0.02
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF 0648/072364 2007/1006111 BSF/648-04 Yes 2005	Sunflower	France North	BAS 540 01 F BAS 505 F: 2 x 0.10	71	0 21 28 35	Plants* Seed Seed Seed	0.38 <0.01 <0.01 <0.01
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF 0648/072364 2007/1006111 BSF/648-05 Yes 2005	Sunflower	Germany	BAS 540 01 F BAS 505 F: 2 x 0.10	75	0 22 29 36	Plants* Seed Seed Seed	1.17 <0.01 <0.01 <0.01

1) Days after last application
2) At last application
* Without roots

Table 6.3.2-10: Residues of dimoxystrobin in sunflower after two applications of BAS 540 01 F in the EU South

Study details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF 0648/072364 2007/1006111 BSF/648-06 Yes 2005	Sunflower	France South	BAS 540 01 F BAS 505 F: 2 x 0.10	69- 71	0 21 28 36	Plants* Seed Seed Seed	1.00 0.01 <0.01 <0.01
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF 0648/072364 2007/1006111 BSF/648-07 Yes 2005	Sunflower	France South	BAS 540 01 F BAS 505 F: 2 x 0.10	71	0 21 28 35	Plants* Seed Seed Seed	0.80 <0.01 <0.01 0.01

1 Days after last application
2 At last application
* Without roots

Report: CA 6.3.2/3
Majzik E.S., 2005a
Determination of the residues of active ingredients of Pictor (Boscalid/BAS 510 F, Dimoxystrobin/BAS 505 F) in sunflower
2005/1035114
Guidelines: none
GLP: yes
(certified by National Institute of Pharmacy, Budapest, Hungary)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Boscalid, dimoxystrobin BAS 510 F, BAS 505 F
Description: BAS 540 01 F (SC)
Lot/Batch #: 1007, Boscalid: 200 g/L; dimoxystrobin: 200 g/L
Purity: Not reported
CAS#: BAS 505 F: 149961-52-4
Development code: Not relevant
Spiking levels: 0.05-0.5 mg/kg

3. **Test Commodity:**
Crop: Sunflower
Type: Oilseeds
Variety: LG/5665, Barolo, Pioneer A82, Arena PR
Botanical name: *Helianthus annuus*
Crop parts(s) or processed Commodity: Sunflower seeds
Sample size: > 1 kg (from >12 heads)

B. STUDY DESIGN AND METHODS

1. Test procedure

In 2005 four trials were conducted in sunflower in Hungary in order to determine the residue levels of BAS 505 F. BAS 540 01 F (200 g/L BAS 505 F and 200 g/L BAS 510 F, SC) was applied twice to sunflower at a rate of 100 g a.s./ha. The first application was made at BBCH stage 16 to 19 and the second at BBCH stage 67 to 81. The spray volume used was approximately 300 L/ha. Sunflower heads were sampled 29 to 30 DALA (days after last application). Samples were stored deep frozen (-16 to -28°C) for less than 2 months.

Table 6.3.2-11: Target application rates and timings for sunflower

Year	No. of trials	No. of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ timing
2005	4	2	F	BAS 540 01 F (SC)	Boscalid Dimoxystrobin	0.100 0.100	300	1st appl: 6-12 BBCH 2nd appl: after flowering, 30 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analysed for BAS 505 F according to PPSCS SOP No R 302FEJ1. BAS 505 F was extracted with acidic methanol-water solution. The extract was cleaned mechanically and subjected to liquid/liquid extraction. The extract was analysed by LC-MS/MS. The limit of quantitation (LOQ) of the method was 0.05 mg/kg for all samples.

Table 6.3.2-12: Summary of recoveries for dimoxystrobin

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 445/0			BAS 505 F	
Seed	0.05/0.50	6	79.2	5.11

N/A Not applicable

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-13, detailed residue levels are shown in Table 6.3.2-14.

Sunflower seeds obtained from heads collected at the intended PHI of 29 to 30 did not show residues of BAS 505 F at or above the LOQ of the analytical method (0.05 mg/kg).

In the control samples no residues of dimoxystrobin at or above the limit of quantitation (LOQ) were found.

Table 6.3.2-13: Summary of dimoxystrobin residues in sunflower

Region	Year	Application	DALA[1] (days)	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
					Matrix	BAS 505 F
EU North	2005	BAS 540 01 F	29-30	68-73	Seed	<0.05

1 Days after last application
2 At last application

Table 6.3.2-14: Residues of dimoxystrobin in sunflower after two applications of BAS 540 01 F in the EU North

Study Details		Crop	Country	Formulation, Application Rate[0] (kg a.s./ha)	GS[2] BBCH	DA LA[1]	Residues Found (mg/kg)	
							Matrix	BAS 505 F
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	05 BASF AA 2005/1035114 05 BASF AA 0701/1 Yes 2005	Sunflower	Hungary	BAS 540 01 F BAS 505 F: 2 x 0.10	67-69	30	Seed	<0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	05 BASF AA 2005/1035114 05 BASF AA 0701/2 Yes 2005	Sunflower	Hungary	BAS 540 01 F BAS 505 F: 2 x 0.10	67-69	30	Seed	<0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	05 BASF AA 2005/1035114 05 BASF AA 0701/3 Yes 2005	Sunflower	Hungary	BAS 540 01 F BAS 505 F: 2 x 0.10	81	29	Seed	<0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	05 BASF AA 2005/1035114 05 BASF AA 0701/4 Yes 2005	Sunflower	Hungary	BAS 540 01 F BAS 505 F: 2 x 0.10	81	29	Seed	<0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application

III. CONCLUSION

At the PHI of 30±1 days, no residues above the limit of quantitation of BAS 505 F were detected.

Report:	CA 6.3.2/4 Blaschke U., 2006a Residue study (decline) with BAS 540 01 F applied to sunflowers in Northern France, The Netherlands, Germany and Southern France in 2005 2006/1018116
Guidelines:	EEC 96/68, EEC 91/414 Annex II (Part A Section 6), EEC 91/414 Annex III (Part A Section 8), EEC 1607/VI/97 rev. 2 10.06.1999
GLP:	yes (certified by Department of Health of the Government of the United Kingdom, United Kingdom)

I. MATERIAL AND METHODS

A. MATERIALS

1.	**Test Material:**	Boscalid, dimoxystrobin BAS 510 F, BAS 505 F
	Description:	BAS 540 01 F (SC)
	Lot/Batch #:	1011, Boscalid: 200.0 g/L nominal; dimoxystrobin: 200.0 g/L nominal
	Purity:	Not reported
	CAS#:	BAS 505 F: 149961-52-4
	Development code:	Not relevant
	Spiking levels:	
3.	**Test Commodity:**	
	Crop:	Sunflower
	Type:	Oilseeds
	Variety:	Aurasol, Orasol, Sunrich Orange, Altesse, Caramba
	Botanical name:	*Helianthus annuus*
	Crop parts(s) or processed commodity:	Sunflower seeds
	Sample size:	> 1 kg (from >12 heads)

B. STUDY DESIGN AND METHODS

1. Test procedure

In 2005 six trials were conducted in sunflower; 4 in the northern (France, The Netherlands, Germany) and 2 in the southern EU (France) in order to determine the residue levels of BAS 505 F. BAS 540 01 F (200 g/L BAS 505 F and 200 g/L BAS 510 F, SC) was applied twice to the sunflower at a rate of 100 g a.s./ha in spray volumes of 60 to 300 L/ha. The targeted time for the applications was 42 DBH for the first and 28 DBH for the second. Plants without roots were harvested at the day of the last application; seeds were collected 21, 28-29 and 35 days after the last application (DALA).

Table 6.3.2-15: Target application rates and timings for sunflower

Year	No. of trials	No. of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ timing
2005	6	2	F	BAS 540 01 F (SC)	Boscalid Dimoxystrobin	0.100 0.100	300	28±1 DBH 42±1 DBH

DBH Days before harvest

2. Description of analytical procedures

The specimens were analysed for BAS 505 F according to BASF method No 445/0. BAS 505 F was extracted using a mixture of methanol, water and hydrochloric acid. An aliquot was centrifuged and partitioned against cyclohexane. The final determination of the analyte was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method was 0.05 mg/kg for all samples

Table 6.3.2-16: Summary of recoveries for dimoxystrobin

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 445/0			Dimoxystrobin (BAS 505 F)	
Plants without roots	0.05/0.50/50	3	85.7	11.5
Seed	0.05/0.50	4	88.3	7.2

N/A Not applicable

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-17, detailed residue levels are shown in Table 6.3.2-18 and Table 6.3.2-19.

Sunflower seeds obtained from heads collected at the intended harvest time contained residues of BAS 505 F between <0.05 and 0.105 mg/kg.

Control samples did not contain BAS 505 residues at or above the LOQ of the analytical method (0.05 mg/kg).

Table 6.3.2-17: Summary of dimoxystrobin residues in sunflower

Region	Year	Application	DALA[1] (days)	Growth stage[2] (BBCH)	Range of residues (mg/kg)	
					Matrix	BAS 505 F
EU North	2005	BAS 540 01 F	0	67-85	Plants without roots	0.855-3.339
			21		Seed	<0.05-0.151
			28-29		Seed	<0.05-0.105
			35		Seed	<0.05-0.163
EU South	2005	BAS 540 01 F	0	67	Plants without roots	1.659-2.924
			21		Seed	<0.05
			28		Seed	<0.05
			35		Seed	<0.05

1) Days after last application
2) At last application

Table 6.3.2-18: Residues of dimoxystrobin in sunflower after two applications of BAS 540 01 F in the EU North

Study details		Crop	Country	Formulation, application rate[0)] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF/643 2006/1018116 BSF/643-01 Yes 2005	Sunflower	France	BAS 540 01 F BAS 505 F: 2 x 0.10	85	0 21 28 35	Plants* Seed Seed Seed	3.116 <0.05 <0.05 <0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF/643 2006/1018116 BSF/643-02 Yes 2005	Sunflower	France	BAS 540 01 F BAS 505 F: 2 x 0.10	67	0 21 28 35	Plants* Seed Seed Seed	3.339 <0.05 <0.05 <0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF/643 2006/1018116 BSF/643-03 Yes 2005	Sunflower	The Netherlands	BAS 540 01 F BAS 505 F: 2 x 0.10	79-81	0 21 29 35	Plants* Seed Seed Seed	0.855 0.151 0.105 0.163
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF/643 2006/1018116 BSF/643-04 Yes 2005	Sunflower	Germany	BAS 540 01 F BAS 505 F: 2 x 0.10	75	0 21 28 35	Plants* Seed Seed Seed	1.014 <0.05 <0.05 <0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

Table 6.3.2-19: Residues of dimoxystrobin in sunflower after two applications of BAS 540 01 F in the EU South

Study details		Crop	Country	Formulation, application rate[0)] (kg a.s./ha)	GS[2] BBCH	DA LA[1]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF/643 2006/1018116 BSF/643-05 Yes 2005	Sunflower	France	BAS 540 01 F BAS 505 F: 2 x 0.10 each	67	0 21 28 35	Plants* Seed Seed Seed	2.924 <0.05 <0.05 <0.05
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	BSF/643 2006/1018116 BSF/643-06 Yes 2005	Sunflower	France	BAS 540 01 F BAS 505 F: 2 x 0.10 each	67	0 21 28 35	Plants* Seed Seed Seed	1.659 <0.05 <0.05 <0.05

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots

III. CONCLUSION

At the proposed PHI of 30 days ±25% sunflower seeds contained residues of BAS 505 F between <0.05 and 0.105 mg/kg.

Report:	CA 6.3.2/5 Erdmann H.-P., 2015a Residue behaviour of BAS 505 F (Dimoxystrobin) and BAS 510 F (Boscalid) in sunflower after application of BAS 540 01 F under field condition in United Kingdom, Northern and Southern France, The Netherlands, Italy, Spain, Greece Germany 2012 2013/1003723
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5, EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Boscalid, dimoxystrobin BAS 510 F, BAS 505 F
   Description: BAS 540 01 F (SC)
   Lot/Batch #: 0003354823, BAS 510 F: 200 g/L nominal; BAS 505 F: 200 g/L nominal
   Purity: Not reported
   CAS#: BAS 505 F: 149961-52-4
   Development code: Not relevant
   Spiking levels: 0.005, 0.05, 0.50, 3.0

3. **Test Commodity:**
   Crop: Sunflower
   Type: Oilseeds
   Variety: Alisson RW, Diamond, Durban, Metharoc, Mon 92 OL, Sinfoni, Imiko, Sikklos
   Botanical name: *Helianthus annuus*
   Crop parts(s) or processed commodity: Whole plant without roots, rest of plant without roots, seeds
   Sample size: >1 kg (from >12 plants)

B. STUDY DESIGN AND METHODS

1. Test procedure

In 2012 eight trials were conducted in sunflower; four in the northern (Germany, France, United Kingdom, The Netherlands) and four in the southern EU (France, Italy, Spain, Greece). After BAS 540 01 F (200 g/L BAS 505 F and 200 g/L BAS 510 F, SC) was applied once to the sunflower crop at a rate of 0.1 kg a.s./ha at BBCH 71 to 75 in a spray volume of 200 L/ha the residue levels of BAS 505 F were determined. For that purpose sunflower heads were sampled 0, 20-22, 29-31 and 30-54 DALA (days after last application). The samples were stored frozen (generally at -18°C) for a maximum of 656 days until analysis.

Table 6.3.2-20: Target application rates and timings for sunflower

Year	No. of trials	No. of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ timing
2012	8	1	F	BAS 540 01 F (SC)	Boscalid Dimoxystrobin	0.100 0.100	200	BBCH 75

DBH days before harvest

2. Description of analytical procedures

The specimens were analyzed for dimoxystrobin and its *Z*-isomer 505M98 using BASF method No L0076/08, quantifying the analytes with a limit of quantitation (LOQ) of 0.005 mg/kg, each. Dimoxystrobin residues were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane before final determination by LC-MS/MS.

Table 6.3.2-21: Summary of procedural recovery data for dimoxystrobin

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0076/05		**Dimoxystrobin (BAS 505 F)**			***Z*-isomer (505M98)**		
Whole plant w/o roots	0.005, 0.05, 0.5, 3.0	5	95.8	5.9	3	93.8	0.65
Rest of plant w/o roots	0.005, 0.05, 0.5, 3.0	8	91.4	7.5	6	82.9	17
Seeds	0.005, 0.05, 0.5	8	90.2	5.8	6	83.8	7.3

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3.2-22, detailed residue levels are shown in Table 6.3.2-23 and Table 6.3.2-24.

Residues of parent BAS 505 F and the Z-isomer 505M98 in sunflower whole plant without seeds after the last application were 0.047-1.70 mg/kg and <LOQ (<0.005 mg/kg), respectively. Residues of parent BAS 505 F and the Z-isomer 505M98 in sunflower seed, the relevant matrix for human consumption, were <0.005-0.04 mg/kg and <LOQ (<0.005 mg/kg) at the intended PHI of 30 days. Residues of parent BAS 505 F and the Z-isomer 505M98 in sunflower rest of plant without seeds ranged from 0.0094-2.10 mg/kg and from <0.005-0.065 mg/kg, respectively.

Control samples did not contain BAS 505 F or 505M98 residues at or above the LOQ of the analytical method (0.005 mg/kg).

Table 6.3.2-22: Summary of dimoxystrobin residues in sunflower

Region	Year	Application	DALA[1] (days)	Growth stage[2] (BBCH)	Range of residues (mg/kg)		
					Matrix	BAS 505 F	505M98
EU North	2012	BAS 540 01 F	0	71-75[Δ]	Plants*	0.47-1.60	<0.005
			20-22		Seed	<0.005-0.0051	<0.005
			29-30		Seed	<0.005-0.005	<0.005
			49-54		Seed	<0.005	<0.005
			20-22		Rest of plants*	0.0099-0.98	<0.005-0.039
			29-30		Rest of plants*	0.0094-0.76	0.016-0.036
			49-54		Rest of plants*	0.0274-0.057	<0.005-0.0075
EU South	2012	BAS 540 01 F	0	75	Plants*	0.57-1.70	<0.005
			21-22		Seed	<0.005-0.021	<0.005
			29-31		Seed	0.0089-0.040	<0.005
			34-47		Seed	<0.005-0.025	<0.005
			21-22		Rest of plants*	0.19-1.70	0.018-0.047
			29-31		Rest of plants*	0.17-1.00	0.012-0.044
			34-47		Rest of plants*	0.22-2.10	0.016-0.065

1 Days after last application
2 At last application
Δ Uneven development of the plants, main stadium of application was BBCH 75
* Without roots

Table 6.3.2-23: Residues of dimoxystrobin in sunflower after two applications of BAS 540 01 F in the EU North

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)		
							Matrix	BAS 505 F	BAS 505M98
Study code:	390015	Sun-flower	Germany	BAS 540 01 F	75	0	Plants*	0.93	<0.005
Doc ID:	2013/1003723			BAS 505 F:		20	Seed	0.0051	<0.005
Trial No:	L120220			1 x 0.10		29	Seed	<0.005	<0.005
GLP:	Yes					54	Seed	<0.005	<0.005
Year:	2012					20	Rest of plants*	0.027	<0.005
						29	Rest of plants*	0.025	<0.005
						54	Rest of plants*	0.027	<0.005
Study code:	390015	Sun-flower	United Kingdom	BAS 540 01 F	71-75^Δ^	0	Plants*	0.47	<0.005
Doc ID:	2013/1003723			BAS 505 F:		21	Seed	<0.005	<0.005
Trial No:	L120221			1 x 0.10		30	Seed	0.0050	<0.005
GLP:	Yes					21	Rest of plants*	0.0099	<0.005
Year:	2012					30	Rest of plants*	0.0094	<0.005
Study code:	390015	Sun-flower	France	BAS 540 01 F	75	0	Plants*	0.88	<0.005
Doc ID:	2013/1003723			BAS 505 F:		22	Seed	<0.005	<0.005
Trial No:	L120222			1 x 0.10		30	Seed	<0.005	<0.005
GLP:	Yes					22	Rest of plants*	0.98	0.039
Year:	2012					30	Rest of plants*	0.76	0.036
Study code:	390015	Sun-flower	The Netherlands	BAS 540 01 F	75	0	Plants*	1.6	<0.005
Doc ID:	2013/1003723			BAS 505 F:		21	Seed	<0.005	<0.005
Trial No:	L120223			1 x 0.10		29	Seed	<0.005	<0.005
GLP:	Yes					49	Seed	<0.005	<0.005
Year:	2012					21	Rest of plants*	0.035	<0.005
						29	Rest of plants*	0.200	0.0160
						49	Rest of plants*	0.057	0.0075

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
Δ Uneven development of the plants, main stadium of application was BBCH 75
* Without roots
_ Underlined values were taken for MRL calculation (see chapter 6.7)

Table 6.3.2-24: Residues of dimoxystrobin in sunflower after two applications of BAS 540 01 F in the EU South

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)		
							Matrix	BAS 505 F	BAS 505M98
Study code:	390015	Sun-flower	Italy	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plants*	0.57	<0.005
Doc ID:	2013/1003723					22	Seed	0.021	<0.005
Trial No:	L120224					30	Seed	0.040	<0.005
GLP:	Yes					37	Seed	0.025	<0.005
Year:	2012					22	Rest of plants*	0.91	0.046
						30	Rest of plants*	0.88	0.044
						37	Rest of plants*	0.44	0.025
Study code:	390015	Sun-flower	France	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plants*	1.4	<0.005
Doc ID:	2013/1003723					21	Seed	<0.005	<0.005
Trial No:	L120225					29	Seed	0.0089	<0.005
GLP:	Yes					47	Seed	<0.005	<0.005
Year:	2012					21	Rest of plants*	0.19	0.018
						29	Rest of plants*	0.17	0.012
						47	Rest of plants*	0.22	0.016
Study code:	390015	Sun-flower	Spain	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plants*	1.7	<0.005
Doc ID:	2013/1003723					20	Seed	0.018	<0.005
Trial No:	L120226					29	Seed	0.015	<0.005
GLP:	Yes					35	Seed	0.010	<0.005
Year:	2012					20	Rest of plants*	1.7	0.047
						29	Rest of plants*	1.0	0.033
						35	Rest of plants*	2.1	0.065
Study code:	390015	Sun-flower	Greece	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plants*	0.71	<0.005
Doc ID:	2013/1003723					20	Seed	0.0080	<0.005
Trial No:	L120227					31	Seed	0.018	<0.005
GLP:	Yes					34	Seed	0.023	<0.005
Year:	2012					20	Rest of plants*	0.92	0.037
						31	Rest of plants*	0.49	0.030
						34	Rest of plants*	0.51	0.029

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots
_ Underlined values were taken for MRL calculation (see chapter 6.7)

III. CONCLUSION

Residues of parent BAS 505 F and the Z-isomer 505M98 in sunflower seed, the relevant matrix for human consumption, were <0.005-0.04 mg/kg and <LOQ (<0.005 mg/kg) at the intended PHI of 30 days±25%, respectively.

Report: CA 6.3.2/6
Erdmann H.-P., 2015b
Residue behaviour of BAS 505 F (Dimoxystrobin) and BAS 510 F (Boscalid) in sunflower after application of BAS 540 01 F under field condition in Germany, Belgium, Northern and Southern France, The Netherlands, Greece, Italy, Spain, 2013
2014/1010807

Guidelines: EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 (July 22 1997), EEC 7525/VI/95 rev. 9 (March 2011)

GLP: yes
(certified by Land Brandenburg Ministerium fuer Umwelt, Gesundheit und Verbraucherschutz, Potsdam, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Boscalid, dimoxystrobin BAS 510 F, BAS 505 F
Description: BAS 540 01 F (SC)
Lot/Batch #: 0003354823, BAS 510 F: 200 g/L nominal; BAS 505 F: 200 g/L nominal
Purity: Not reported
CAS#: BAS 505 F: 149961-52-4
Development code: Not relevant
Spiking levels: 0.005, 0.05, 0.5, 5.0, 50.0

3. **Test Commodity:**
Crop: Sunflower
Type: Oilseeds
Variety: P64LE25, PR64A31, Violetta, H41, MAS 85 OL, Safira, Sikklos
Botanical name: *Helianthus annuus*
Crop parts(s) or processed commodity: Whole plants without roots, rest of plants without roots, seeds
Sample size: >1 kg (from >12 plants)

B. STUDY DESIGN AND METHODS

1. Test procedure

In 2013 eight trials were conducted in sunflower; four in the northern (Germany, France, United Kingdom, The Netherlands) and four in the southern EU (France, Italy, Spain, Greece). BAS 540 01 F (200 g/L BAS 505 F and 200 g/L BAS 510 F, SC) was applied once to sunflower at a rate of 0.1 kg a.s./ha at BBCH 75, in a spray volume of 200 L/ha. The residue levels of BAS 505 F and its Z-isomer 505M98 were determined in sunflower heads sampled at the day of treatment, 20-22, 29-31 DALA (days after last application) and at BBCH 89 if crop maturity was not reached after 30 ± 1 DALA. The samples were stored for a maximum of 457 days until analysis.

Table 6.3.2-25: Target application rates and timings for sunflower

Year	No. of trials	No. of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/ timing
2013	8	2	F	BAS 540 01 F (SC)	Boscalid Dimoxystrobin	0.100 0.100	200	BBCH 75

2. Description of analytical procedures

The specimens were analyzed for dimoxystrobin and its *Z*-isomer 505M98 using BASF method No L0076/08, quantifying the analytes with a limit of quantitation (LOQ) of 0.005 mg/kg, each. Dimoxystrobin residues were extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned against cyclohexane before final determination by LC-MS/MS.

Table 6.3.2-26: Summary of procedural recovery data for dimoxystrobin

Matrix	Fortification Level (mg/kg)	Summary Recoveries					
		n	Mean (%)	RSD (%)	n	Mean (%)	RSD (%)
BASF method No L0076/0		**Dimoxystrobin (BAS 505 F)**			***Z*-isomer (505M98)**		
Whole plant w/o roots	0.005, 5.0, 50.0	3	92.6	1.6	3	96.7	1.1
Rest of plant w/o roots	0.005, 0.5, 5.0	9	88.8	5.2	9	88.1	5.6
Seeds	0.005, 0.05, 0.5	9	92.1	6.1	9	89.7	5.2

II. RESULTS AND DISCUSSION

A summary of dimoxystrobin residues is shown in Table 6.3.2-27, detailed results are presented in Table 6.3.2-28 and Table 6.3.2-29.

Residues of parent BAS 505 F and the Z-isomer 505M98 in sunflower whole plant without seeds after the last application were 0.50-1.20 mg/kg and <LOQ (<0.005 mg/kg), respectively. Residues of parent BAS 505 F and the Z-isomer 505M98 in sunflower seed, the relevant matrix for human consumption, were <0.005-0.018 mg/kg and <LOQ (<0.005 mg/kg) at the intended PHI of 30 days. Residues of parent BAS 505 F and the Z-isomer 505M98 in sunflower rest of plant without seeds ranged from 0.017-4.90 mg/kg and from <0.005-0.17 mg/kg, respectively.

Control samples did not contain BAS 505 or 505M98 residues at or above the LOQ of the analytical method (0.005 mg/kg).

Table 6.3.2-27: Summary of dimoxystrobin residues in sunflower

Region	Year	Application	DALA[1] (days)	Growth stage[2] (BBCH)	Range of residues (mg/kg)		
					Matrix	BAS 505 F	505M98
EU North	2013	BAS 540 01 F	0	75	Plants*	0.50-1.20	<0.005
			22		Seed	<0.005	<0.005
			29-31		Seed	<0.005-0.013	<0.005
			46-70		Seed	<0.005	<0.005
			22		Rest of plants*	0.042-0.16	<0.005-0.011
			29-31		Rest of plants*	0.017-0.09	<0.005-0.08
			46-70		Rest of plants*	0.055-0.057	<0.005-0.05
EU South	2013	BAS 540 01 F	0	75	Plants*	0.85-1.10	<0.005
			20-22		Seed	<0.005-0.022	<0.005
			30-31		Seed	<0.005-0.018	<0.005
			43-64		Seed	<0.005	<0.005
			21-22		Rest of plants*	0.017-1.50	<0.005-0.045
			30-31		Rest of plants*	0.018-1.10	<0.005-0.045
			43-64		Rest of plants*	0.017-4.90	<0.005-0.17

1 Days after last application
2 At last application
* Without roots

Table 6.3.2-28: Residues of dimoxystrobin in sunflower after application of BAS 540 01 F in the EU North

Study details		Crop	Country	Formulation, application rate[0] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)		
							Matrix	BAS 505 F	BAS 505M98
Study code:	390016	Sun-flower	Germany	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plants*	0.84	<0.005
Doc ID:	2014/101087					22	Seed	<0.005	<0.005
Trial No:	L130078					29	Seed	<0.005	<0.005
GLP:	Yes					70	Seed	<0.005	<0.005
Year:	2013					22	Rest of plants*	0.042	0.005
						29	Rest of plants*	0.043	0.005
						70	Rest of plants*	0.057	0.005
Study code:	390016	Sun-flower	Belgium	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plants*	1.10	<0.005
Doc ID:	2014/101087					22	Seed	<0.005	<0.005
Trial No:	L130079					30	Seed	0.013	<0.005
GLP:	Yes					22	R. of pl. w/o roots	0.16	0.011
Year:	2013					30	R. of pl. w/o roots	0.09	0.008
Study code:	390016	Sun-flower	France	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plants*	0.50	<0.005
Doc ID:	2014/101087					22	Seed	<0.005	<0.005
Trial No:	L130080					29	Seed	<0.005	<0.005
						46	Seed	<0.005	<0.005
GLP:	Yes					22	Rest of plants*	0.081	0.006
Year:	2013					29	Rest of plants*	0.017	<0.005
						46	Rest of plants*	0.055	<0.005
Study code:	390016	Sun-flower	The Netherlands	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plants*	1.2	<0.005
Doc ID:	2014/101087					22	Seed	<0.005	<0.005
Trial No:	L130081					31	Seed	<0.005	0.005
GLP:	Yes					22	Rest of plants*	0.053	<0.005
Year:	2013					31	Rest of plants*	0.019	<0.005

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots
_ Underlined values were taken for MRL calculation (see chapter M-CA 6.7)

Table 6.3.2-29: Residues of dimoxystrobin in sunflower after application of BAS 540 01 F in the EU South

Study details		Crop	Country	Formulation, application rate[0)] (kg a.s./ha)	GS[2] BBCH	DALA[1]	Residues found (mg/kg)		
							Matrix	BAS 505 F	BAS 505M98
Study code:	390016	Sun-flower	Italy	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plant w/o roots	0.85	<0.005
Doc ID:	2014/101087					22	Seed	<0.005	<0.005
Trial No:	L130082					30	Seed	<0.005	<0.005
GLP:	Yes					64	Seed	<0.005	<0.005
Year:	2013					22	R. of pl. w/o roots	0.017	<0.005
						30	R. of pl. w/o roots	0.018	<0.005
						64	R. of pl. w/o roots	0.017	<0.005
Study code:	390016	Sun-flower	France	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plant without roots	1.10	<0.005
Doc ID:	2014/101087					21	Seed	<0.005	<0.005
Trial No:	L130083					30	Seed	<0.005	<0.005
GLP:	Yes					43	Seed	<0.005	<0.005
Year:	2013					21	R. of pl. w/o roots	0.57	0.031
						30	R. of pl. w/o roots	0.29	0.019
						43	R. of pl. w/o roots	0.71	0.047
Study code:	390016	Sun-flower	Spain	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plant without roots	1.1	<0.005
Doc ID:	2014/101087					21	Seed	0.012	<0.005
Trial No:	L130084					30	Seed	0.013	<0.005
GLP:	Yes					51	Seed	<0.005	<0.005
Year:	2013					21	R. of pl. w/o roots	0.94	0.021
						30	R. of pl. w/o roots	1.1	0.028
						51	R. of pl. w/o roots	4.9	0.17
Study code:	390016	Sun-flower	Greece	BAS 540 01 F BAS 505 F: 1 x 0.10	75	0	Plant without roots	1.1	<0.005
Doc ID:	2014/101087					20	Seed	0.022	<0.005
Trial No:	L130085					31	Seed	0.018	<0.005
GLP:	Yes					20	R. of pl. w/o roots	1.5	0.045
Year:	2013					31	R. of pl. w/o roots	0.78	0.045

0 Actual application rates varied by 10% at most
1 Days after last application
2 At last application
* Without roots
_ Underlined values were taken for MRL calculation (see chapter 6.7)

III. CONCLUSION

At the proposed PHI of 30±2 days residues of parent BAS 505 F and the Z-isomer 505M98 in sunflower seed, the relevant matrix for human consumption, were <0.005-0.018 mg/kg and <LOQ (<0.005 mg/kg) at the intended PHI of 30 days.

CA 6.4 Feeding studies

Data/information on a lactating ruminant feeding study for dimoxystrobin were reviewed during the Annex I inclusion process and were considered to be acceptable. The suitability of the study was recently confirmed by EFSA during the re-evaluation of the established MRLs according to Regulation 396/2005, Art. 12.
The following passage was taken directly from the EFSA Reasoned Opinion 2013: 'Considering the calculated values for dietary burden, it is not necessary to define residue of dimoxystrobin in poultry and pigs.'

CA 6.4.1 Poultry

A feeding study in poultry:

(1) is only required where intake is above 0.004 mg/kg bw/day, except in cases where the residue, that is to say the active substance, its metabolites or breakdown products, as defined in the residue definition for risk assessment, tends to accumulate.

and

(2) shall be provided where metabolism studies indicate that residues at levels of above 0.01 mg/kg may occur in edible animal tissue, milk, eggs or fish, taking into account the residue levels in potential feeding stuffs, obtained at the 1 × dose rate, calculated on the dry weight basis.

None of the above prerequisites is fulfilled:

(1) the maximum feed burden calculated (with the OECD calculator) for poultry using feeding stuffs produced under cGAP conditions is 0.00058 mg/kg bw/d and 0.0082 mg/kg feed on a DM basis (see chapter 6.7).

(2) in the metabolism study on poultry (please refer to chapter M-CA 6.2) only one dose level was investigated (0.83 mg/kg bw/d) which was significantly higher than the calculated feed burden of 0.00058 mg/kg bw/d (see chapter M-CA 6.7). Residues in the metabolism study above the trigger of 0.01 mg/kg (LOQ) were only found in liver tissue (0.354 mg/kg; benzyl label). Taking into account the overdosing factor in the metabolism study of 1549 times (12.7 mg/kg DM/0.0082 mg/kg DM) compared to the dose level expected in feed items resulting from the cGAP it is concluded, that no residues are to be expected in poultry products.

It is therefore concluded that no feeding study in laying hens is required.

CA 6.4.2 Ruminants

A feeding study on ruminants:

(1) shall not be required where intake is below 0.004 mg/kg bw/day, except in cases where the residue, that is to say the active substance, its metabolites or breakdown products, as defined in the residue definition for risk assessment, tends to accumulate.

and

(2) shall be provided where metabolism studies indicate that residues at levels of above 0.01 mg/kg may occur in edible animal tissue, milk, eggs or fish, taking into account the residue levels in potential feeding stuffs, obtained at the 1 × dose rate, calculated on the dry weight basis.

None of the above prerequisites is fulfilled:

(1) the maximum feed burden calculated for beef (worst case using EFSA PROFile) using plant feed stuffs produced under cGAP conditions is 0.00066 mg/kg bw/d and 0.0153 mg/kg on a DM basis (see chapter M-CA 6.7).

(2) In the cow feeding study three dose levels were investigated, the lowest was (0.07 mg/kg bw/d) which was about 100 times higher than the dose level of the calculated feed burden. No residues above LOQ (0.025 mg/kg) were found in any ruminant matrix. Thus residues in the feeding study above 0.01 mg/kg are not expected when the overdosing factor of 106 is taken into account (0.07 mg/kg bw/d / 0.00066 mg/kg bw/d).

Therefore no ruminant feeding study is triggered by the residues generated by the present cGAPs. Nevertheless, a feeding study in lactating cows had been submitted to the authorities during the EU review process of dimoxystrobin and has been found adequate.

The following passage is taken directly from the Article 12 evaluation (Reasoned Opinion 2013): 'The RMS also reported a livestock feeding study on lactating cows, which is considered sufficient for deriving MRLs in ruminants.'

CA 6.4.3 Pigs

A feeding study in pigs is only required, if the metabolic pathways differ significantly in pigs as compared to ruminants. This is not the case, since there was no significant difference found between the metabolic pathways in rats and goats (please refer to chapter M-CA 6.2).

Moreover, this passage was taken directly from the EFSA Reasoned Opinion 2013: 'Considering the calculated values for dietary burden, it is not necessary to define residue of dimoxystrobin in poultry and pigs.' Therefore a pig feeding study is not required.

CA 6.4.4 Fish

A feeding study on fish is only required

(1) where significant residues (≥ 0.1 mg/kg of the total diet as received, except special cases, such as active substances which accumulate) occur in crops or part of the crops fed to fish,

and

(2) the log Pow is >3.

No supplementary nature of the residue study in fish is required for dimoxystrobin (BAS 505 F) since:

the calculated maximum feed burden for fish is below the trigger (0.03 mg/kg feed DM for carp, 0.02 mg/kg feed DM for trout; see also M-CA chapter 6.7). Due to this fact, but also driven by the lack of any suitable EU guideline/guidance document for the conduct of fish feeding studies a study was not performed.

CA 6.5 Effects of Processing

A high temperature hydrolysis study was already reviewed during the previous Annex I process. The suitability of the original study was recently confirmed by EFSA during the re-evaluation of the established MRLs according to Reg. 396/2005, Art. 12. As this study did not include testing at 120°C (simulating sterilization), an additional high temperature hydrolysis study has been conducted to complete the existing information (see below report CA 6.5.1/1). Dimoxystrobin was hydrolytically stable under all conditions tested in this study.
A separate high temperature hydrolysis study for 505M09 (proposed residue definition in animal matrices for monitoring and risk assessment) is not considered necessary for the following reasons: i) Commission Regulation EU No. 283/2013 states that a study on the nature of the residue is not required if the level of the residue is below 0.01 mg/kg. While being a considerable part of the residue in ruminant metabolism studies neither 505M09 nor its parent dimoxystrobin were detected in a massively overdosed (106fold at the lowest level) cow feeding study with a LOQ of 0.025 mg/kg. Taking the overdosing factor into account 505M09 is far below the trigger value of 0.01 mg/kg. ii) 505M09s chemical structure differs from its parent dimoxystrobin only by the conversion of one methyl group of the dimethylphenyl moiety into a carboxylic acid. As the hydrolysis susceptible structural elements (amide, oxime), located in dimoxystrobins sidechain, were stable under all tested conditions in the high temperature hydrolysis studies it is highly unlikely that 505M09 will react differently. Therefore it can be considered as covered by the previous high temperature hydrolysis studies.

As they were not submitted during the previous Annex I process, two studies investigating the residues in processed fractions of oilseed rape and sunflowers are also included (see chapter M-CA 6.5.3; reports CA 6.5.3/1 and CA 6.5.3/2). The studies confirm that residues do not accumulate in sunflower and oilseed rape processed products.

CA 6.5.1 Nature of the residue

Report: CA 6.5.1/1
Traub M., 2013a
14C-Dimoxystrobin (BAS 505 F): Simulated processing - Hydrolysis at 90°C, 100°C and 120°C
2013/1400490

Guidelines: EEC 7035/VI/95 rev. 5, OECD Test Guideline 507 - Nature of the residues in processed commodities - High temperature hydrolysis

GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test item

Internal code: BAS 505 F
Reg.No.: 285028
Chem. name: (E)-o-(2,5-dimethylphenoxymethyl)-2-methoxyimino-N-methylphenylacetamide
Molar mass: 326.39 g/mol (unlabeled)
Lot/Batch #: 597-1401, (phenyl-U-14C): 98.5
Specific act.: 4.7 MBq/mg
Radiochem. Purity: 98.9%

2. Test system

An application solution of ^{14}C-labeled test item was prepared with acetonitrile and suspended in aqueous buffer solutions of different pH-values, to give a final concentration of ca. 1.38 µg/mL. The solvent volume did not exceed 1% of the buffer solution.

B. STUDY DESIGN

pH 4 and 90°C - pasteurization
The test solutions were pasteurized in an oil bath for 20 minutes at 90°C. The pH-value remained constant with a pH of 4.02 at the beginning and 4.00 at the end of the test. The test was performed in the dark with three independent samples.

pH 5 and 100°C - baking, brewing, boiling
The test solutions were treated in an oil bath at 100°C for 60 minutes. The pH-value remained constant with a pH of 4.96 at the beginning and at the end of the test. The test was performed in the dark with three independent samples.

pH 6 and 120°C - sterilization
Sterilization of the samples was performed at about 120°C in an autoclave for 20 minutes. The pH-value remained constant with a pH of 5.97 at the beginning and at the end of the test. The test was performed in the dark with three independent samples.

Prior to processing a subsample of the respective test solution was taken out of each test vessel and stabilized with acetonitrile. Upon completion of each of the hydrolytic test conditions, the samples were equilibrated to ambient temperature, and the respective test solution stabilized with acetonitrile. The stabilized samples were analyzed by LSC, HPLC and LC-MS procedures to quantify the amount of test item and to verify the distribution of the test item and possible degradation products.

II. RESULTS & DISCUSSION

The recovery of applied radioactivity was 99-102% (see Table 6.5.1-1) after incubation compared to the radioactivity before incubation.

Table 6.5.1-1: Recovery after processing simulation tests with ^{14}C-BAS 505 F

Process represented	Test conditions	Recovery %
		Total radioactivity*
Pasteurization (1)	pH 4, 90°C, 20 minutes	102
Baking (2)/ brewing (2)/ boiling (2)	pH 5, 100°C, 60 minutes	101
Sterilization (3)	pH 6, 120°C, 20 minutes	99

* Means of three tests

In addition, no significant change in sample weight was obtained following processing. The HPLC and LC-MS results showed that no significant hydrolysis or reaction products were formed under conditions 1 (representative of pasteurization), 2 (representative of baking, brewing, boiling) and 3 (representative of sterilization). Dimoxystrobin was stable under all tested conditions. The isomer distribution of dimoxystrobin did not change under all tested conditions; the ratio of the isomers was stable. The E:Z isomer distribution was approximately 96:4 before and after processing. The radio-HPLC recovery was independently determined twice (102.62% and 99.96%).

III. CONCLUSION

It was demonstrated that no hydrolysis or reaction products were formed under conditions representative of pasteurization, baking, brewing, boiling and sterilization. Furthermore the isomer distribution of dimoxystrobin did not change under all tested conditions. The E:Z isomer distribution was approximately 96:4 before and after processing.

CA 6.5.2 Distribution of the residue in inedible peel and pulp

The distribution between peel and pulp is not applicable for oilseed rape and sunflower crops.

CA 6.5.3 Magnitude of residues in processed commodities

The two studies investigating the residues in processed fractions of oilseed rape and sunflowers are summarized below.

Report: CA 6.5.3/1
Versoi P.L.,Abdel-Baky S., 2001b
The magnitude of BAS 505 F residues in canola seed processed fractions
2001/5000050
Guidelines: EPA 860.1520, PMRA 98-02 Section 10, EEC 7035/VI/95
GLP: yes
(certified by United States Environmental Protection Agency)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Dimoxystrobin, Wettol LF 700 BAS 50501F
Description: BAS 50501F
Lot/Batch #: 97-1: 169.7 g/L
Purity: Not relevant
CAS#: 149961-52-4
Development code: Not reported
Spiking levels: Not reported

2. **Test Commodity:**
Crop: Oilseed rape
Type: Oilseeds
Variety: Hyola 401, Canterra 1867RR, Quest, Golden Ready
Botanical name: *Brassica napus*
Crop parts(s) or processed commodity: Seed RAC, oil, meal, soapstock

B. STUDY DESIGN AND METHODS

1. Test procedure

Canola plants at four locations in Minnesota (1 site), North Dakota (1 site), and the Canadian province of Manitoba (2 sites) were treated with a SC formulation of BAS 505 F in order to determine the magnitude of the residues of the fungicide in canola processed fractions. Each trial, or site, consisted of two plots, one untreated control plot (Treatment 1), and a treated plot (Treatment 3). At each site, two sequential foliar applications of BAS 505 01 F were made to the treated plot targeting an exaggerated rate of 0.302 kg a.s./ha for each application, resulting in an exaggerated seasonal target rate of 0.605 kg a.s./ha; 3 times the proposed EU application rate. Applications were made at a targeted 26 (±1) and 21 (±1) days prior to the anticipated harvest date. All sprays were applied in combination with a locally available spray adjuvant. Canola plants were cut at normal plant maturity, 20 to 22 days after the last application. The plants were allowed to dry in the field for 0 to 7 days before the canola seed (RAC) specimens were collected and placed in frozen storage. Canola seed from the control plots at two sites and from all four 3X treated plots were processed according to simulated commercial procedures into cleaned seed, expeller crude oil, meal, solvent extracted crude oil, refined oil and soapstock. All specimens were held frozen after collection for a maximum of 3-5 months prior to analysis. Available storage stability data indicates residues of BAS 505 F to be stable in various frozen plant matrices, including canola seed, for at least 12 months.

2. Description of analytical procedures

Canola seed RAC and processed commodity samples were analyzed by BASF method Number D9908. Method D9908 measures residues of BAS 505 F as the parent compound, the residue of concern in plants. Residues of BAS 505 F are extracted from canola seed, meal, and soapstock with a 70:25:5 methanol/water/2N HCI mixture. An aliquot of the extract is removed and cleaned by liquid/liquid partition. For oil matrices, residues are extracted by liquid/liquid partitioning using an acetonitrile/hexane mixture. An aliquot of the extract is removed and cleaned by Polar Plus® C_{18} micro column chromatography. The oil extracts are further cleaned-up using the optional Silica Gel Speedisk® micro-column. The final chromatography analysis of BAS 505 F residues is determined by LC-MS/MS. The limit of quantitation (LOQ) for method D9908 for BAS 505 F residues is 0.05 mg/kg. This is the lowest level for which this method has been tested and good fortification recovery data obtained.

II. RESULTS AND DISCUSSION

The efficiency of the method was determined by fortifying control samples of canola seed and processed fractions with BAS 505 F. The overall average recovery from all matrices was 83±11% (n=12). A summary of the residues of BAS 505 F in canola processed commodities derived from canola seed treated at 3X (2 x 0.302 kg a.s./ha) is given in Table 6.5.3-1.

Table 6.5.3-1: Summary of dimoxystrobin (BAS 505 F) residues in oilseed rape RAC and processed fractions

Commodity	BAS 505 residues [mg/kg]	Processing factor [1]	Average processing factor
Canola seed RAC			Not applicable
Site 1	<0.05	Not applicable	
Site 2	0.08	Not applicable	
Site 3	0.09	Not applicable	
Site 4	<0.05	Not applicable	
Cleaned seed			0.9X
Site 1	<0.05	1.00X	
Site 2	<0.05	0.63X	
Site 3	0.09	1.00X	
Site 4	<0.05	1.00X	
Expeller crude oil			0.8X
Site 1	<0.05	1.00X	
Site 2	<0.05	0.63X	
Site 3	<0.05	0.56X	
Site 4	<0.05	1.00X	
Solvent extracted crude oil			0.9X
Site 1	<0.05	1.00X	
Site 2	<0.05	0.63X	
Site 3	<0.05	1.00X	
Site 4	0.09	1.00X	
Meal			0.8X
Site 1	<0.05	1.00X	
Site 2	<0.05	0.63X	
Site 3	<0.05	0.56X	
Site 4	0.05	1.00X	
Refined oil			0.9X
Site 1	<0.05	1.00X	
Site 2	<0.05	0.63X	
Site 3	0.07	0.78X	
Site 4	<0.05	1.00X	
Soapstock			0.9X
Site 1	<0.05	1.00X	
Site 2	<0.05	0.63X	
Site 3	0.10	1.11X	
Site 4	<0.05	1.00X	

1 Processing factor = $\frac{\text{Residue in processed fraction}}{\text{Residue in RAC}}$

III. CONCLUSION

In the final consumer product, refined oil, residue values of dimoxystrobin (BAS 505 F) were found ranging from <0.05 to 0.07 mg/kg with a mean transfer factor of 0.9. Dimoxystrobin residues did not concentrate in any of the processed oilseed rape fractions.

Report: CA 6.5.3/2
Harant H., 2007b
Determination of residues of BAS 510 F (Boscalid) and BAS 505 F (Dimoxystrobin) in sunflower seed and its processed products after two applications of BAS 540 01 F in Germany
2007/1006110

Guidelines: IVA Guideline I-III (1992), BBA IV 3-3, EEC 7029/VI/95 rev. 5, Working document of the Commission of the European Communities Directorate General for Agriculture VI B II-1 Appendix B

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Boscalid, dimoxystrobin BAS 540 01 F
   Description: BAS 540 01 F
   Lot/Batch #: 1011, Boscalid: 200.0 g/L; dimoxystrobin: 200.0 g/L
   Purity: Not relevant
   CAS#: 149961-52-4 dimoxystrobin
   Development code: Not reported
   Spiking levels: Not reported

2. **Test Commodity:**
   Crop: Sunflower
   Type: Oilseeds
   Variety: San Luca, Pegasol
   Botanical name: *Helianthus annuus*
   Crop parts(s) or processed commodity: Seed RAC, raw oil, press cake, soapstock, refined oil
   Sample size: Not reported

B. STUDY DESIGN AND METHODS

1. Test procedure
During the growing season 2006, four field trials were conducted in representative sunflower growing areas in Germany in order to determine the magnitude of the residues of dimoxystrobin (BAS 505 F) in sunflower plants without roots and seed as well as after processing into raw oil, press cake, soapstock after neutralisation and refined oil.

The test item BAS 540 01 F, a SC formulation of boscalid (BAS 510 F) and dimoxystrobin (BAS 505 F) was foliar applied twice at an exaggerated target rate of 1.5 L product/ha (nominal a.s./ha: 0.30 kg BAS 510 F and 0.30 kg BAS 505 F) for each application, resulting in a seasonal target rate of 0.60 kg a.s./ha each. This exaggerated rate compared to the normal rate according to EU label was used in an attempt to generate residue levels sufficiently above the method limit of quantitation (LOQ) in the raw agricultural commodity. The applications were made 42±1 days and 28±1 days before the planned harvest date, using a spray volume of 300 L/ha. Plants without roots were sampled at the day of the last application and at harvest for determination of the residues in the RAC and for further processing into raw oil, press cake, soapstock and refined oil.

2. Description of analytical procedures
All specimens were analyzed with BASF method No 535/2 which quantifies the residues of dimoxystrobin and boscalid with a limit of quantitation of 0.01 mg/kg for each analyte. The results of procedural recovery experiments averaged at about 99% for BAS 505 F at fortification levels between 0.01 and 1.0 mg/kg.
Dimoxystrobin was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of the analytes was performed by HPLC-MS/MS. The limit of quantitation (LOQ) of the method for both analytes is 0.01 mg/kg each.

II. RESULTS AND DISCUSSION

Directly after the application, sunflower plants without roots contained dimoxystrobin between 3.49 and 5.45 mg/kg. Sunflower seed sampled 28 days after application showed residues of dimoxystrobin between 0.02 and 0.08 mg/kg. In raw oil, residues were found to be slightly higher than in the RAC ranging between 0.01 and 0.12 mg/kg. In the fractions press cake and soapstock the residues were lower than in the RAC.

In the final consumer product, which is the refined oil, dimoxystrobin was found between <0.01 and 0.05 mg/kg. Table 6.5.3-2 provides an overview of the analytical results.

Table 6.5.3-2: Summary of dimoxystrobin (BAS 505 F) residues and transfer factors

Commodity	BAS 505 residues [mg/kg]	Transfer factor [1]	Average processing factor
Plant without roots - 0 DALA			
Site 1	5.45	Not applicable	Not applicable
Site 2	3.49	Not applicable	
Site 3	4.86	Not applicable	
Site 4	3.92	Not applicable	
Seed, RAC			
Site 1	**0.02**	1.00X	1.00X
Site 2	**0.08**	1.00X	
Site 3	**0.04**	1.00X	
Site 4	**0.03**	1.00X	
Raw oil			
Site 1	0.05	2.50X	1.40X
Site 2	0.12	1.50X	
Site 3	0.01	0.25X	
Site 4	0.04	1.33X	
Press cake			
Site 1	0.01	0.50X	0.37X
Site 2	0.03	0.38X	
Site 3	< 0.01[1]	0.25X	
Site 4	0.01	0.33X	
Soapstock [2]			
Site 1	< 0.01[1]	0.50X	0.30X
Site 2	0.01	0.13X	
Site 3	< 0.01[1]	0.25X	
Site 4	< 0.01[1]	0.33X	
Refined oil			
Site 1	0.02	1.00X	0.55X
Site 2	0.05	0.63X	
Site 3	< 0.01[1]	0.25X	
Site 4	0.01	0.33X	

[1] For calculation purposes <0.01 is set 0.01
[2] Soapstock after neutralization

III. CONCLUSION

In the final consumer product, refined oil, residue values of dimoxystrobin (BAS 505 F) were found ranging from <0.01 to 0.05 mg/kg with a mean transfer factor of 0.55.

CA 6.6 Residues in Rotational Crops

According to the soil degradation studies (evaluated during the previous EU Review of the active substance), the DT90 value of dimoxystrobin is expected to exceed 1 year which is higher than the trigger value of 100 days (EFSA Scientific Report, 2005). According to the European guidelines on rotational crops (EC, 1997b), further investigation of residues in rotational crops was relevant.

A confined rotational crop study (DocID 2000/1012415) was peer-reviewed during the last Annex I-process. The residue levels and the nature of residues were investigated in three different succeeding crops (radish, lettuce, wheat) at an application rate of 600 g a.s./ha (3 to 6X of actual cGAPs). In the study phenyl-U-^{14}C and benzyl-U-^{14}C labelled dimoxystrobin was applied to bare soil. The study is considered to be scientifically valid and is meeting the requirements established in OECD guideline 502. The assessment was recently confirmed by EFSA during the re-evaluation of the established MRLs according to Reg. 396/2005, Art. 12.

The following conclusion is directly copied from the Reasoned Opinion (see EFSA Journal 2013; 11(11):3464):

Considering the overdosing factor of the above study (ca. 1.5 N) and the fact that dimoxystrobin was applied to bare soil (interception of dimoxystrobin is expected in practice) dimoxystrobin residue levels in commodities from rotational crops are not expected to exceed 0.01 mg/kg. Therefore, a specific residue definition for rotational crops in these crops is not deemed necessary due to the low residue levels expected.

For the sake of completeness a short summary and evaluation of metabolism and magnitude of residues in rotational crops is provided under chapter M-CA 6.6.1 and 6.6.2.

CA 6.6.1 Metabolism in rotational crops

The metabolism of dimoxystrobin in rotational crops - radish, lettuce, wheat - has been evaluated in a confined rotational crop study investigating the nature of residues following different plant-back intervals.

Dimoxystrobin (BAS 505 F) is intensively metabolized in the system soil/plant resulting in several metabolites and a relatively low level of parent. The metabolism of dimoxystrobin in rotational crops involves oxidations of the phenyl ring and of its methyl groups, followed by conjugation on the formed hydroxyl groups with glycosides. Cleavage of parent dimoxystrobin at the connecting ether bridge followed by conjugation was another observed metabolic pathway. In addition, demethylation and oxidation reactions on the side chain and subsequent conjugation reactions were detected in feed items.

Consequently, on the basis of the above findings, EFSA concluded that the metabolic pathway in rotational crops is more extensive than the one observed in primary crops. Considering the overdosing factor of the above study (3 to 6X compared to the actual cGAPs for oilseed rape and sunflower) and the fact that dimoxystrobin was applied to a bare soil (interception of dimoxystrobin by the plants is expected in practice), dimoxystrobin residue levels in commodities from rotational crops are not expected to exceed 0.01 mg/kg. Therefore, a specific residue definition for rotational crops in these crops is not deemed necessary due to the low residue levels expected.

CA 6.6.2 Magnitude of residues in rotational crops

According to Commission Regulation (EC) 283/2013, studies on the magnitude of residues in rotational crops are required under the following circumstances:

If the metabolism studies indicate that residues of the active substance or of relevant metabolites or breakdown products either from plant or soil metabolism may occur (>0.01 mg/kg), limited field studies and, if necessary, field trials shall be carried out.

Studies shall not be required in the following cases:

- no metabolism studies on rotational crops are to be performed, or
- metabolism studies on rotational crops show that no residues of concern are to be expected in rotational crops

Due to the favorable residue behavior of dimoxystrobin in succeeding crops which has been demonstrated with different ring labels and under worst case conditions (bare soil application with exaggerated rates), no residues are expected. Consequently, no higher tier study is required.

CA 6.7 Proposed residue definitions and maximum residue levels

CA 6.7.1 Proposed residue definitions

The residue definitions currently established in the EU and supported in future are compiled in Table 6.7.1-1. In the subsequent section, detailed justifications for BASF's proposal are provided. The proposal is based on a careful evaluation of all studies being available at the time point of submission. Consequently it includes considerations for all crops with established EU MRLs and is not limited to the representative uses in oilseeds only.

Table 6.7.1-1: Residue definitions - dimoxystrobin

End-Point	**Active substance: Dimoxystrobin**	
	EU agreed endpoints (Reasoned opinion on the review of the existing maximum residue levels (MRLs) for dimoxystrobin according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(11):3464, 41 pp.)	Residue definitions proposed in the context of this dossier
Residue definition in plant matrices for risk assessment	Parent compound (dimoxystrobin)	Parent compound (dimoxystrobin)
Residue definition in plant matrices for monitoring	Parent compound (dimoxystrobin)	Parent compound (dimoxystrobin)
Residue definition in animal matrices for risk assessment	Metabolite 505M09, expressed as dimoxystrobin For liver: sum of metabolites 505M09 and 505M76, expressed as dimoxystrobin	Metabolite 505M09, expressed as dimoxystrobin For liver: sum of metabolites 505M09 and 505M76, expressed as dimoxystrobin
Residue definition in animal matrices for monitoring	Dimoxystrobin or alternatively metabolite 505M09, expressed as dimoxystrobin	Metabolite 505M09, expressed as dimoxystrobin
Conversion factors between residue definitions (animal)	Not applicable	Not applicable

For deriving appropriate residue definitions for monitoring and risk assessment purposes the principles described in the following document were considered:

- OECD GUIDANCE DOCUMENT ON THE DEFINITION OF RESIDUE (as revised in 2009), SERIES ON TESTING AND ASSESSMENT No. 63 and SERIES ON PESTICIDES No. 31 (ENV/JM/MONO(2009)30)
- EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment, EFSA Journal 2012;10(07): 2799

The first document covers both aspects whereas the purpose of the PPR Scientific Opinion is limited to the residue definition for risk assessment purposes. The corresponding EU guidance document is in preparation and will be available earliest by end of 2015.

Plant Matrices

For proposing a suitable residue definition in plant and animal matrices, multiple investigations were performed:

- Crop metabolism studies
- Confined rotational crop studies
- Hydrolysis studies at exaggerated temperatures simulating processing

In general dimoxystrobin is metabolized by the following key transformation steps:

- Demethylation/oxidation of the side chain
- Hydroxylation of the aromatic ring system
- Oxidation of the methyl groups at the phenyl ring system
- Cleavage of the ether bridge between the two ring systems

oxidation
cleavage
hydroxylation
demethylation
oxidation
oxidation/demethylation

The contribution of these reactions followed by subsequent conjugation leads to a large number of metabolites (see document N3 for a detailed list). In almost all samples investigated in the course of the metabolism studies parent dimoxystrobin forms by far the predominant residue. Available metabolism studies do not indicate a change in the ratio of E-/Z-isomers.

The representative crops in this dossier are oilseed rape and sunflower and associated with the crop group of pulses & oilseeds. As registration is sought for one crop group only the presentation of metabolism studies in the respective crop group is sufficient. For deriving a suitable residue definition for food of plant origin, two crop metabolism studies in oilseed rape have been conducted. The older one (using two labels: benzyl-U-^{14}C and phenyl-U-^{14}C) was evaluated during the Article 12 review under Regulation (EU) 396/2005 but not during the EU review process for the active substance. This study, and an additional study in oilseed rape (using a phenyl-U-^{14}C label) are presented in this dossier.
A confined rotational crop study was already peer-reviewed during the EU review process for the active substance. It was concluded that if dimoxystrobin is used according to GAP no significant residue levels of dimoxystrobin or metabolites are to be expected. As the study still fulfills the guideline requirements, no additional information will be provided. A field rotational crop study is not required.

A study on the effect of processing on the nature of the residue in oilseeds was evaluated in the context of Article 12 review under Regulation (EU) 396/2005 and during the EU review for the active substance. In a complementary high temperature hydrolysis study included in the present dossier it was demonstrated that dimoxystrobin is also stable under sterilization conditions. The isomer distribution of dimoxystrobin did not change under the tested processing conditions (96:4 before and after processing).

Residue definition for monitoring purposes

According to the OECD Guidance Document, the residue definition for tolerance/MRL enforcement purposes should focus on those analytes which would indicate a possible misuse of the pesticide and which can be easily detected/measured by a broad base of national laboratories (use of a multi-residue method). The analyte(s) to be selected for monitoring purposes should occur in large quantities, and should be common to all commodities in which residues are expected. Ideally, a monitoring method should be based on one single analyte ('marker or indicator compound').

In case of dimoxystrobin and its metabolites in food of plant origin, there is only one component which meets all criteria listed in the OECD guidance document. Based on the plant studies available, in which parent BAS 505 F was the most prominent residue the following residue definition is proposed for monitoring purposes in plant commodities (including process fractions thereof):

Dimoxystrobin, parent only

Residue definition for data generation / risk assessment purposes

The derivation of a suitable residue definition for risk assessment purposes is much more complex; according to the OECD guidance document the contribution of each metabolite/degradate to a potential dietary risk needs to be considered.

In general, two factors must be addressed:

- **Potential for exposure** to the metabolite/degradate in the human diet
- **Relative toxicity** of the metabolite/degradate to the parent

Metabolites/degradates with higher potential exposures and toxicities are more likely to be included in the dietary assessment. The OECD guidance document provides a first hint on how an indicative risk assessment can be performed if metabolites are not readily available as reference substances. For such cases, the document recommends to calculate parent/metabolite ratios from the metabolism studies and to apply these ratios in a second step to the residue level being measured during e.g. supervised field trials. The approach is described much more in detail in the EFSA Scientific Opinion 2799 (2012) which also includes the concept of the threshold of toxicological concern (TTC) as screening tool for pesticide metabolites. Main "purpose" of the TTC concept is to check whether there is negligible exposure.

In order to propose a suitable residue definition for risk assessment purposes, the dimoxystrobin metabolites found in metabolism studies were assigned to five different groups (for details see M-CA 6.9):

- Group 1: Cleaved metabolites 505M01 and 505M80
- Group 2: Hydroxylated metabolites
- Group 3: Carboxylated metabolites 505M09 and 505M81
- Group 4: Metabolites with modified sidechain
- Group 5: Hydroxylated and carboxylated metabolites

The dietary exposure for each metabolite was assessed separately for identifying the contributions of the plant metabolites to the total dietary risk. The assessments were limited to those crops from which a contribution to the dietary risk could be expected.

As no residue data from field trials is available for the different metabolites all assessments are based on metabolite/parent ratios. The relevant chronic and acute exposure assessments for plant commodities are summarized in more detail in M-CA 6.9. The assessments are based on acute and chronic Cramer class III endpoints after proving the absence of genotoxicity (see M-CA 5.8). The individual contributions of the metabolites to the dietary risk are summarized in the tables below.

Table 6.7.1-2: Contribution of dimoxystrobin plant metabolites to chronic dietary risk

Group/Metabolite	ADI used (mg/kg bw/d)	ADI utilization (%)
1-505M01	0.0015 (TTC – Cramer Class III)	0.1
2-505M06	0.0015 (TTC – Cramer Class III)	0.1
5-505M33	0.0015 (TTC – Cramer Class III)	0.4
1-505M80	0.0015 (TTC – Cramer Class III)	4.0
2-505M91	0.0015 (TTC – Cramer Class III)	0.1
2-505M93	0.0015 (TTC – Cramer Class III)	0.1
2-505M95	0.0015 (TTC – Cramer Class III)	0.2

Table 6.7.1-3: Contribution of dimoxystrobin plant metabolites to acute dietary risk

Group/Metabolite	ARfD used (mg/kg bw/d)	ARfD utilization (%)
1-505M01	0.005 (TTC – Cramer Class III)	Max. 1.7 (Scarole)
2-505M06	0.005 (TTC – Cramer Class III)	Max. 3.5 (Scarole)
5-505M33	0.005 (TTC – Cramer Class III)	Max. 2.5 (Carrots)
1-505M80	0.005 (TTC – Cramer Class III)	Max. 1.7 (Scarole)
2-505M91	0.005 (TTC – Cramer Class III)	Max. 1.7 (Scarole)
2-505M93	0.005 (TTC – Cramer Class III)	Max. 3.5 (Scarole)
2-505M95	0.005 (TTC – Cramer Class III)	Max. 5.2 (Scarole)

The data show that the contributions of dimoxystrobin metabolites in plants to the dietary risk are small even under unrealistic worst case assumptions. None of the metabolites should be included in the residue definition for dietary risk assessment. Due to the favorable outcome, no further refinement was performed.

Following an indicative assessment based on an in-depth analysis of all metabolism, residue and processing fraction studies, the residue definition shown below is proposed for risk assessment in plant commodities (including process fractions thereof):

Dimoxystrobin, parent only

Animal matrices

For deriving a suitable residue definition in animal matrices, the following studies were considered:

- Metabolism studies in goats (ruminants)
- Metabolism study in hens
- Magnitude of residue study (cow feeding study)

For dimoxystrobin all relevant studies are available.

In general, dimoxystrobin follows a common pathway in different livestock species, which is comparable to the one observed in rats. As in plant matrices, the following metabolic conversion steps were observed in the relevant studies:

- Demethylation/oxidation of the side chain
- Hydroxylation of the aromatic ring system
- Oxidation of the methyl groups at the aromatic ring system
- Cleavage of the ether bridge between the two ring systems

The available metabolism studies do not indicate a change in the ratio of E-/Z-isomers. Although the major components differed from tissue to tissue, 505M09 was found as a major metabolite in goat milk, liver and kidney, and 505M76 in goat liver and milk. Especially in goat liver, these two metabolites were in the range of 0.05 mg/kg. In poultry there was no major metabolite common to all tissues. Unchanged parent compound was found in all analysed matrices, but except for chicken fat, where it was a major component, only in subordinate amounts. Based on the low absolute and relative amounts of individual metabolites found in the livestock metabolism studies and based on residue transfer calculations, it could be excluded that detectable residues would occur in poultry.

In a livestock feeding study lactating cows were dosed with dimoxystrobin (30 days; 2.5 mg/kg, 7.5 mg/kg and 25 mg/kg dimoxystrobin in the feed corresponding to 1x, 3x and 10x of the expected feed residue level) and analysed for residues of dimoxystrobin and the metabolites 505M76 and 505M09. Residues in all edible matrices were individually below the LOQ of 0.025 mg/kg (and 0.010 mg/kg for milk). It is considered to be unlikely that quantifiable residues will occur in ruminants when fed with feeding stuffs produced in accordance to GAP.

Residue definition for monitoring purposes

For the residue definition in animal commodities the same criteria apply as for plants. The analyte(s) to be selected for monitoring purposes should occur in large quantities, and should be common to all commodities in which residues are expected. Ideally, the monitoring method should be based on one single analyte ('marker or indicator compound').

Based on the studies where metabolite 505M09 formed a considerable part of the residue (milk for small children, meat/fat for all subpopulation groups), the following residue definition is proposed for monitoring purposes in animal commodities:

Metabolite 505M09, expressed as dimoxystrobin

Residue definition for data generation / risk assessment purposes

As in plants, the derivation of a suitable residue definition for risk assessment purposes is much more complex; according to the OECD guidance document the contribution of each metabolite/degradate to a potential dietary risk need to be considered.

In order to propose a suitable residue definition for risk assessment purposes, the dimoxystrobin metabolites found in metabolism studies and in the confined rotational crop study (feed items) were grouped into five groups, following the same selection principles as for plant metabolites. Feed items were included in livestock metabolite assessment based on the rationale that exposure of consumers to feed item metabolites will predominantly occur via livestock commodities.

- Group 1: Cleaved metabolites 505M01 and 505M80
- Group 2: Hydroxylated metabolites
- Group 3: Carboxylated metabolites 505M09 and 505M81
- Group 4: Metabolites with modified sidechains
- Group 5: Hydroxylated and carboxylated metabolites

The dietary exposure to each metabolite was assessed separately for identifying the contributions of the livestock metabolites to the total dietary risk. For deriving residue levels to be used in chronic and acute exposure assessments, the following studies were additionally considered:

- Magnitude of residue study in cows

As no residue data from magnitude of residue studies are available for the majority of the different metabolites most assessments are based on metabolite/parent ratios. This can be considered a worst-case scenario as the LOQ of the analytical method used in the feeding study is used as a factor in deriving the input values for the risk assessment calculations. This procedure results in a significant overestimation of the potential dietary burden. The relevant chronic and acute exposure assessments for livestock commodities are summarized more in detail in section 6.9. The assessments are based on acute and chronic Cramer class III endpoints after proving the absence of genotoxicity (see M-CA 5.8). The individual contributions of the livestock and feed item metabolites to the dietary risk according to the TTC concept are summarized in the tables below.

Table 6.7.1-4: Contribution of dimoxystrobin livestock metabolites to chronic dietary risk

Group/Metabolite	ADI used (mg/kg bw/d)	ADI utilization (%)
2-505M02[a]	0.0015 (TTC – Cramer Class III)	not relevant
2-505M04	0.0015 (TTC – Cramer Class III)	26.4
3-505M09	0.0015 (TTC – Cramer Class III)	21.8
2-505M49	0.0015 (TTC – Cramer Class III)	1.1
2-505M50	0.0015 (TTC – Cramer Class III)	1.5
2-505M51	0.0015 (TTC – Cramer Class III)	0.6
2-505M63[a]	0.0015 (TTC – Cramer Class III)	not relevant
5-505M76	0.0015 (TTC – Cramer Class III)	21.7
2-505M78	0.0015 (TTC – Cramer Class III)	1.2
2-505M79	0.0015 (TTC – Cramer Class III)	2.2
3-505M81	0.0015 (TTC – Cramer Class III)	1.2
2-505M84	0.0015 (TTC – Cramer Class III)	0.7
2-505M86	0.0015 (TTC – Cramer Class III)	26.4
4-505M88[a]	0.0015 (TTC – Cramer Class III)	not relevant
4-505M89[a]	0.0015 (TTC – Cramer Class III)	not relevant
4-505M94[a]	0.0015 (TTC – Cramer Class III)	not relevant
5-505M105	0.0015 (TTC – Cramer Class III)	0.9
2-505M107[a]	0.0015 (TTC – Cramer Class III)	not relevant

[a] metabolite only found in feed items, calculated consumer exposure values assuming dietary intake via products of animal origin are < genotoxicity trigger (0.0000025 mg/kg bw/d)

Table 6.7.1-5: Contribution of Dimoxystrobin livestock metabolites to acute dietary risk

Metabolite	ARfD used (mg/kg bw/d)	ARfD utilization (%])
2-505M02[a]	0.005 (TTC – Cramer Class III)	not relevant
2-505M04	0.005 (TTC – Cramer Class III)	Max 24.8 (bovine milk products)
3-505M09	0.005 (TTC – Cramer Class III)	Max 24.8 (bovine milk products)
2-505M49	0.005 (TTC – Cramer Class III)	Max 16.9 (bovine kidney)
2-505M50	0.005 (TTC – Cramer Class III)	Max 16.3 (bovine liver)
2-505M51	0.005 (TTC – Cramer Class III)	Max 6.1 (bovine liver)
2-505M63[a]	0.005 (TTC – Cramer Class III)	not relevant
5-505M76	0.005 (TTC – Cramer Class III)	Max 24.8 (bovine milk products)
2-505M78	0.005 (TTC – Cramer Class III)	Max 15.1 (bovine kidney)
2-505M79	0.005 (TTC – Cramer Class III)	Max 32.0 (bovine kidney)
3-505M81	0.005 (TTC – Cramer Class III)	Max 12.4 (bovine liver)
2-505M84	0.005 (TTC – Cramer Class III)	Max 9.4 (bovine kidney)
2-505M86	0.005 (TTC – Cramer Class III)	Max 24.8 (bovine milk products)
4-505M88[a]	0.005 (TTC – Cramer Class III)	not relevant
4-505M89[a]	0.005 (TTC – Cramer Class III)	not relevant
4-505M94[a]	0.005 (TTC – Cramer Class III)	not relevant
5-505M105	0.005 (TTC – Cramer Class III)	Max. 9.4 (bovine liver)
2-505M107[a]	0.005 (TTC – Cramer Class III)	not relevant

[a] metabolite only found in feed items, calculated consumer exposure values assuming dietary intake via products of animal origin are < genotoxicity trigger (0.0000025 mg/kg bw/d)

The data show that contributions of the individual metabolites to the dietary risk are small even under unrealistic worst case assumptions used in the assessment. The proposal below is based on the indicative TTC assessment and on the metabolism studies available wherein metabolites 505M09 (liver, kidney, milk) and 505M76 (liver only) formed a considerable part of the residue in food items.

Accordingly, the following residue definitions are proposed for risk assessment in animal commodities:

Risk assessment residue definition in all animal matrices except liver:

Metabolite 505M09, expressed as dimoxystrobin

Risk assessment residue definition for liver:

Metabolites 505M09 + 505M76, expressed as dimoxystrobin.

These risk assessment residue definitions are in alignment with the latest evaluation of dimoxystrobin according to Article 12 Regulation (EC) No 396/2005 (EFSA Journal 2013;11(11):3464, 41 pp) published by EFSA.

CA 6.7.2 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed

The following table shows EU MRLs for dimoxystrobin that entered into force in October, 2012 (Commission Regulation (EU) No 897/2012) (source: http://ec.europa.eu/sanco_pesticides/public/?event=activesubstance.detail&language=EN&selectedID=1251). These MRLs are complemented by the MRLs published after the Article 12 review for dimoxystrobin (see SANCO/11973/2014) and MRLs proposed by BASF in the context of this AIR submission.

Table 6.7.2-1: EU MRLs set[a]/pending[b]/proposed[c] for the uses of dimoxystrobin

Code number	Groups and examples of individual products to which the MRLs apply	Dimoxystrobin
100000	FRUITS, FRESH or FROZEN; TREE NUTS	0.01*[a/b]
200000	VEGETABLES, FRESH or FROZEN	0.01*[a/b]
210000	Root and tuber vegetables	0.01*[a/b]
220000	Bulb vegetables	0.01*[a/b]
230000	Fruiting vegetable	0.01*[a/b]
240000	Brassica vegetables (excluding brassica roots and brassica baby leaf crops)	0.01*[a/b]
250000	Leaf vegetables, herbs and edible flowers	
251000	(a) lettuces and salad plants	0.01*[a/b]
252000	(b) spinaches and similar leaves	0.01*[a/b]
253000	(c) grape leaves and similar species	0.01*[a/b]
254000	(d) watercresses	0.01*[a/b]
255000	(e) witloofs/Belgian endives	0.01*[a/b]
256000	(f) herbs and edible flowers	0.01*[a]/0.02*[b]
260000	Legume vegetables	0.01*[a/b]
270000	Stem vegetables	0.01*[a/b]
280000	Fungi, mosses and lichens	0.01*[a/b]
290000	Algae and prokaryotes organisms	0.01*[a/b]
300000	PULSES, DRY	0.01*[a/b]
400000	OILSEEDS AND OIL FRUITS	
401000	Oilseeds	
401010	Linseeds	0.01*[a/b]/0.2[c]
401020	Peanuts/groundnuts	0.01*[a/b]
401030	Poppy seeds	0.01*[a/b]/0.2[c]
401040	Sesame seeds	0.01*[a/b]
401050	Sunflower seeds	0.3[a/b]
401060	Rapeseeds/canola seeds	0.05[a]/0.05*[b]/0.2[c]
401070	Soyabeans	0.01*[a/b]
401080	Mustard seeds	0.05[a]/0.05*[b]/0.2[c]
401090	Cotton seeds	0.01*[a/b]
401100	Pumpkin seeds	0.01*[a/b]
401110	Safflower seeds	0.01*[a/b]
401120	Borage seeds	0.01*[a/b]
101130	Gold of pleasure seeds	0.01*[a/b]/0.2[c]
401140	Hemp seeds	0.01*[a/b]
401150	Castor beans	0.01*[a/b]
401990	Others (2)	0.01*[a/b]
402000	Oil fruits	0.01*[a/b]

Table 6.7.2-1: EU MRLs set[a]/pending[b]/proposed[c] for the uses of dimoxystrobin

Code number	Groups and examples of individual products to which the MRLs apply	Dimoxystrobin
500000	CEREALS	
500010	Barley	0.1[a]/0.01*[b]
500020	Buckwheat and other pseudo-cereals	0.01*[a/b]
500030	Maize/corn	0.01*[a/b]
500040	Common millet/proso millet	0.01*[a/b]
500050	Oat	0.01*[a/b]
500060	Rice	0.01*[a/b]
500070	Rye	0.08[a/b]
500080	Sorghum	0.01*[a/b]
500090	Wheat	0.1[a]/0.08[b]
500990	Others (2)	0.01*[a/b]
600000	TEAS, COFFEE, HERBAL INFUSION, COCOA AND CAROBS	0.01*[a]/0.05*[b]
700000	HOPS	0.01*[a]/0.05*[b]
800000	SPICES[1]	0.01*[a]/0.05*[b]
900000	SUGAR PLANTS	0.01*[a/b]
1000000	PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	
1010000	Tissues from swine, bovine, sheep, goat, equine, poultry, other farmed terrestrial animals	0.05*[a]/ 0.03*[b]
1020000	Milk	0.01*[a/b]
1030000	Birds' eggs	0.05*[a]/0.02*[b]
1040000	Honey and other apiculture products	0.05*[a/b]
1050000	Amphibians and reptiles	0.05*[a]/0.03*[b]
1060000	Terrestrial invertebrate animals	0.05*[a]/0.03*[b]
1070000	Wild terrestrial vertebrate animals	0.05*[a]/0.03*[b]

* Indicates lower limit of analytical determination
a MRLs according to Reg. (EU) No 897/2012
b MRLs according to SANCO/11973/2014 (pending EU Commission approval), MRLs published in Reasoned opinion on the review of the existing maximum residue levels (MRLs) for dimoxystrobin according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(11):3464, 41 pp.
c MRLs proposed by BASF in the context of this AIR3 submission
1 Pending MRL of 0.05 mg/kg applies for all spices except horseradish, where existing MRL is 0.01 mg/kg according to SANCO/11973/2014

Plant Matrices

For dimoxystrobin in plant and animal matrices EU MRLs have been established according to Reg. (EU) No 897/2012. New MRLs are pending based on SANCO/11973/2014, summarizing the outcome of the recent evaluation according to Article 12 of Reg 396/2005.

MRLs proposed in this dossier are based on residue trials performed after the EU review of dimoxystrobin.
Full sets of additional residue trials have been performed for the representative uses, oilseed rape and sunflower, with the new critical GAPs (see Table 6.7.2-3). Since the new GAP for oilseed rape is more critical than the GAP used before (see Table 6.7.2-2; extended application time period; LOQ decreased from 0.05 mg/kg to 0.005 mg/kg) it was agreed with the RMS Hungary to only use the new residue trials for MRL derivation. The same reasoning applies for sunflower. Although the number of applications decreased from two to one, the timing is later than scheduled in the old GAP and this may impact residue levels critically.

For the sake of completeness, older residue trials that have not yet been evaluated are presented as well. In these trials all residues in oilseed rape were below the LOQ of the analytical method used for data generation at the time (0.05 mg/kg). All residues of the older trials/the residues submitted for Article 12 evaluation (see Table 6.7.2-4) are covered by the MRLs calculated based on the new trial data.

Table 6.7.2-2: GAP for older residue data/data submitted for Article 12 evaluation

GAP Old													
Crop	Outdoor/ Indoor	Formulation			Application data								PHI (days)
Common name		Type	Content		Growth stage		Number		Interval (days)		Min. rate g a.s./ha	Max. rate g a.s./ha	
			Conc g/L	Method	From BBCH	Until BBCH	Min.	Max.	Min.	Max.			
Oilseed rape	Outdoor	SC	200	Foliar spray	14	39	1	2				100	N/A
Sunflower	Outdoor	SC	200	Foliar spray	20	65	1	2				100	28

Table 6.7.2-3: GAP for new oilseed rape and sunflower residue trials submitted in the present dossier

GAP New													
Crop	Outdoor/ Indoor	Formulation			Application data								PHI (days)
Common name		Type	Content		Growth stage		Number		Interval (days)		Min. rate g a.s./ha	Max. rate g a.s./ha	
			Conc g/L	Method	From BBCH	Until BBCH	Min.	Max.	Min.	Max.			
Oilseed rape	Outdoor	SC	200	Foliar spray	20	75	1	2	28	42		100	N/A
Sunflower	Outdoor	SC	200	Foliar spray	51	75	1	1				100	30

The data shown in Table 6.7.2-4 were taken from the 'Reasoned opinion on the review of the existing maximum residue levels (MRLs) for dimoxystrobin according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(11):3464, 41 pp' without alterations. Based on these data the EU MRLs were established (see Table 6.7.2-1).

Table 6.7.2-4: Overview of dimoxystrobin residue data evaluation under Article 12

Commodity	Residue region (a)	Outdoor /Indoor	Individual trial results (mg/kg)		Median residue (mg/kg) (b)	Highest residue (mg/kg) (c)	MRL proposal (mg/kg)	Median CF (d)	Comments
			Enforcement (dimoxystrobin)	Risk assessment (dimoxystrobin)					
Sunflower seed	NEU	Outdoor	5x<0.01; 3x<0.05; 8x<0.1;0.163	5x<0.01; 3x<0.05; 8x<0.1;0.163	0.10	0.16	0.30	1.00	Trials compliant with GAP (EFSA, 2012). MRL OECD=0.26 Rber=0.2 Rmax=0.18
	SEU	Outdoor	<0.01; 0.01; 2x<0.05	<0.01; 0.01; 2x<0.05	0.03	0.05	0.15	1.00	Trials compliant with GAP (EFSA, 2012). MRL OECD=0.12 Rber=0.1 Rmax=0.15
Rape seed	NEU	Outdoor	5x<0.05	5x<0.05	0.05	0.05	0.05	1.00	Trials on rapeseed compliant with critical GAP on mustard seed (EFSA, 2012); trials also acceptable to support less critical GAP on rape seed. MRL OECD= 0.05 Rber= - Rmax= -
	SEU	Outdoor	2x<0.05	2x<0.05	0.05	0.05	0.05	1.00	Trials on rapeseed conducted with a more critical GAP than GAP for rape seed (France, 2013); no authorised use on mustard seed in SEU. MRL OECD= - Rber= - Rmax= -

(a): NEU (Northern and Central Europe), SEU (Southern Europe and Mediterranean), EU (i.e outdoor use) or Import (country code) (EC, 2011).
(b): Median value of the individual trial results according to the enforcement residue definition.
(c): Highest value of the individual trial results according to the enforcement residue definition.
(d): The median conversion factor for enforcement to risk assessment is obtained by calculating the median of the individual conversion factors for each residues trial.

In the chapters below, the new data are evaluated using statistical means and compared with the data being included in the most recent EFSA Reasoned Opinions. For sunflower seeds, the MRL assessments performed in context of the Reasoned Opinion are covering the new data. However, in the new oilseed rape trials higher residues were detected which are not covered by the existing MRL and a new MRL is therefore proposed.

Oilseed rape

Eight trials in oilseed rape were conducted in the EU (8 in the EU North, 8 in the EU South in the period from 2012 to 2014) according to the new critical GAP. Formulation BAS 540 01 F was applied twice at a rate of 0.1 kg dimoxystrobin/ha at target BBCH stages 20 and 75 for the first and second application. The targeted harvest time was determined by plant maturity. The residue results at 28 DALA±25%, as listed below, were used for MRL derivation. If residues where higher at a later than the targeted harvest time these values were used.

EU North outdoor (n=8): <0.005 (6x), 0.0093, 0.013 mg/kg

EU South outdoor (n=8): <0.005 (2x), 0.0092, 0.034, 0.053, 0.063, 0.092, 0.093 mg/kg

Table 6.7.2-5: MRL calculation for dimoxystrobin in oilseed rape based on parent residues (mg/kg)

	Dimoxystrobin [mg/kg]	
	North	**South**
STMR	0.005	0.044
HR	0.013	0.093
OECD	**0.02**	**0.2**

The current **EU MRL** of **0.05 mg/kg** is not sufficient to cover the residues generated by the new cGAP. Therefore it is proposed to raise the EU MRL to

0.2 mg/kg for dimoxystrobin in oilseed rape seed.

According to "Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs" (SANCO 7525/VI/95 rev. 9, March 2011) it is further suggested to extrapolate this MRL to

linseed, mustard seed, poppy seed, gold of pleasure.

Sunflower

Eight trials in sunflower were conducted in the EU (8 in the EU North, 8 in the EU South in the period from 2012 to 2014) according to the new critical GAP. Formulation BAS 540 01 F was applied once at a rate of 0.1 kg dimoxystrobin/ha at target BBCH application stages between 51 and 75. The intended PHI was 30 days. The residue results at PHI 30 days±25%, as listed below, were used for MRL derivation. If residues where higher at a later harvest time these values were used.

EU North outdoor (n=8): <0.005 (6x), 0.005, 0.013 mg/kg

EU South outdoor (n=8): <0.005 (2x), 0.0089, 0.013, 0.015, 0.018, 0.023, 0.04 mg/kg

The current **EU MRL** of **0.3 mg/kg** dimoxystrobin covers all residue values (including HR) originating from the cGAP supported in this dossier for sunflower. No new MRL proposal is needed.

Animal matrices

A worst case diet was derived for different livestock species according the comparison of the feed burdens calculated with the EFSA PROFile and the OECD feed burden calculators. Generally, it is assumed that from each group of crops/commodities the item with the highest potential residue contribution on a dry matter basis is chosen. Then, the total diet is composed beginning with the group representing the highest contribution and filling the rest with feed from the other groups in descending order.

The selection of input commodities is based on the recent EFSA calculations (EFSA Journal 2013;11(11):3464, 41 pp) and the following input values were used for calculation of the maximum dietary burden:

Table 6.7.2-6: Input values of dimoxystrobin for the maximum dietary burden calculations

Commodity	STMR/ $STMR_P$ [mg/kg]	HR / HR_P [mg/kg]	Origin
Oilseed rape seed	0.044	N/A	STMR based on residue trials according to new intended GAP
Oilseed rape meal	0.036	N/A	$STMR_P$ (based on residue trials according to new intended GAP) =0.044 mg/kg x PF 0.82*
Canola meal	0.036	N/A	$STMR_P$ (based on residue trials according to new intended GAP) =0.044 mg/kg x PF 0.82*
Sunflower seed	0.014	N/A	$STMR_P$ = value in seed of new residue trials (according to current cGAP)
Sunflower seed meal	0.005	N/A	$STMR_P$ = value in seed of new residue trials (according to current cGAP) 0.014 mg/kg x 0.36*

* Processing factors taken from EFSA Conclusion 2013; EFSA Journal 2013;11(11):3464, 41 pp

Table 6.7.2-7: Estimated maximum dietary burden calculation of dimoxystrobin residues for ruminants

Commodity	Dry matter content (%)	Residue level (STMR/ $STMR_P$ mg/kg)	Dairy cattle*		Beef cattle**		Lamb***	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
Oilseed meals								
Rape/canola	86[1]/88[2]	0.044/ 0.036	30[1]/10[2]	0.00056[1]/ 0.00016[2]	30[1]/20[2]	0.00066[1]/ 0.00020[2]	15[2]	0.00026[2]
Dietary burden:	mg/kg bw/day		0.00056[1]/0.00016[2]		0.00066[1]/0.00020[2]		0.00026[2]	
	mg/animal/day		0.28[1]/0.10[2]		0.23[1]/0.10[2]		0.01[2]	
	mg/kg total feed (DM)		0.0153[1]/0.0041[2]		0.0153[1]/0.0082[2]		0.0061[2]	

* EFSA PROFile: Feed intake 20 kg DM, body weight (bw) 550 kg; OECD: Feed intake 25 kg DM, body weight 650 kg
** EFSA PROFile: Feed intake 15 kg DM, body weight (bw) 350 kg, OECD: Feed intake 12 kg DM, body weight 500 kg
*** OECD: Feed intake 1.7 kg, body weight 40 kg
1 Calculated with the EFSA PROFile feed burden calculator
2 Calculated with the OECD feed burden calculator

Table 6.7.2-8: Estimated maximum dietary burden calculation of dimoxystrobin residues for poultry and pigs

Commodity	Dry matter content (%)	Residue level (STMR/ STMRP mg/kg)	Poultry*		Pigs**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
Oilseed meals						
Rape/canola seed	86[1]/88[2]	0.044/0.036	10[1]/20[2]	0.00032[1]/0.00058[2Δ]	20[1]/20[2]	0.00041[1]/0.00025[2]
Dietary burden:		mg/kg bw/day	0.00032[1]/0.00058[2]		0.00041[1]/0.00025[2]	
		mg/animal/day	0.00061[1]/0.0041[2]		0.041[1]/0.025[2]	
		mg/kg total feed (DM)	0.0051[1]/0.0082[2]		0.010[1]/0.0082[2]	

* EFSA PROFile: Feed intake 0.12 kg DM, body weight (bw) 1.7 kg, OECD: Feed intake turkey 0.5 kg DM, body weight 7 kg

** EFSA PROFile: Feed intake swine 3 kg DM, body weight (bw) 75 kg, OECD: Feed intake finishing swine 3 kg DM, body weight 100 kg

1 Calculated with the EFSA PROFile feed burden calculator

2 Calculated with the OECD feed burden calculator

Proposed animal MRLs

Thus, the doses (mg/kg bw/d) calculated for ruminants and pigs with the EFSA PROFile dietary burden calculator and for poultry with the OECD calculator result in the most critical values and will consequently be used for estimating the maximum residues in products of animal origin:

dairy cattle	0.00056 mg/kg bw/d (0.0153 mg/kg DM)
beef	0.00066 mg/kg bw/d (0.0153 mg/kg DM)
poultry	0.00058 mg/kg bw/d (0.0082 mg/kg DM)
pigs	0.00041 mg/kg bw/d (0.0100 mg/kg DM)

Ruminants

A residue transfer study with dimoxystrobin was conducted in cows, the actual dose levels were 45.6, 113.7, and 435.4 mg BAS 505 F/animal/day which corresponds to 0.07, 0.18, and 0.64 mg/kg bw/day. The maximum dietary burden values calculated for beef were 0.00066 mg/kg bw/d and for dairy cow were 0.00056 mg/kg bw/d. These values are 106 and 125 times lower than the lowest dose level of the cow feeding study. When samples were analysed for BAS 505 F and metabolites 505M09 (BF 505-8) and 505M76 (BF 505-11), no residues were detected in any sample (LOQ: 0.01 mg/kg for milk and 0.025 mg/kg for tissues), even at the highest dose level. No residue at or above the LOQ in any matrix is anticipated when the dilution factor between the calculated feed burden and the actual dose level in the feeding study of 106 is taken into account. Therefore, no residues above the pending MRL of 0.03 mg/kg for ruminant tissues/offals and 0.01 mg/kg for milk (SANCO/11973/2014) are anticipated. No new MRLs are proposed.

Poultry

No chicken feeding study is required as justified in MCA 6.4: The calculated feed burden is <0.004 mg/kg bw/d, the residues in the total diet are <0.01 mg/kg feed DM (0.008 mg/kg DM) and no significant residues (above the limit of determination) occurred in in any edible animal tissue in metabolism studies at the 1X dose rate.
Therefore, no residues above the pending MRLs of 0.03 mg/kg in poultry tissues/offals and 0.02 mg/kg in eggs (SANCO/11973/2014) are anticipated. No new MRLs are proposed.

Pig

Given the similar metabolic pathways in rats, hens and goats no separate pig feeding study had to be performed. The residue situation in pig products can be extrapolated from the cow feeding study instead. Here, the minimum dose level was 45.6 mg/animal/day which corresponded to 0.07 mg/kg bw/day. This is about 171 times the anticipated dietary burden for pigs (0.00041 mg/kg) based on maximum residues from the intended uses in this dossier. When samples from the cow feeding were analyzed for unchanged BAS 505 F and metabolites 505M09 (BF 505-8) and 505M76 (BF 505-11) according to method D006 sample (LOQ: 0.025 mg/kg), no residues were detected in any sample, even at the highest feeding level. No residue at or above the LOQ in any swine matrix is anticipated when the dilution factor between the calculated feed burden and the actual dose level in the feeding study are taken into account.
Therefore, no residues above the pending MRLs of 0.03 mg/kg (SANCO/11973/2014) in swine products are anticipated. No new MRLs are proposed.

Fish

For calculation purposes, the procedure specified in the EU Working Document SANCO/11187/2013 on the nature of pesticide residues in fish (as of 31 January 2013) has been used. According to the working document, fish diet for trout and carp mainly consists of cereals, pulses, oilseeds and processed fractions thereof. The input values for calculation of the fish feed burden are shown in Table 6.7.2-6. Additionally, for vegetable oil an input value of 0.039 mg/kg (based on STRM oilseed rape seed 0.044 mg/kg x PF 0.9 for refined oil taken from EFSA Reasoned Opinion 2013) was used.

The calculation resulted in the following approximate maximum feed burdens:

- Trout: 0.02 mg /kg feed DM
- Carp: 0.03 mg/kg feed DM

The calculated feed burden is below the trigger for fish metabolism and fish feeding studies of 0.1 mg/kg DM and therefore no proposal of a future EU MRL for fish commodities is provided.

Honey

Four field trials were conducted in representative oilseed rape growing areas in Germany to determine the magnitude of the residues in oilseed rape honey (see also MCA 6.10). Formulation BAS 540 01 F (SC) was foliar applied to winter oilseed rape once at a target rate of 0.1 kg dimoxystrobin/ha. The application was conducted during full flowering at BBCH 65, the most critical time for honey production. The residue results of honey specimens sampled are listed below.

EU North outdoor: <0.01 (4x) mg/kg

All residues in honey are well covered by the established **EU MRL** of **0.05 mg/kg**.

CA 6.7.3 Proposed maximum residue levels (MRLs) and justification of the acceptability of the levels proposed for imported products (import tolerance)

No imported products containing dimoxystrobin residues are to be considered at the time of AIR3 dossier submission.

CA 6.8 Proposed safety intervals

Dimoxystrobin is intended for post emergence use (BBCH 20-75 for oilseed rape and BBCH 51-75 for sunflower). Residue trials have been conducted with applications made at the latest recommended crop growth stage with harvest taking place at the time of crop maturity following good agricultural practice.

Pre-harvest interval

In oilseed rape, applications are intended at growth stages BBCH 20-75. No specific pre-harvest interval is defined; the interval between last application and crop harvest is defined by crop maturity. In sunflower, one post emergence application at the growth stage BBCH 51-75 is intended with a pre-harvest interval of 30 days.

Re-entry period (in days) for livestock, to areas to be grazed

Because dimoxystrobin is not intended to be used in areas to be grazed, no re-entry period for livestock has to be defined.

Re-entry period for man to crops, buildings or spaces treated

Re-entry assessments are provided for the representative uses in the supplemental product dossier (see M-CP 7.2). It was concluded that there is no unacceptable risk anticipated for workers wearing adequate working clothing when re-entering crops treated with BAS 540 01 F after the spray dilute has dried.

Withholding period (in days) for animal feeding stuffs

The products of treated sunflower and oilseed rape (referred to as meal) may be used as fodder for livestock. Dimoxystrobin derived residues in those feed items are assessed in this dossier by providing the respective calculations of livestock dietary burdens MRLs (see M-CA 6.7) for animal products covering the intended uses. There is no additional withholding period needed for animal feeds with regard to dimoxystrobin derived residues.

Waiting period between last application and sowing or planting

No waiting period is necessary since dimoxystrobin containing products are intended for post emergence use.

Waiting periods between application and handling treated products

Not relevant since a post-harvest treatment is not intended.

Waiting period before sowing/planting succeeding crops

Due to the fact that no accumulation of dimoxystrobin or its degradation products were observed in the confined rotational crop study (see M-CA 6.6), no limitation concerning succeeding crops is necessary.

CA 6.9 Estimation of the potential and actual exposure through diet and other sources

Assessments of the potential chronic and acute dietary consumer risk due to exposure to residues of dimoxystrobin were performed using the EFSA model for chronic and acute risk assessment - rev.2.0 (PRIMo). The EFSA model was used since it considers all the different diets and consumer groups in the EU. The toxicological profile of dimoxystrobin was evaluated in the framework of Directive 91/414/EEC, the revised ADI and ARfD values based on this review are summarized in Table 6.9-1.

Table 6.9-1: Toxicological endpoints - dimoxystrobin

Endpoint	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.02 mg/kg bw/d	Rat (adult), 7-day study; 18-month mouse	200*,**	Data presented in this dossier (M-CA 5.2), EFSA Conclusion (EFSA Scientific Report (2005) 46, 1-82, Conclusion on the peer review of dimoxystrobin),
Acute Reference Dose (ARfD)	0.04 mg/kg bw	Rat (adult), 7-day study	100*	

* During the peer-review, experts agreed to base the toxicological reference values on the NOAEL of 4 mg/kg bw/day and applied a safety factor of 1000 based on assumed 10-fold higher iron utilization in younger animals. From a new mechanistic study in young Wistar rats (21 days old; 7 days administration of dimoxystrobin via the diet), there is no evidence, that young animals are more sensitive than adults (with regard to serum iron levels and anemia). Furthermore, a new enhanced one-generation reproduction toxicity study supports that the NOAEL in young and adult rats is comparable. The same NOAELs for young and adults have been derived from these studies. Therefore, no additional safety factor is warranted based on susceptibility of young animals (for details see M-CA 5.2).
** additional safety factor of 2 due to an assumption that there is variability of iron requirements amongst humans

The ADI and ARfD values used for metabolites 505M09 and 505M76 (being part of the residue definition for risk assessment in animal matrices) are included in Table 6.9-2. Based on their structural similarity to BAS 505 F, the use of the parent ADI and ARfD is feasible (see evaluation according to article 12 of Regulation (EC) No 396/2005, EFSA Journal 2013,11(11), 3464). However, it was agreed with the RMS Hungary to use the stricter Cramer Class III TTC endpoints in the dietary risk assessment of all dimoxystrobin metabolites. The risk assessment for 505M09 and 505M76 according to the TTC concept is provided within the indicative risk assessment (see below).

Table 6.9-2: Toxicological endpoints – Metabolites

Group No.	Metabolite Code	Acceptable Daily Intake (ADI)	Study	Safety factor	Acute Reference Dose (ARfD)	Study	Safety factor	Reference
Group 1	505M01 505M80	0.0015 mg/kg bw/d	None	Not relevant	0.005 mg/kg bw	None	Not relevant	TTC
Group 2	505M02* 505M04 505M06 505M49 505M50 505M51 505M63* 505M78 505M79 505M84 505M86 505M91 505M93 505M95 505M107*	0.0015 mg/kg bw/d	None	Not relevant	0.005 mg/kg bw	None	Not relevant	TTC
Group 3	505M09 505M81	0.0015 mg/kg bw/d	None	Not relevant	0.005 mg/kg bw	None	Not relevant	TTC
Group 4	505M88* 505M89* 505M94*	-	-	-	-	-	-	-
Group 5	505M33 505M76 505M105	0.0015 mg/kg bw/d	None	Not relevant	0.005 mg/kg bw	None	Not relevant	TTC

* feed item. Included in the table for clarification purposes

Acceptable Daily Intake (ADI) and Dietary Exposure Calculation

Dimoxystrobin

TMDI calculation

A chronic exposure assessment was performed, for which all crops and maximum residue levels used are summarized in Table 6.9-3. The risk assessment was based on established MRLs listed in Regulation (EU) No 897/2012, supplemented by MRLs pending according to the Article 12 evaluation (SANCO/11973/2014) and by MRLs proposed by BASF in the present dossier. For the assessment, an ADI of 0.02 mg/kg bw/day was used. Using PRIMo the TMDI for dimoxystrobin has been simultaneously calculated for all relevant consumer groups in the different EU countries.

Table 6.9-3: Dimoxystrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	Dimoxystrobin
100000	FRUITS, FRESH or FROZEN; TREE NUTS	0.01*[a/b]
200000	VEGETABLES, FRESH or FROZEN	0.01*[a/b]
210000	Root and tuber vegetables	0.01*[a/b]
220000	Bulb vegetables	0.01*[a/b]
230000	Fruiting vegetable	0.01*[a/b]
240000	Brassica vegetables (excluding brassica roots and brassica baby leaf crops)	0.01*[a/b]
250000	Leaf vegetables, herbs and edible flowers	
251000	(a) lettuces and salad plants	0.01*[a/b]
252000	(b) spinaches and similar leaves	0.01*[a/b]
253000	(c) grape leaves and similar species	0.01*[a/b]
254000	(d) watercresses	0.01*[a/b]
255000	(e) witloofs/Belgian endives	0.01*[a/b]
256000	(f) herbs and edible flowers	0.01*[a]/0.02*[b]
260000	Legume vegetables	0.01*[a/b]
270000	Stem vegetables	0.01*[a/b]
280000	Fungi, mosses and lichens	0.01*[a/b]
290000	Algae and prokaryotes organisms	0.01*[a/b]
300000	PULSES, DRY	0.01*[a/b]
400000	OILSEEDS AND OIL FRUITS	
401000	Oilseeds	
401010	Linseeds	0.01*[a/b]/0.2[c]
401020	Peanuts/groundnuts	0.01*[a/b]
401030	Poppy seeds	0.01*[a/b]/0.2[c]
401040	Sesame seeds	0.01*[a/b]
401050	Sunflower seeds	0.3[a/b]
401060	Rapeseeds/canola seeds	0.05[a]/0.05*[b]/0.2[c]
401070	Soyabeans	0.01*[a/b]
401080	Mustard seeds	0.05[a]/0.05*[b]/0.2[c]
401090	Cotton seeds	0.01*[a/b]
401100	Pumpkin seeds	0.01*[a/b]
401110	Safflower seeds	0.01*[a/b]
401120	Borage seeds	0.01*[a/b]
101130	Gold of pleasure seeds	0.01*[a/b]/0.2[c]
401140	Hemp seeds	0.01*[a/b]
401150	Castor beans	0.01*[a/b]
401990	Others (2)	0.01*[a/b]
402000	Oil fruits	0.01*[a/b]
500000	CEREALS	
500010	Barley	0.1[a]/0.01*[b]
500020	Buckwheat and other pseudo-cereals	0.01*[a/b]
500030	Maize/corn	0.01*[a/b]
500040	Common millet/proso millet	0.01*[a/b]
500050	Oat	0.01*[a/b]
500060	Rice	0.01*[a/b]
500070	Rye	0.08[a/b]
500080	Sorghum	0.01*[a/b]
500090	Wheat	0.1[a]/0.08[b]
500990	Others (2)	0.01*[a/b]

Table 6.9-3: Dimoxystrobin - MRL values used for risk assessment

Code number	Groups and examples of individual products to which the MRLs apply	Dimoxystrobin
600000	TEAS, COFFEE, HERBAL INFUSION, COCOA AND CAROBS	0.01*[a]/0.05*[b]
700000	HOPS	0.01*[a]/0.05*[b]
800000	SPICES[1]	0.01*[a]/0.05*[b]
900000	SUGAR PLANTS	0.01*[a/b]
1000000	PRODUCTS OF ANIMAL ORIGIN -TERRESTRIAL ANIMALS	
1010000	Tissues from swine, bovine, sheep, goat, equine, poultry, other farmed terrestrial animals	0.05*[a]/ 0.03*[b]
1020000	Milk	0.01*[a/b]
1030000	Birds' eggs	0.05*[a]/0.02*[b]
1040000	Honey and other apiculture products	0.05*[a/b]
1050000	Amphibians and reptiles	0.05*[a]/0.03*[b]
1060000	Terrestrial invertebrate animals	0.05*[a]/0.03*[b]
1070000	Wild terrestrial vertebrate animals	0.05*[a]/0.03*[b]

* Indicates lower limit of analytical determination
a MRLs according to Regulation (EU) No 897/2012, entered into force on October 22, 2012
b Reasoned opinion on the review of the existing maximum residue levels (MRLs) for dimoxystrobin according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2013;11(11):3464, 41 pp.
c MRLs proposed by BASF in the context of this AIR3 submission

The summary of the long term risk assessment is presented in Table 6.9-4. With the EFSA model for chronic exposure the ADI utilization ranges from 0.5% to 6.9%. The diet with the highest TMDI is "WHO Cluster diet B" with 6.9% of the ADI. For this diet, the highest contributors are wheat and sunflower seed with 3.4% and 1.1% of the ADI, respectively. The diet with the second highest TMDI is “DK child” with 6.0% of the ADI, in which wheat and rye are the major contributors with 2.2% and 1.8% of the ADI, respectively.

For the sake of completeness a chronic exposure assessment using only the representative crops is reported in Table 6.9-6. The ADI utilization ranges from 0% to 1.1%. The diet with the highest TMDI is "WHO Cluster diet B" with 1.1% of the ADI. For this diet, the highest contributor are sunflower seeds with 1.1%. The diet with the second highest TMDI is “WHO Cluster diet E” with 1.1% of the ADI, in which rape and sunflower seeds are the major contributors with 0.6% and 0.5% of the ADI, respectively.

According to the presented TMDI calculation a chronic intake of dimoxystrobin residues is unlikely to present a public health concern. A similar outcome was obtained using the lower ADI values published in the recent conclusion on the peer review of dimoxystrobin (EFSA Scientific Report (2005) 46, 1-82).

Metabolites

The main purpose of the information presented below is to support the development of a robust residue definition for risk assessment purposes. Therefore, the data being summarized are going far beyond the scope of this dossier which supports the uses in oilseed rape and sunflower. The indicative assessment is based on the available exposure data. This includes extrapolations for feed item metabolites and the use of metabolism studies, rotational crop studies and magnitude of the residue studies for a comprehensive evaluation of residue input values.

For facilitating the evaluation of the performed exposure assessment, the relevant data for the metabolites are derived in a separate report.

Report: CA 6.9/1
Funk D.,Schaffert D., 2015a
Supplementary information - Dimoxystrobin BAS 505 F - Application of the TTC-Concept to BAS 505 F metabolites in plant and animal commodities
2015/1158034
Guidelines: none
GLP: no

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** BAS 505 F (Dimoxystrobin, Reg. No. 285 028)
Description: not relevant
Lot/Batch #: not relevant
Purity: not relevant
CAS#: 149961-52-4
Development code: not applicable
Spiking levels: not relevant

3. **Test Commodity:**
Crop: not relevant
Type: not relevant
Variety: not relevant
Botanical name: not relevant
Crop parts(s) or processed
Commodity: not relevant
Sample size: not relevant

B. STUDY DESIGN AND METHODS

In order to assess the contribution of dimoxystrobin metabolites to the chronic dietary risk, the metabolites being present in the plant and livestock metabolism studies were assigned to five groups. The grouping was performed based on structural elements.

- Group 1: contains the cleavage products 505M01 and 505M80 (conjugate of 505M01)
- Group 2: contains the majority of dimoxystrobin metabolites with hydroxylations at the aromatic ring system (including methyl groups) and their conjugates
- Group 3: contains all carboxylated metabolites (via oxidation of methyl groups of the aromatic ring system) and their conjugates.
- Group 4: contains metabolites with modifications at the sidechain and their conjugates.
- Group 5: contains metabolites with hydroxylations and carboxylation at the aromatic ring system and their conjugates

Depending on the availability of exposure data, the input data for the exposure assessment are derived differently for the individual groups.

Group 1:
Input values were derived from confined rotational crop and livestock metabolism studies.

Group 2:
Input values were derived from a cow feeding study, a confined rotational crop study and livestock metabolism studies.

Group 3:
Input values for group 3 metabolites were derived from livestock metabolism studies and a cow feeding study.

Group 4:
Input values for metabolites from group 4 were derived from a confined rotational crop study.

Group 5:
Input values were derived from a confined rotational crop study, livestock metabolism studies and a cow feeding study.

II. RESULTS AND DISCUSSION

Group 1 – Cleavage products

Group 1 – Metabolite 505M01
With the current EFSA model the chronic risk assessment ranges from 0 to 0.1% of ADI. The diet with the highest TMDI is "NL child" with 0.1% of ADI. For this diet the highest contributors are leaf vegetables & fresh herbs.

Group 1 – Metabolite 505M80
With the current EFSA model the chronic risk assessment ranges from 0.3 to 4.0% of ADI. The diet with the highest TMDI is "WHO Cluster Diet B" with 4.0% of ADI. For this diet cereals are the highest contributors.

Group 2 – Hydroxylation products

Group 2 – Metabolite 505M02
Metabolite 505M02 is only found in feed items. The estimation of the exposure to feed metabolites through consumption of products of animal origin resulted in residue amounts below the genotoxicity trigger (0.0000025 mg/kg bw/d). Due to this negligible exposure, no chronic risk assessment was performed.

Group 2 – Metabolite 505M04
With the current EFSA model the chronic risk assessment ranges from 1.8 to 26.4% of ADI. The diet with the highest TMDI is "FR toddler" with 26.4% of ADI. For this diet the highest contributing group are cattle dairy products with 26.4% of ADI.

Group 2 – Metabolite 505M06
With the current EFSA model the chronic risk assessment ranges from 0 to 0.1% of ADI. The diet with the highest TMDI is "NL child" with 0.1% of ADI. For this diet the highest contributors are leaf vegetables & fresh herbs.

Group 2 – Metabolite 505M49
With the current EFSA model the chronic risk assessment ranges from 0 to 1.1% of ADI. The diet with the highest TMDI is "WHO Cluster Diet B" with 1.1% of ADI. For this diet the highest contributor is bovine kidney with 1.1% of ADI.

Group 2 – Metabolite 505M50
With the current EFSA model the chronic risk assessment ranges from 0.1 to 1.5% of ADI. The diet with the highest TMDI is "IE Adult" with 1.5% of ADI. For this diet the highest contributors is sheep liver.

Group 2 – Metabolite 505M51
With the current EFSA model the chronic risk assessment ranges from 0 to 0.6% of ADI. The diet with the highest TMDI is "IE adult" with 0.6% of ADI. For this diet the highest contributor is sheep liver with 0.6% of ADI.

Group 2 – Metabolite 505M63
Metabolite 505M63 is only found in feed items. The estimation of the exposure to feed metabolites through consumption of products of animal origin resulted in residue amounts below the genotoxicity trigger (0.0000025 mg/kg bw/d). Due to this negligible exposure, no chronic risk assessment was performed.

Group 2 – Metabolite 505M78
With the current EFSA model the chronic risk assessment ranges from 0 to 1.2% of ADI. The diet with the highest TMDI is "WHO Cluster Diet B" with 1.2% of ADI. For this diet the highest contributor is bovine liver with 1.0% of ADI.

Group 2 – Metabolite 505M79
With the current EFSA model the chronic risk assessment ranges from 0 to 2.2% of ADI. The diet with the highest TMDI is "WHO Cluster Diet B" with 2.2% of ADI. For this diet the highest contributor is bovine kidney with 2.1% of ADI.

Group 2 – Metabolite 505M84
With the current EFSA model the chronic risk assessment ranges from 0 to 0.7% of ADI. The diet with the highest TMDI is "WHO Cluster Diet B" with 0.7% of ADI. For this diet the highest contributor is bovine kidney with 0.6% of ADI.

Group 2 – Metabolite 505M86
With the current EFSA model the chronic risk assessment ranges from 1.8 to 26.4% of ADI. The diet with the highest TMDI is "FR toddler" with 26.4% of ADI. For this diet the highest contributing group are dairy products with 26.4% of ADI.

Group 2 – Metabolite 505M91
With the current EFSA model the chronic risk assessment ranges from 0 to 0.1% of ADI. The diet with the highest TMDI is "NL child" with 0.1% of ADI. For this diet the highest contributors are leaf vegetables & fresh herbs.

Group 2 – Metabolite 505M93
With the current EFSA model the chronic risk assessment ranges from 0 to 0.1% of ADI. The diet with the highest TMDI is "NL child" with 0.1% of ADI. For this diet the highest contributors are leaf vegetables & fresh herbs.

Group 2 – Metabolite 505M95
With the current EFSA model the chronic risk assessment ranges from 0 to 0.2% of ADI. The diet with the highest TMDI is "NL child" with 0.2% of ADI. For this diet the highest contributors are leaf vegetables & fresh herbs.

Group 2 – Metabolite 505M107
Metabolite 505M107 is only found in feed items. The estimation of the exposure to feed metabolites through consumption of products of animal origin resulted in residue amounts below the genotoxicity trigger (0.0000025 mg/kg bw/d). Due to this negligible exposure, no chronic risk assessment was performed.

Group 3 – Carboxylation Products

Group 3 – Metabolite 505M09
With the current EFSA model the chronic risk assessment ranges from 0.0 to 21.8% of ADI. The diet with the highest TMDI is "NL child" with 21.8% of ADI. For this diet the highest contributing group are cattle dairy products with 19.5% of ADI.

Group 3 – Metabolite 505M81
With the current EFSA model the chronic risk assessment ranges from 0.1 to 1.2% of ADI. The diet with the highest TMDI is "IE Adult" with 1.2% of ADI. For this diet the highest contributor is sheep liver with 1.2% of ADI.

Group 4 – Products with modifications at side chains

Group 4 covers metabolites found in feed items. The estimation of the exposure to feed metabolites through consumption of products of animal origin resulted in residue amounts below the genotoxicity trigger (0.0000025 mg/kg bw/d). Due to this negligible exposure, no chronic risk assessment was performed for group 4.

Group 5 – Hydroxylation and carboxylation products

Group 5 – Metabolite 505M33
With the current EFSA model the chronic risk assessment ranges from 0.1 to 0.4% of ADI. The diet with the highest TMDI is "FR Infant" with 0.4% of ADI. For this diet the highest contributing group is "other root & tuber vegetables".

Group 5 – Metabolite 505M76
With the current EFSA model the chronic risk assessment ranges from 0.2 to 21.7% of ADI. The diet with the highest TMDI is "NL child" with 21.7% of ADI. For this diet the highest contributing group are cattle dairy products with 21.7% of ADI.

Group 5 – Metabolite 505M105
With the current EFSA model the chronic risk assessment ranges from 0.1 to 0.9% of ADI. The diet with the highest TMDI is "IE Adult" with 0.9% of ADI. For this diet the highest contributor is sheep liver with 0.9% of ADI.

III. CONCLUSION

Dimoxystrobin follows a common pathway in crops and livestock. In general, it is metabolized by four metabolic key transformation steps:

- Hydroxylation of the aromatic ring system
- Oxidation of the methyl groups at the aromatic ring system
- Cleavage of the ether bridge between the two ring systems
- Demethylation/modification of the side chain

To perform an indicative metabolite assessment according to the TTC concept the metabolites were first grouped according to their chemical substructures. In a second step the exposure to each metabolite was estimated based on all available data (metabolism studies, feeding studies and confined rotational crop studies). Subsequently, chronic and acute dietary exposure assessments were performed to identify the possible contribution of the metabolites to the dietary risk. The feed item metabolites 505M02, 505M63, 505M88, 505M89, 505M94 and 505M107 were not further considered as their potential contribution to consumer diet was significantly lower than the TTC genotoxicity trigger.
The exposure estimates applying worst case assumptions did not indicate any dietary concern. The calculation of ADI utilization resulted in values far below 100%. No significant contribution to the chronic exposure can be expected from any plant or livestock metabolite.

Acute Reference Dose (ARfD) and Dietary Exposure Calculation

Dimoxystrobin

IESTI calculation

The acute reference dose has been set to 0.04 mg/kg bw. An acute exposure assessment was performed, for which all crops and maximum residue levels (either the established MRL value, the pending value or if different the proposed MRL) used are summarized in Table 6.9-3. The summary of the calculation using the EFSA model rev 2.0 is presented in Table 6.9-5.

The ARfD utilization was highest for consumption of potatoes (RAC) of children with 3.8% followed by 3.8% ARfD for melons. Highest ARfD utilization for adults comes about by wheat and pumpkin consumption (1.6 and 1.3%, respectively).

For the sake of completeness an acute exposure assessment using only the representative crops is reported in Table 6.9-7. Highest ARfD utilization is caused by sunflower seeds with 2.3% while oilseeds contribute 0.6% for adults and children.

According to the presented IESTI calculation an acute intake of dimoxystrobin residues is unlikely to present a public health concern. A similar outcome was obtained using the lower ARfD values published in the recent conclusion on the peer review of dimoxystrobin (EFSA Scientific Report (2005) 46, 1-82).

Metabolites

The main purpose of the information presented below is to support the establishment of a robust residue definition for risk assessment purposes. Therefore, the data being summarized are going far beyond the scope of this dossier which supports the uses in oilseed rape and sunflower. The safety of dimoxystrobin parent residues in crops has been already assessed by EFSA during the establishment of MRLs. In the re-evaluation according to Regulation 396/2005, Art. 12, the previous assessments have been re-confirmed (EFSA Journal 2013, 11(11):3464, 41 pp. doi:10.2903/j.efsa.2013.3464).

In order to assess the contribution of the metabolites to the acute dietary risk, the metabolites being present in the plant and livestock metabolism studies were assigned to five groups. The grouping was performed based on structural elements. In general, they are identical for chronic and for acute assessment:

- Group 1: contains the cleavage products 505M01 and 505M80 (conjugate of 505M01)
- Group 2: contains the majority of dimoxystrobin metabolites with hydroxylations at the aromatic ring system and their conjugates
- Group 3: contains all carboxylated metabolites (via oxidation of methyl groups of the aromatic ring system) and their conjugates.
- Group 4: contains metabolites with modifications at the sidechain and their conjugates.
- Group 5: contains metabolites with hydroxylations and carboxylation at the aromatic ring system and their conjugates

The exposure estimates applying worst case assumptions did not indicate any acute dietary concern. The calculation of the % ARfD utilizations resulted in values far below 100%. No significant contribution to the acute exposure can be expected from any plant or livestock metabolite.

Group 1 – Cleavage products

Group 1 – Metabolite 505M01
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for scarole (1.7%), followed by witloof (0.9%).

Group 1 – Metabolite 505M80
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for scarole (1.7%), followed by wheat (1.4%).

Group 2 – Hydroxylation products

Group 2 – Metabolite 505M02
505M02 is only found in feed items. The estimation of the exposure to feed metabolites through consumption of products of animal origin resulted in residue amounts below the genotoxicity trigger (0.0000025 mg/kg bw/d). Due to this negligible exposure, no acute risk assessment was performed for metabolite 505M02.

Group 2 – Metabolite 505M04
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for cattle milk and milk products (24.8%), followed by goat milk and milk products (4.8%).

Group 2 – Metabolite 505M06
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for scarole (3.5%), followed by witloof (1.9%).

Group 2 – Metabolite 505M49
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for bovine kidney (16.9%), followed by bovine liver (1.6%).

Group 2 – Metabolite 505M50
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for bovine liver (16.1%), followed by bovine kidney (6.0%).

Group 2 – Metabolite 505M51
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for bovine liver (6.1%), followed by bovine kidney (4.5%).

Group 2 – Metabolite 505M63
Metabolite 505M63 is only found in feed items. The estimation of the exposure to feed metabolites through consumption of products of animal origin resulted in residue amounts below the genotoxicity trigger (0.0000025 mg/kg bw/d). Due to this negligible exposure, no acute risk assessment was performed.

Group 2 – Metabolite 505M78
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for bovine kidney (15.1%), followed by bovine liver (6.5%).

Group 2 – Metabolite 505M79
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for bovine kidney (32.0%), followed by bovine liver (5.3%).

Group 2 – Metabolite 505M84
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for bovine kidney (9.4%), followed by bovine liver (1.8%).

Group 2 – Metabolite 505M86
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for cattle milk and milk products (24.8%), followed by goat milk and milk products (4.8%).

Group 2 – Metabolite 505M91
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for scarole (1.7%), followed by witloof (0.9%).

Group 2 – Metabolite 505M93
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for scarole (3.5%), followed by witloof (1.9%).

Group 2 – Metabolite 505M95
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for scarole (5.2%), followed by witloof (1.9%).

Group 2 – Metabolite 505M107
Metabolite 505M107 only found in feed items. The estimation of the exposure to feed metabolites through consumption of products of animal origin resulted in residue amounts below the genotoxicity trigger (0.0000025 mg/kg bw/d). Due to this negligible exposure, no acute risk assessment was performed for metabolite 505M107.

Group 3 – Carboxylation Products

Group 3 – Metabolite 505M09
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for cattle milk and milk products (24.8%), followed by cattle meat (6.4%).

Group 3 – Metabolite 505M81
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for bovine liver (12.4%), followed by bovine kidney (7.5%).

Group 4 – Products with modifications at side chains

Group 4 covers metabolites found in feed items. The estimation of the exposure to feed metabolites through consumption of products of animal origin resulted in residue amounts below the genotoxicity trigger (0.0000025 mg/kg bw/d). Due to this negligible exposure, no acute risk assessment was performed for group 4.

Group 5 – Hydroxylation and carboxylation products

Group 5 – Metabolite 505M33
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for carrots (2.5%), followed by celeriac (2.2%).

Group 5 – Metabolite 505M76
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for cattle milk and milk products (24.8%), followed by cattle meat (6.4%).

Group 5 – Metabolite 505M105
The calculation used the default variability factors. The evaluation resulted in ARfD utilizations well below 100% for all commodities. Highest ARfD utilization was observed for bovine liver (9.4%).

Table 6.9-4: Dimoxystrobin (BAS 505 F): TMDI calculation using EFSA PRIMo based on input values listed in Table 6.9-3

Dimoxystrobin BAS 505F

Status of the active substance:		Code no.	
proposed LOQ (mg/kg bw):			
Toxicological end points			
ADI (mg/kg bw/day):	**0.02**	ARfD (mg/kg bw):	**0.04**
Source of ADI:	**proposed in this dossier**	Source of ARfD:	**proposed in this dossier**

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum	
	1	7
No of diets exceeding ADI	---	

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
6.9	WHO Cluster diet B	3.4	Wheat	1.1	Sunflower seed	0.5	Meat, preparations of meat,	
6.0	DK child	2.2	Wheat	1 8	Rye	0.6	Milk and cream,	
5.4	NL child	1.9	Wheat	1 5	Milk and cream,	0.7	FRUIT (FRESH OR FROZEN);	
5.1	FR toddler	2.0	Milk and cream,	1 0	Wheat	0.6	FRUIT (FRESH OR FROZEN);	
4.8	UK Toddler	1.6	Wheat	1.1	SUGAR PLANTS	1.0	Milk and cream,	
4.8	DE child	1.6	Wheat	1 2	FRUIT (FRESH OR FROZEN);	0.7	Milk and cream,	
4.6	UK Infant	1.9	Milk and cream,	1 0	Wheat	0.5	SUGAR PLANTS	
4.5	WHO cluster diet E	1.6	Wheat	0 6	Rape seed	0.5	Sunflower seed	
4.1	ES child	1.8	Wheat	0.7	Meat, preparations of meat, offals,	0.6	Milk and cream,	
3.6	WHO Cluster diet F	1.4	Wheat	0.4	Meat, preparations of meat, offals,	0.3	Rape seed	
3.6	SE general population 90th percentile	1.3	Wheat	0.7	Meat, preparations of meat, offals,	0.6	Milk and cream,	
3.3	IE adult	0.9	Wheat	0 5	FRUIT (FRESH OR FROZEN);	0.4	Meat, preparations of meat,	
3.2	WHO regional	1.2	Wheat	0 6	Meat, preparations of meat, offals,	0.2	Milk and cream,	
3.2	FR infant	1.3	Milk and cream,	0 8	FRUIT (FRESH OR FROZEN);	0.4	Root and tuber vegetables	
3.2	IT toddler	2.7	Wheat	0 2	FRUIT (FRESH OR FROZEN);	0.1	Fruiting vegetables	
2.8	PT General population	1.6	Wheat	0.4	Sunflower seed	0.3	FRUIT (FRESH OR FROZEN);	
2.7	FR all population	1.3	Wheat	0 5	Sunflower seed	0.3	FRUIT (FRESH OR FROZEN);	
2.3	ES adult	0.9	Wheat	0.4	Meat, preparations of meat, offals,	0.2	Milk and cream,	
2.1	IT adult	1.7	Wheat	0.1	FRUIT (FRESH OR FROZEN);	0.1	Fruiting vegetables	
2.0	NL general	0.8	Wheat	0 3	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN);	
2.0	DK adult	0.8	Wheat	0 3	Meat, preparations of meat, offals,	0.3	Rye	
1.8	LT adult	0.4	Rye	0.4	Wheat	0.3	Meat, preparations of meat,	
1.6	UK vegetarian	0.8	Wheat	0 2	SUGAR PLANTS	0.2	FRUIT (FRESH OR FROZEN);	
1.6	UK Adult	0.7	Wheat	0 2	SUGAR PLANTS	0.2	Meat, preparations of meat,	
1.5	FI adult	0.4	Wheat	0 3	Milk and cream,	0.3	Rye	
0.5	PL general population	0.2	Root and tuber vegetables	0 2	FRUIT (FRESH OR FROZEN);	0.1	Fruiting vegetables	

Conclusion:

The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of Dimoxystrobin BAS 505F is unlikely to present a public health concern.

Table 6.9-5: Dimoxystrobin (BAS 505 F): IESTI calculation using EFSA PRIMo based on input values listed in Table 6.9-3

Acute risk assessment /children | **Acute risk assessment / adults / general population**

The acute risk assessment is based on the ARfD.

For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation.

In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used.
In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3.

Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.

Unprocessed commodities

Children: No of commodities for which ARfD/ADI is exceeded (IESTI 1)		---	Children: No of commodities for which ARfD/ADI is exceeded (IESTI 2)		---	Adults: No of commodities for which ARfD/ADI is exceeded (IESTI 1)		---	Adults: No of commodities for which ARfD/ADI is exceeded (IESTI 2)		---
IESTI 1	*)	**)	**IESTI 2**	*)	**)	**IESTI 1**	*)	**)	**IESTI 2**	*)	**)
Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
3.8	Potatoes	0.01 / -	3.8	Melons	0.01 / -	1.6	Wheat	0.08 / -	1.6	Wheat	0.08 / -
3.8	Melons	0.01 / -	3.5	Watermelons	0.01 / -	1.3	Pumpkins	0.01 / -	1.3	Pumpkins	0.01 / -
3.5	Watermelons	0.01 / -	3.1	Milk and milk	0.01 / -	1.1	Watermelons	0.01 / -	1.1	Watermelons	0.01 / -
3.3	Oranges	0.01 / -	2.9	Wheat	0.08 / -	1.0	Melons	0.01 / -	1.0	Melons	0.01 / -
3.1	Milk and milk products: Cattle	0.01 / -	2.7	Potatoes	0.01 / -	1.0	Rye	0.08 / -	1.0	Rye	0.08 / -
No of critical MRLs (IESTI 1)			---			**No of critical MRLs (IESTI 2)**			---		

Processed commodities

Children: No of commodities for which ARfD/ADI is exceeded		---	Adults: No of commodities for which ARfD/ADI is exceeded		---
		***)			***)
Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)
2.4	Wheat flour	0.08 / -	0.9	Bread/pizza	0.08 / -
1.3	Apple juice	0.01 / -	0.3	Orange juice	0.01 / -
1.2	Orange juice	0.01 / -	0.2	Apple juice	0.01 / -
1.1	Carrot, juice	0.01 / -	0.1	Wine	0.01 / -
0.8	Grape juice	0.01 / -	0.1	Pineapples preserved	0.01 / -

*) The results of the ESTI calculations are reported for at least 5 commodities. If the ARfD is exceeded for more than 5 commodities, all ESTI values > 90% of ARfD are reported.
**) pTMRL: provisional temporary MRL
***) pTMRL: provisional temporary MRL for unprocessed commodity

Conclusion:

For Dimoxystrobin BAS 505F ESTI 1 and IESTI 2 were calculated for food commodities for which pTMRLs were submitted and for which consumption data are available.
No exceedance of the ARfD/ADI was established for any unprocessed commodity.

For processed commodities, no exceedance of the ARfD/ADI was identified.

Table 6.9-6: Dimoxystrobin (BAS 505 F): TMDI calculation using EFSA PRIMo based on input values of representative uses (see Table 6.9-3)

Dimoxystrobin BAS 505F

Status of the active substance:		Code no.	
proposed LOQ (mg/kg bw):			
Toxicological end points			
ADI (mg/kg bw/day):	**0.02**	ARfD (mg/kg bw):	**0.04**
Source of ADI:	**proposed in this dossier**	Source of ARfD:	**proposed in this dossier**

Explain choice of toxicological reference values.

The risk assessment has been performed on the basis of the MRLs collected from Member States in April 2006. For each pesticide/commodity the highest national MRL was identified (proposed temporary MRL = pTMRL).
The pTMRLs have been submitted to EFSA in September 2006.

Chronic risk assessment

TMDI (range) in % of ADI minimum - maximum	1
No of diets exceeding ADI	---

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
1.1	WHO Cluster diet B	1.1	Sunflower seed	0 0	Rape seed		FRUIT (FRESH OR FROZEN);	
1.1	WHO cluster diet E	0.6	Rape seed	0 5	Sunflower seed		FRUIT (FRESH OR FROZEN);	
0.5	FR all population	0.5	Sunflower seed	0 0	Rape seed		FRUIT (FRESH OR FROZEN);	
0.4	WHO Cluster diet F	0.3	Rape seed	0.1	Sunflower seed		FRUIT (FRESH OR FROZEN);	
0.4	PT General population	0.4	Sunflower seed		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
0.3	WHO regional European diet	0.2	Sunflower seed	0.1	Rape seed		FRUIT (FRESH OR FROZEN);	
0.3	IE adult	0.3	Sunflower seed		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
0.2	FR toddler	0.2	Sunflower seed		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
0.2	ES child	0.2	Sunflower seed		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
0.2	ES adult	0.2	Sunflower seed		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
0.1	DE child	0.1	Sunflower seed	0 0	Rape seed		FRUIT (FRESH OR FROZEN);	
0.1	LT adult	0.1	Sunflower seed		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
0.0	FR infant	0.0	Sunflower seed		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
0.0	IT toddler	0.0	Sunflower seed		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
0.0	NL child	0.0	Sunflower seed	0 0	Rape seed		FRUIT (FRESH OR FROZEN);	
0.0	IT adult	0.0	Sunflower seed		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
0.0	PL general population	0.0	Sunflower seed		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
0.0	NL general	0.0	Sunflower seed	0 0	Rape seed		FRUIT (FRESH OR FROZEN);	
0.0	DK adult	0.0	Sunflower seed	0 0	Rape seed		FRUIT (FRESH OR FROZEN);	
	FI adult		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
	FI adult		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
	FI adult		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
	FI adult		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
	FI adult		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
	FI adult		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	
	FI adult		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);		FRUIT (FRESH OR FROZEN);	

Conclusion:

The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of Dimoxystrobin BAS 505F is unlikely to present a public health concern.

Table 6.9-7: Dimoxystrobin (BAS 505 F): IESTI calculation using EFSA PRIMo based on input values of representative uses (see Table 6.9-3)

	Acute risk assessment /children						Acute risk assessment / adults / general population					
	The acute risk assessment is based on the ARfD.											
	For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation.											
	In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used. In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3.											
	Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.											
Unprocessed commodities	**No of commodities for which ARfD/ADI is exceeded (IESTI 1)**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2)**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 1)**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2)**		---
	IESTI 1	*)	**)	**IESTI 2**	*)	**)	**IESTI 1**	*)	**)	**IESTI 2**	*)	**)
	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
	2.3	Sunflower seed	0.3 / -	2.3	Sunflower seed	0 3 / -	0.8	Sunflower seed	0.3 / -	0.8	Sunflower seed	0.3 / -
	0.6	Rape seed	0.2 / -	0.6	Rape seed	0 2 / -			#NV			#NV
			#NV			#NV			#NV			#NV
			#NV			#NV			#NV			#NV
			#NV			#NV			#NV			#NV
	No of critical MRLs (IESTI 1)			---			**No of critical MRLs (IESTI 2)**			---		
Processed commodities	**No of commodities for which ARfD/ADI is exceeded**		---				**No of commodities for which ARfD/ADI is exceeded**		---			
			***)						***)			
	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)				Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)			
			#NV						#NV			
			#NV						#NV			
			#NV						#NV			
			#NV						#NV			
			#NV						#NV			
	*) The results of the ESTI calculations are reported for at least 5 commodities. If the ARfD is exceeded for more than 5 commodities, all ESTI values > 90% of ARfD are reported. **) pTMRL: provisional temporary MRL ***) pTMRL: provisional temporary MRL for unprocessed commodity											
	Conclusion:											
	For Dimoxystrobin BAS 505F ESTI 1 and IESTI 2 were calculated for food commodities for which pTMRLs were submitted and for which consumption data are available.											
	No exceedance of the ARfD/ADI was established for any unprocessed commodity.											
	For processed commodities, no exceedance of the ARfD/ADI was identified.											

CA 6.10 Other studies

A study determining the residues in rape honey after application of BAS 540 01 F is summarized in the following.

CA 6.10.1 Effect on the residue level in pollen and bee products

As rape is a relevant crop for the production of honey a residue study has been performed. The objective of this study is to determine dimoxystrobin residues in pollen and bee products for human consumption resulting from residues taken up by honeybees from rape at blossom.

Report: CA 6.10.1/1
Braun D., 2008a
Determination of residues of BAS 505 F in rape honey after one application of BAS 540 01 F in oilseed rape during full flowering (BBCH 65) in Germany
2008/1068951

Guidelines: EEC 7029/VI/95 rev. 5, Working document of the Commission of the European Communities Directorate General for Agriculture VI B II-1 Appendix B, BBA IV 3-3, IVA Guideline IA-II (1992)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: BAS 540 01 F (SC)
   Lot/Batch #: FRE-000569, dimoxystrobin (BAS 505 F): 200.0 g/L nominal; boscalid (BAS 510 F): 200.0 g/L nominal
   Purity:
   CAS#: 149961-52-4, dimoxystrobin
   Development code:
   Spiking levels: 0.01-10 mg/kg

2. Test Commodity:

Crop: Oilseed rape / Honey bee (bee colonies with at least 1 brood body and 1 honey body)
Type: Oilseeds
Variety: Lorenz, NK Fair, Elektra, Baldur
Botanical name: *Brassica napus* / *Apis mellifera*
Crop parts(s) or processed commodity: Whole plant without root, honey
Sample size: Whole plant without root: >1 kg; from composite honey samples (24-35 kg) analysis samples of 0.58-0.74 kg were taken

B. STUDY DESIGN

1. Test procedure

During the 2008 growing season, four field trials were conducted in different representative oilseed rape growing areas in Germany to determine the magnitude of the residues in oilseed rape honey. The fungicidal product BAS 540 01 F (SC), containing boscalid and dimoxystrobin (concentration of 200 g/L for each active substance), was foliar applied to winter oilseed rape once at a target rate corresponding to 0.100 kg/ha of dimoxystrobin in a spray volume of 300 L/ha. The application was conducted during full flowering at BBCH 65. Three hives with one brood body each were placed at the border of each field 2-3 days before planned application for adaptation. Immediately before application (day 0) one honey body with empty honey combs was set up on each brood body. Whole plant without roots specimens were sampled directly before and after application and the honey specimens were sampled 20±5 days after application. All specimens were frozen immediately after specimen preparation and remained frozen (≤-18°C) until analysis including transportation. The maximum storage interval from harvest until extraction for analysis of dimoxystrobin was 44 days.

Table 6.10.1-1: Target application rates and timings for oilseed rape (honey production)

Year	No of trials	No of appl.	F P or G	Test item	Active substance	Appl. rate (kg a.s./ha)	Water volume (L/ha)	Target date/BBCH
2008	4	1	F	BAS 540 01 F (SC)	BAS 505 F BAS 510 F	0.100 0.100	300	BBCH 65

2. Description of analytical procedures

Samples were analyzed for dimoxystrobin residues using BASF method No 535/2 (L0076/02). Dimoxystrobin (BAS 505 F) was extracted with a mixture of methanol, water and hydrochloric acid. An aliquot of the extract was centrifuged and partitioned at alkaline conditions against cyclohexane. The final determination of the analyte was performed by HPLC-MS/MS with a limit of quantitation (LOQ) of 0.01 mg/kg.

Table 6.10.1-2: Summary of procedural recovery data for dimoxystrobin

Matrix	Fortification level (mg/kg)	Summary recoveries		
		n	Mean (%)	RSD (%)
BASF method No 535/2; LOQ = 0.01 mg/kg		**Dimoxystrobin**		
Whole plant w/o root	0.01, 0.1, 10.0	3	85	4.7
Honey	0.01, 0.1, 1.0	13	84	5.9

II. RESULTS AND DISCUSSION

The residue ranges of dimoxystrobin treated with formulation BAS 540 01 F are summarized in Table 6.10.1-3. Details are presented in Table 6.10.1-4.
Dimoxystrobin residues in whole plant without root specimens at 0 DALA (BBCH 65) ranged between 0.37-0.78 mg/kg. Residues in honey 15-18 DALA were <0.01 mg/kg.
No residues of dimoxystrobin were found in the control samples above the LOQ.

Table 6.10.1-3: Summary of residues in oilseed rape treated with BAS 540 01 F

Crop	Year	DALA[1]	Growth stage[2] (BBCH)	Dimoxystrobin (mg/kg)	
				Matrix	BAS 505 F
Oilseed rape	2008	0	65	Whole plant without roots	0. 37-0.78
		15-18	69	Honey	<0.01

1 Days after last application
2 At sampling

III. CONCLUSION

The residues of dimoxystrobin in oilseed rape honey were below the LOQ of 0.01 mg/kg.

Table 6.10.1-4: Residues of dimoxystrobin in oilseed rape honey after application of BAS 540 01 F in Northern Europe

Trial details		Crop	Country	Formulation, Application rate[0] (kg a.s./ha)	Crop growth stage[2] (BBCH)	DALA[1)]	Residues found (mg/kg)	
							Matrix	BAS 505 F
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	336847 2008/1068951 L080077 Yes 2008	Oilseed rape	Germany	BAS 540 01 F 1 x 0.100	65	0 18	Whole plant without root Honey	0.74 <0.01
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	336847 2008/1068951 L080078 Yes 336847	Oilseed rape	Germany	BAS 540 01 F 1 x 0.100	65	0 15	Whole plant without root Honey	0.78 <0.01
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	336847 2008/1068951 L080079 Yes 336847	Oilseed rape	Germany	BAS 540 01 F 1 x 0.100	65	0 16	Whole plant without root Honey	0.37 <0.01
Study code: **Doc ID:** **Trial No:** **GLP:** **Year:**	336847 2008/1068951 L080080 Yes 336847	Oilseed rape	Germany	BAS 540 01 F 1 x 0.100	65	0 17	Whole plant without root Honey	0.73 <0.01

0 Actual application rates varied by 10% at most
1 Days after last application
2 At application
_ Underlined values were used for MRL derivation

Appendix: Tier 1 Summaries of the Supervised Field Residue Trials

Oilseeds

Oilseed rape

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	None (tank mix with BAS 510 01 F, 500 g/L boscalid)
Content of active substance (g/kg or g/L)	500 g/L		
Formulation (e.g. WP)	SC (BAS 505 02 F)	**Residues calculated as:**	BAS 505 F

1	2	3	4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**		
NEU/FR/02/00 2001/1006138 3290 Schaffen Belgium (AGR/02/00)	SO 0495 Colosse	1. 01.09.99 2. 19.04.-03.05.00 3. 06.07.00	Foliar spray	0.033	300	0.100	2 26.04.00	65	Plant without roots Seed	0.59 <0.05	0 71	BASF method No 445/0 LOQ = 0.05 mg/kg
NEU/FR/02/00 2001/1006138 5500 Middelfart Denmark (ALB/01/00)	SO 0495 Capitol	1. 23.08.99 2. 02.05.-25.05.00 3. 25.07.00	Foliar spray	0.033	300	0.100	2 15.05.00	65	Plant without roots Seed	1.52 <0.05	0 70	
NEU/FR/02/00 2001/1006138 24625 Grossharrie Germany (D05/02/00)	SO 0495 Artus	1. 01.09.99 2. 29.04.-16.05.00 3. 24.07.00	Foliar spray	0.033	300	0.100	2 04.05.00	65	Plant without roots Seed	0.99 <0.05	0 81	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	None (tank mix with BAS 510 01 F, 500 g/L boscalid)
Content of active substance (g/kg or g/L)	500 g/L		
Formulation (e.g. WP)	SC (BAS 505 02 F)	**Residues calculated as:**	BAS 505 F

1	2	3	4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**		
NEU/FR/02/00 2001/1006138 67459 Böhl-Iggelheim Germany (DU2/11/00)	SO 0495 Express	1. 28.08.99 2. 20.04.-30.05.00 3. 01.07.00	Foliar spray	0.033	300	0.100	2 28.04.00	65	Plant without roots Seed	0.91 <0.05	0 62	BASF method No 445/0 LOQ = 0.05 mg/kg
NEU/FR/02/00 2001/1006138 CV33 9QB Leamington United Kingdom (OAT/02/00)	SO 0495 Apex	1. 04.09.99 2. 13.04.-09.05.00 3. 21.07.00	Foliar spray	0.033	300	0.100	2 27.04.00	65	Plant without roots Seed	1.04 <0.05	0 83	
72551 2002/1007081 5458 AB, Siebengewald Netherlands (AGR/17/01)	SO 0495 Lisabeth	1. 26.08.2000 2. 27.04.-11.05.2001 3. 12.07.-13.07.2001	Foliar spray	0.033	300	0.100	2 04.05.2001	65	Plant without roots Seed	0.43 <0.05	0 69	BASF method No 445/0 LOQ = 0.05 mg/kg
72551 2002/1007081 DK-5500 Middelfart Denmark (ALB/04/01)	SO 0495 Cymbal	1. 25.08.00 2. 12.05.-10.06.2001 3. 27.07.-06.08.2001	Foliar spray	0.033	300	0.100	2 21.05.2001	65	Plant without roots Seed	1.51 <0.05	0 73	
72551 2002/1007081 72800 Luche-Pringe France (FBM/03/01)	SO 0495 Madrigal	1. 12.09.00 2. 10.04.-28.04.2001 3. 09.07.-11.07.2001	Foliar spray	0.033	300	0.100	2 17.04.2001	65	Plant without roots Seed	0.79 <0.05	0 76	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	None (tank mix with BAS 510 01 F, 500 g/L boscalid)
Content of active substance (g/kg or g/L)	500 g/L		
Formulation (e.g. WP)	SC (BAS 505 02 F)	**Residues calculated as:**	BAS 505 F

1	2	3	4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**		
72551 2002/1007081 Raunds NN9 6BX Northhamptonshire United Kingdom (OAT/03/01)	SO 0495 Escort	1. 28.08.00 2. 03.05.-03.06.2001 3. 21.07.-22.07.2001	Foliar spray	0.033	300	0.100	2 11.05.2001	65	Plant without roots Seed	1.68 <0.05	0 70	BASF method No 445/0 LOQ = 0.05 mg/kg

0 Actual application rates varied by 10% at most, except where noted otherwise
1 Days after last application
2 At last treatment

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 KA F)	**Residues calculated as:**	BAS 505 F

1	2	3	4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**		
72551 2002/1007081 5458 AB, Siebengewald Netherlands (AGR/17/01)	SO 0495 Lisabeth	1. 26.08.2000 2. 27.04.-11.05.2001 3. 12.07.-13.07.2001	Foliar spray	0.033	300	0.100	2 04.05.2001	65	Plant without roots Seed	0.68 <0.05	0 69	BASF method No 445/0 LOQ = 0.05 mg/kg
72551 2002/1007081 DK-5500 Middelfart Denmark (ALB/04/01)	SO 0495 Cymbal	1. 25.08.00 2. 12.05.-10.06.2001 3. 27.07.-06.08.2001	Foliar spray	0.033	300	0.100	2 21.05.2001	65	Plant without roots Seed	1.23 <0.05	0 73	
72551 2002/1007081 72800 Luche-Pringe France (FBM/03/01)	SO 0495 Madrigal	1. 12.09.00 2. 10.04.-28.04.2001 3. 09.07.-11.07.2001	Foliar spray	0.033	300	0.100	2 17.04.2001	65	Plant without roots Seed	0.91 <0.05	0 76	
72551 2002/1007081 Raunds NN9 6BX Northhamptonshire United Kingdom (OAT/03/01)	SO 0495 Escort	1. 28.08.00 2. 03.05.-03.06.2001 3. 21.07.-22.07.2001	Foliar spray	0.033	300	0.100	2 11.05.2001	65	Plant without roots Seed	1.78 <0.05	0 70	

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 00 F)	**Residues calculated as:**	BAS 505 F

1	2	3	4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**		
150304 2004/1015921 5458 Siebengewald Germany (AGR/05/03)	SO 0495 Lion	1. 27.08.2002 2. 29.04.-15.05.2003 3. 07.07.- 08.07.2003	Foliar spray	0.033	300	0.100	2 26.05.2003	75	Plant without roots Plant without roots Seed Seed Seed	0.87 <0.05 <0.05 <0.05 <0.05	0 21 28 35 42	BASF method No 445/0 LOQ = 0.05 mg/kg
150304 2004/1015921 5500 Middelfart, Denmark (ALB/02/03)	SO 0495 Canberra	1. 26.08.2002 2. 10.05.-05.06.2003 3. 20.07.-30.07.2003	Foliar spray	0.033	300	0.100	2 17.06.2003	75	Plant without roots Plant without roots Seed Seed Seed	0.87 <0.05 <0.05 <0.05 <0.05	0 21 29 34 42	
150304 2004/1015921 67459 Böhl Iggelheim Germany (DU4/09/03)	SO 0495 Expreß	1. 31.08.2002 2. 07.04.-02.05.2003 3. 21.07.2003	Foliar spray	0.033	300	0.100	2 17.06.2003	75	Plant without roots Seed Seed Seed Seed	1.12 <0.05 <0.05 <0.05 <0.05	0 21 27 35 43	
150304 2004/1015921 67160 Seebach France (FAN/07/03)	SO 0495 Pollen	1. 06.09.2002 2. 01.05 -20.05.2003 3. 04.07.2003	Foliar spray	0.033	300	0.100	2 23.05.2003	75	Plant without roots Plant without roots Seed Seed Seed	0.82 <0.05 <0.05 <0.05 <0.05	0 20 27 35 41	
150304 2004/1015921 MK18, 4AB Radclive United Kingdom (OAT/05/03)	SO 0495 Borneo	1. 12.09.2002 2. 17.04.-06.05.2003 3. 12.07.-13.07.2003	Foliar spray	0.033	300	0.100	2 29.05.2003	75	Plant without roots Plant without roots Rest of plant Pods with seed Seed Seed	1.41 <0.05 <0.05 <0.05 <0.05 <0.05	0 21 28 28 34 41	

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No location (trial No)	**Commodity/ variety**	**1. Date of sowing / planting 2. flowering 3. harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	***Z*-isomer (505M98)**		
390013 2013/1003732 16833 Brunne Germany (L120374)	SO 0495 RNX3922	1. 24.08.2011 2. 20.04.-11.05.2012 3. 24.07.2012	Foliar spray	0.050	200	0.100	2 04.06.2012	75	Whole plant* Rest of plant* Seed	0.41 0.048 <0.005	<0.005 <0.005 <0.005	0 50 50	BASF method No L0076/08 LC-MS/MS LOQ = 0.005 mg/kg
390013 2013/1003732 CO11 2NF Manningtree United Kingdom (L120375)	SO 0495 Castille	1. 15.09.2011 2. 30.04.-28.05.2012 3. 25.07.2012	Foliar spray	0.050	200	0.100	2 25.06.2012	75	Whole plant* Rest of plant* Seed	1.1 0.026 <0.005	<0.005 <0.005 <0.005	0 30 30	
390013 2013/1003732 51110 Bourgogne France (L120376)	SO 0495 Dynastie	1. 01.09.2011 2. 20.04.-15.05.2012 3. 18.07.2012	Foliar spray	0.050 0.059	200 225	0.100 0.113	2 30.05.2012	77	Whole plant* Rest of plant* Seed	1.5 0.0058 <0.005	<0.005 <0.005 <0.005	0 50 50	
390013 2013/1003732 6561 Groesbeck The Netherlands (L120377)	SO 0495 Billy	1. 24.08.2011 2. 08.05.-24.05.2012 3. 23.07.2012	Foliar spray	0.050	200	0.100	2 05.06.2012	75	Whole plant* Rest of plant* Seed	1.1 0.019 <0.005	<0.005 <0.005 <0.005	0 48 48	
390014 2014/1010806 16845 Manker Germany (L130070)	SO 0495 Sherpa	1. 28.08.2012 2. 05.05.-25.05.2013 3. 26.07.2013	Foliar spray	0.050 0.050	20022 200	0.111 0.100	2 10.06.2013	75	Whole plant* Rest of plant* Seed	0.95 0.0066 <0.005	<0.005 <0.005 <0.005	0 46 46	
390014 2014/1010806 3891 Gingelom France (L130071)	SO 0495 Arsenal	1. 27.08.2012 2. 02.05.-24.05.2013 3. 23.07.2013	Foliar spray	0.050	200	0.100	2 31.05.2013	75	Whole plant* Rest of plant* Seed	1.3 0.035 0.0093	<0.005 <0.005 <0.005	0 53 53	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No location (trial No)	**Commodity/ variety**	**Date of 1. sowing / planting 2. flowering 3. harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	***Z*-isomer (505M98)**		
390014 2014/1010806 51450 Betheny France (L130072)	SO 0495 Deca Expertise	1. 27.08.2012 2. 28.04.-27.05.2013 3. 03.08.2013	Foliar spray	0.050	200	0.100	2 01.06.2013	75	Whole plant* Rest of plant* Seed	0.94 <0.005 <0.005	<0.005 <0.005 <0.005	0 61 61	BASF method No L0076/08 LC-MS/MS LOQ = 0.005 mg/kg
390014 2014/1010806 6573 Beek The Netherlands (L130073)	SO 0495 Visby	1. 01.09.2012 2. 03.05.-25.05.2013 3. 25.07.2013	Foliar spray	0.050	200	0.100	2 04.06.2013	75	Whole plant* Rest of plant* Seed	1.5 0.20 0.013	0.014 0.0079 <0.005	0 51 51	

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment
* Without roots

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	None (tank mix with BAS 510 01 F, 500 g/L boscalid)
Content of active substance (g/kg or g/L)	500 g/L		
Formulation (e.g. WP)	SC (BAS 505 02 F)	**Residues calculated as:**	BAS 505 F

1	2	3	4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**		
72551 2002/1007081 26750 St-Paul les Romans France FBD/02/01	VL 0495 SO 0495	1. 24.08.01 2. 09.04.01-20.04.01 3. 03.07.01	Foliar spray	0.033	300	0.100	2 12.04.01	65	Plant without roots Seed	0.99 <0.05	0 68	BASF method No 445/0 LOQ = 0.05 mg/kg

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 KA F)	**Residues calculated as:**	BAS 505 F

1	2	3	4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**		
72551 2002/1007081 26750 St-Paul les Romans France FBD/02/01	VL 0495 SO 0495	1. 24.08.01 2. 09.04.01-20.04.01 3. 03.07.01	Foliar spray	0.033	300	0.100	2 12.04.01	65	Plant without roots Seed	1.53 <0.05	0 68	BASF method No 445/0 LOQ = 0.05 mg/kg

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 00 F)	**Residues calculated as:**	BAS 505 F

1	2	3	4	5			6	7	8	9	10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**	**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**		
150304 2004/1015921 26750 St-Paul les Romans France (FBD/03/03)	SO 0495 Parabole	1. 23.08.2002 2. 10.04.-24.04.2003 3. 12.06.-13.06.2003	Foliar spray	0.033	300	0.100	2 15.05.2003	75	Plant without roots Seed Seed Seed Seed	0.84 <0.05 <0.05 <0.05 <0.05	0 21 27 34 42	BASF method No 445/0 LOQ = 0.05 mg/kg
150304 2004/1015921 82170 Pornpignan France FTL/04/03	SO 0495 Banjo	1. 29.08.2002 2. 15.04.-30.04.2003 3. 23.06.-30.06.2003	Foliar spray	0.033	300	0.100	2 13.05.2003	75	Plant without roots Seed Seed Seed Seed	1.69 <0.05 <0.05 <0.05 <0.05	0 22 29 35 42	

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No location (trial No)	**Commodity/ variety**	**Date of 1. sowing / planting 2. flowering 3. harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	***Z*-isomer (505M98)**		
390013 2013/1003732 17800 Saint-Leger France (L120378)	SO 0495 Albatros	1. 23.08.2011 2. 10.04.-05.05.2012 3. 24.07.2012	Foliar spray	0.050	200	0.100	2 29.05.2012	77	Whole plant* Rest of plant* Seed	1.2 0.0077 <0.005	<0.005 <0.005 <0.005	0 48 48	BASF method No 445/0 LOQ = 0.05 mg/kg
390013 2013/1003732 Nea Mesivria 57003 Greece (L120379)	SO 0495 PR46W31	1. 30.11.2011 2. 10.04.-30.04.2012 3. 10.06.-25.06.2012	Foliar spray	0.050	200	0.100	2 11.05.2012	75	Whole plant* Rest of plant* Seed	1.6 0.15 0.0092	0.0065 <0.005 <0.005	0 32 32	
390013 2013/1003732 40051 Altedo Italy (L120380)	SO 0495 Avenir	1. 21.09.2011 2. 06.04.-20.04.2012 3. 11.06.2012	Foliar spray	0.050	200	0.100	2 17.05.2012	75	Whole plant* Rest of plant* Seed	1.2 0.31 0.063	<0.005 0.016 <0.005	0 25 25	
390013 2013/1003732 41710 Utrera Spain (L120381)	SO 0495 Kabel	1. 07.03.2012 2. 06.04.-20.04.2012 3. 11.06.2012	Foliar spray	0.050	200	0.100	2 04.06.2012	75	Whole plant* Rest of plant* Seed	0.84 1.3 0.053	<0.005 0.087 <0.005	0 36 36	
390014 2014/1010806 17800 Biron France (L130074)	SO 0495 Extorm	1. 20.09.2012 2. 10.04.-03.05.2013 3. 30.07.2013	Foliar spray	0.050	200	0.100	2 27.05.2013	75	Whole plant* Rest of plant* Seed	0.43 <0.005 <0.005	<0.005 <0.005 <0.005	0 64 64	
390014 2014/1010806 62100 Pralimnio Greece (L130075)	SO 0495 Nelson	1. 10.10.2012 2. 20.03.-15.04.2013 3. 20.05.-10.06.2013	Foliar spray	0.050	200	0.100	2 14.05.2013	75	Whole plant* Rest of plant* Seed	0.68 0.11 0.034	<0.005 0.012 <0.005	0 30 30	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	-
Crop/crop group:	Oilseed rape/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No location (trial No)	**Commodity/ variety**	**Date of 1. sowing / planting 2. flowering 3. harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	***Z*-isomer (505M98)**		
390014 2014/1010806 40014 Crevalcore Italy (L130076)	SO 0495 Excalibur	1. 24.09.2012 2. 22.04.-02.05.2013 3. 24.06.2013	Foliar spray	0.050	200	0.100	2 25.05.2013	75	Whole plant* Rest of plant* Seed	1.6 0.83 0.093	<0.005 0.028 <0.005	0 30 30	BASF method No 445/0 LOQ = 0.05 mg/kg
390014 2014/1010806 41710 Utrera Spain (L130077)	SO 0495 Salsa CL	1. 01.02.2013 2. 01.05.-10.06.2013 3. 22.07.2013	Foliar spray	0.050	200	0.100	2 05.06.2013	75	Whole plant* Rest of plant* Seed	1.0 2.9 0.092	<0.005 0.11 <0.005	0 47 47	

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment

Sunflower

Northern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	Pictor SC
Crop/crop group:	Sunflower/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	***Z*-isomer (505M98)**		
BSF 0648/072364 2007/1006111 1495 Marbais Belgium (BSF/648-01)	SO 0702 LG 54.50 HO	1. 08.05.2006 2. n.r. 3. 11.09.2006	Foliar spray	0.033	300	0.100	2 14.08.2006	73	Plant without roots Seed Seed Seed	0.69 <0.01 <0.01 <0.01	-	0 21 28 35	BASF method No 535/2 LOQ = 0.01 mg/kg
BSF 0648/072364 2007/1006111 6662 NP Elst Netherlands (BSF/648-02)	SO 0702 Sunrich Orange	1. 01.04.2006 2. n.r. 3. 05.09.2006	Foliar spray	0.033	300	0.100	2 07.08.2006	68-69	Plant without roots Seed Seed Seed	0.91 <0.01 <0.01 <0.01	-	0 22 29 35	
BSF 0648/072364 2007/1006111 51140 Treslon France (BSF/648-03)	SO 0702 Aria	1. 10.05.2006 2. n.r. 3. 11.09.2006	Foliar spray	0.033	300	0.100	2 14.08.2006	71	Plant without roots Seed Seed Seed	0.28 <0.01 0.01 0.02	-	0 21 28 35	
BSF 0648/072364 2007/1006111 51110 Aumenancourt France (BSF/648-04)	SO 0702 Aurasol	1. 11.04.2006 2. n.r. 3. 11.09.2006	Foliar spray	0.033	300	0.100	2 14.08.2006	71	Plant without roots Seed Seed Seed	0.38 <0.01 <0.01 <0.01	-	0 21 28 35	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	Pictor SC
Crop/crop group:	Sunflower/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**1. Date of Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	**Z-isomer (505M98)**		
BSF 0648/072364 2007/1006111 76703 Oberdingen Germany (BSF/648-05)	SO 0702 Heliaroc	1. 24.04.2006 2. 18.07.-30.07.2006 3. 05.09.2006	Foliar spray	0.033	300	0.100	2 08.08.2006	75	Plant without roots Seed Seed Seed	1.17 <0.01 <0.01 <0.01	-	0 22 29 36	BASF method No 535/2 LOQ = 0.01 mg/kg
05 BASF AA 0701 2005/1035114 Mezõkeresztes Hungary (05 BASF AA 0701/1)	SO 0702 LG 5665	1. 03.04.-04.04.2005 2. n.r. 3. 27.08.2005	Foliar spray	0.033	300	0.100	2 28.07.2005	67-69	Seed Seed Seed	<0.05 <0.05 <0.05	-	30 30 30	PPSCS SOP No R 302FEJ1 LOQ = 0.05 mg/kg
05 BASF AA 0701 2005/1035114 Boldva Hungary (05 BASF AA 0701/2)	SO 0702 Barolo	1. 25.04.2005 2. n.r. 3. 09.09.2005	Foliar spray	0.033	300	0.100	2 10.08.2005	67-69	Seed Seed Seed	<0.05 <0.05 <0.05	-	30 30 30	
05 BASF AA 0701 2005/1035114 Hantos Hungary (05 BASF AA 0701/3)	SO 0702 Pioneer A82	1. 12.04.2005 2. n.r. 3. 24.08.2005	Foliar spray	0.033- 0.040	250-300	0.100	2 26.07.2005	81	Seed Seed Seed	<0.05 <0.05 <0.05	-	29 29 29	
05 BASF AA 0701 2005/1035114 Pázmánd Hungary (05 BASF AA 0701/4)	SO 0702 Aréna PR	1. 24.04.2005 2. n.r. 3. 27.08.2005	Foliar spray	0.040- 0.125	80-250	0.100	2 29.07.2005	81	Seed Seed Seed	<0.05 <0.05 <0.05	-	29 29 29	
BSF 643/062202 2006/1018116 49700 St. Georges France (BSF/643-01)	SO 0702 Aurasol	1. 22.04.2005 2. n.r. 3. 20.09.2005	Foliar spray	0.033	300	0.100	2 23.08.2005	85	Plant without roots Seed Seed Seed	3.116 <0.05 <0.05 <0.05	-	0 21 28 35	BASF method No 445/0 LOQ = 0.05 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	Pictor SC
Crop/crop group:	Sunflower/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	**Z-isomer (505M98)**		
BSF 643/062202 2006/1018116 71290 Ratenelle France (BSF/643-02)	SO 0702 Orasol	1. 09.05.2005 2. n.r. 3. 14.09.2005	Foliar spray	0.033	300	0.100	2 17.08.2005	67	Plant without roots Seed Seed Seed	3.339 <0.05 <0.05 <0.05	-	0 21 28 35	BASF method No 445/0 LOQ = 0.05 mg/kg
BSF 643/062202 2006/1018116 Arnhern 6800 Netherlands (BSF/643-03)	SO 0702 Sunrich Orange	1. 22.04.2005 2. n.r. 3. 04.10.2005	Foliar spray	0.033	300	0.100	2 05.09.2005	79-81	Plant without roots Seed Seed Seed	0.855 0.151 0.105 0.163	-	0 21 29 35	
BSF 643/062202 2006/1018116 76646 Bruchsal Germany (BSF/643-04)	SO 0702 Altesse	1. 12.04.2005 2. n.r. 3. 30.08.2005	Foliar spray	0.033	300	0.100	2 09.08.2005	75	Plant without roots Seed Seed Seed	1.014 <0.05 <0.05 <0.05	-	0 21 28 35	
390015 2013/1003723 16845 Manker Germany (L120220)	SO 702 Alisson RW	1. 17.04.2012 2. 09.07.-20.07.2012 3. 17.09.2012	Foliar spray	0.050	200	0.100	1 25.07.2012	75	Plants* Seed Seed Seed Rest of plants* Rest of plants* Rest of plants*	0.93 0.0051 <0.005 <0.005 0.027 0.025 0.027	<0.005 <0.005 <0.005 <0.005 <0.005 <0.005 <0.005	0 20 29 54 20 29 54	BASF method No L0076/08 LOQ = 0.005 mg/kg
390015 2013/1003723 OX27 9AS Stratton Audley United Kingdom (L120221)	SO 702 Diamond	1. 18.05.2012 2. 22.08.-14.09.2012 3. 31.10.2012	Foliar spray	0.050	200	0.100	1 01.10.2012	71-75	Plants* Seed Seed Rest of plants* Rest of plants*	0.47 <0.005 0.0050 0.0099 0.0094	<0.005 <0.005 <0.005 <0.005 <0.005	0 21 30 21 30	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	Pictor SC
Crop/crop group:	Sunflower/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	***Z*-isomer (505M98)**		
390015 2013/1003723 72500 Saint Pierre de Chevillé France (L120222)	SO 702 Durban	1. 11.05.2012 2. 12.07.-25.07.2012 3. 20.09.2012	Foliar spray	0.050	200	0.100	1 21.08.2012	75	Plants* Seed Seed Rest of plants* Rest of plants*	0.88 <0.005 <0.005 0.98 0.76	<0.005 <0.005 <0.005 0.039 0.036	0 22 30 22 30	BASF method No L0076/08 LOQ = 0.005 mg/kg
390015 2013/1003723 6599 CJ Ven-Zelderheide The Netherlands (L120223)	SO 702 Metharoc	1. 22.04.2012 2. 25.06.-16.07.2012 3. 20.09.-26.09.2012	Foliar spray	0.050	200	0.100	1 08.08.2012	75	Plants* Seed Seed Seed Rest of plants* Rest of plants* Rest of plants*	1.6 <0.005 <0.005 <0.005 0.035 0.200 0.057	<0.005 <0.005 <0.005 <0.005 <0.005 0.0160 0.0075	0 21 29 49 21 29 49	
390016 2014/1010807 16845Manker Germany (L130078)	SO 702 P64LE25	1. 25.04.2013 2. 16.07.-26.07.2013 3. 08.10.2013	Foliar spray	0.050	200	0.100	1 30.07.2013	75	Plants* Seed Seed Seed Rest of plants* Rest of plants* Rest of plants*	0.84 <0.005 <0.005 <0.005 0.042 0.043 0.057	<0.005 <0.005 <0.005 <0.005 0.005 0.005 0.005	0 22 29 70 22 29 70	BASF method No L0076/08 LOQ = 0.005 mg/kg
390016 2014/1010807 3534 Halen Belgium (L130079)	SO 702 PR64A31	1. 19.04.2013 2. 20.06.-05.07.2013 3. 11.09.2013	Foliar spray	0.050	200	0.100	1 12.08.2013	75	Plants* Seed Seed Rest of plants* Rest of plants*	1.10 <0.005 0.013 0.16 0.09	<0.005 <0.005 <0.005 0.011 0.008	0 22 30 22 30	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	Pictor SC
Crop/crop group:	Sunflower/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	**Z-isomer (505M98)**		
390016 2014/1010807 72500 Saint Pierre de Chevillé (L1300780)	SO 702 Violetta	1. 25.04.2013 2. 14.07.-10.08.2013 3. 08.10.2013	Foliar spray	0.050	177	0.088	1 12.08.2013	75	Plants* Seed Seed Seed Rest of plants* Rest of plants* Rest of plants*	0.50 <0.005 <0.005 <0.005 0.081 0.017 0.055	<0.005 <0.005 <0.005 <0.005 0.006 <0.005 <0.005	0 22 29 46 22 29 46	BASF method No L0076/08 LOQ = 0.005 mg/kg
390016 2014/1010807 6599 AV Ven-Zelderheide The Netherlands (L1300781)	SO 702 PR64A31	1. 17.04.2013 2. 21.06.-16.07.2013 3. 13.09.2013	Foliar spray	0.050	200	0.100	1 13.08.2013	75	Plants* Seed Seed Rest of plants* Rest of plants*	1.2 <0.005 <0.005 0.053 0.019	<0.005 <0.005 0.005 <0.005 <0.005	0 22 31 22 31	

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment
n.r. Not reported
* Without roots

Southern Europe

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	Pictor SC
Crop/crop group:	Sunflower/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	***Z*-isomer (505M98)**		
04 BASF 01 01 2005/1025862 Pusztaszölö Hungary (04 BASF 01 01/1)	SO 0702 Matis	1. n.r. 2. n.r. 3. 14.09.2004	Foliar spray	0.033	300	0.100	3 17.08.2004	85-87	Seed	<0.05	-	28	BASF method No 445/0 LOQ = 0.05 mg/kg
04 BASF 01 01 2005/1025862 Köröstetétlen Hungary (04 BASF 01 01/2)	SO 0702 Arena	1. n.r. 2. n.r. 3. 31.08.2004	Foliar spray	0.033-0.167	60-300	0.100	3 03.08.2004	81-83	Seed	<0.05	-	28	
04 BASF 01 01 2005/1025862 Nagykarácsony Hungary (04 BASF 01 01/3)	SO 0702 Alexandra	1. n.r. 2. n.r. 3. 30.08.2004	Foliar spray	0.033-0.040	250-300	0.100	3 02.08.2004	85	Seed	<0.05	-	28	
04 BASF 01 01 2005/1025862 Kápolnásnyék Hungary (04 BASF 01 01/4)	SO 0702 Pioneer/ 310448/ YF-3312	1. n.r. 2. n.r. 3. 20.09.2004	Foliar spray	0.033	300	0.100	3 23.08.2004	87	Seed	<0.05	-	28	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	Pictor SC
Crop/crop group:	Sunflower/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	***Z*-isomer (505M98)**		
BSF 0648/072364 2007/1006111 17240 St Fort sur Gironde France (BSF/648-06)	SO 0702 LG 5435	1. 15.05.2006 2. 28.07.-07.08.2006 3. 04.09.2006	Foliar spray	0.033	300	0.100	2 07.08.2006	69-71	Plant without roots Seed Seed Seed	1.00 0.01 <0.01 <0.01	-	0 21 28 36	BASF method No 535/2 LOQ = 0.01 mg/kg
BSF 0648/072364 2007/1006111 17210 Montlieu la Garde France (BSF/648-07)	SO 0702 Prodisol	1. 28.04.2006 2. 23.07.-05.08.2006 3. 04.09.2006	Foliar spray	0.033	300	0.100	2 07.08.2006	71	Plant without roots Seed Seed Seed	0.80 <0.01 <0.01 0.01	-	0 21 28 35	
BSF 643/062202 2006/1018116 01290 Biziat France (BSF/643-05)	SO 0702 Orasol	1. 05.05.2005 2. n.r. 3. 31.08.2005	Foliar spray	0.033	300	0.100	2 03.08.2005	67	Plant without roots Seed Seed Seed	2.924 <0.05 <0.05 <0.05	-	0 21 28 35	BASF method No 445/0 LOQ = 0.05 mg/kg
BSF 643/062202 2006/1018116 01090 Francheleins France (BSF/643-06)	SO 0702 Caramba	1. 01.05.2005 2. n.r. 3. 30.08.2005	Foliar spray	0.033	300	0.100	2 03.08.2005	67	Plant without roots Seed Seed Seed	1.659 <0.05 <0.05 <0.05	-	0 21 28 35	
390015 2013/1003723 40054 Bagnarola di Budrio Italy (L120224)	SO 0702 Mon 92 OL	1. 16.03.2012 2. 12.06.-27.06.2012 3. 09.08.2012	Foliar spray	0.05	200	0.100	1 03.07.2012	75	Plants* Seed Seed Seed Rest of plants* Rest of plants* Rest of plants*	0.57 0.021 0.040 0.025 0.91 0.88 0.44	<0.005 <0.005 <0.005 <0.005 0.046 0.044 0.025	0 22 30 37 22 30 37	BASF method No L0076/08 LOQ = 0.005 mg/kg

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	Pictor SC
Crop/crop group:	Sunflower/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	***Z*-isomer (505M98)**		
390015	SO 0702	1. 02.04.2012	Foliar	0.05	200	0.100	1	75	Plants*	1.4	<0.005	0	BASF method
2013/1003723	Sinfoni	2. 01.07.-14.07.2012	spray				19.07.2012		Seed	<0.005	<0.005	21	No L0076/08
32130		3. 06.09.2012							Seed	0.0089	<0.005	29	LOQ = 0.005 mg/kg
Cazaux Saves									Seed	<0.005	<0.005	47	
France									Rest of plants*	0.19	0.018	21	
(L120225)									Rest of plants*	0.17	0.012	29	
									Rest of plants*	0.22	0.016	47	
390015	SO 0702	1. 14.03.2012	Foliar	0.05	200	0.100	1	75	Plants*	1.7	<0.005	0	
2013/1003723	Imiko	2. 19.06.-02.07.2012	spray				12.07.2012		Seed	0.018	<0.005	20	
41710 Utrera		3. 17.08.2012							Seed	0.015	<0.005	29	
Spain									Seed	0.010	<0.005	35	
(L120226)									Rest of plants*	1.7	0.047	20	
									Rest of plants*	1.0	0.033	29	
									Rest of plants*	2.1	0.065	35	
390015	SO 0702	1. 12.06.2012	Foliar	0.05	200	0.100	1	75	Plants*	0.71	<0.005	0	
2013/1003723	Sikklos	2. 25.07.-05.08.2012	spray				24.08.2012		Seed	0.0080	<0.005	20	
59032 Platanos		3. 15.09.-30.09.2012							Seed	0.018	<0.005	31	
Greece									Seed	0.023	<0.005	34	
(L120227)									Rest of plants*	0.92	0.037	20	
									Rest of plants*	0.49	0.030	31	
									Rest of plants*	0.51	0.029	34	
390016	SO 0702	1. 09.04.2013	Foliar	0.05	200	0.100	1	75	Plants*	0.85	<0.005	0	BASF method
2014/1010807	H41	2. 08.06.-24.06.2013	spray				09.07.2013		Seed	<0.005	<0.005	22	No L0076/08
48124 San Michele		3. 11.09.2013							Seed	<0.005	<0.005	30	LOQ = 0.005 mg/kg
di Ravenna									Seed	<0.005	<0.005	64	
Italy									Rest of plants*	0.017	<0.005	22	
(L130082)									Rest of plants*	0.018	<0.005	30	
									Rest of plants*	0.017	<0.005	64	

RESIDUE DATA SUMMARY FROM SUPERVISED TRIALS (SUMMARY)

Active substance (common name)	BAS 505 F (Dimoxystrobin)	**Commercial Product (name)**	Pictor SC
Crop/crop group:	Sunflower/Oilseeds	**Producer of commercial product**	BASF SE
Responsible body for reporting (name, address)	BASF SE, 67117 Limburgerhof	**Indoor/Glasshouse/Outdoor**	Outdoor
Country	Germany	**Other active substance in the formulation (common name and content)**	BAS 510 F (Boscalid), 200 g/L
Content of active substance (g/kg or g/L)	200 g/L		
Formulation (e.g. WP)	SC (BAS 540 01 F)	**Residues calculated as:**	BAS 505 F, *Z*-isomer 505M98

1	2	3	4	5			6	7	8	9		10	11
Report-No Location (Trial No)	**Commodity/ Variety**	**Date of 1. Sowing / Planting 2. Flowering 3. Harvest**	**Method of treatment**	**Application rate[0] per treatment**			**No of treatm. and last date**	**Growth stage (BBCH)[2]**	**Portion analyzed**	**Residues (mg/kg)**		**DALA[1]**	**Remarks**
				kg a.s./hL	**Water (L/ha)**	**kg a.s./ha**				**BAS 505 F**	***Z*-isomer (505M98)**		
390016 2014/1010807 32220 St Lizier du Plante France (L130083)	SO 0702 MA S 85 OL	1. 23.04.2013 2. 15.07.-25.07.2013 3. 27.09.2013	Foliar spray	0.05	200	0.100	1 12.08.2013	75	Plants* Seed Seed Seed Rest of plants* Rest of plants* Rest of plants*	1.10 <0.005 <0.005 <0.005 0.57 0.29 0.71	<0.005 <0.005 <0.005 <0.005 0.031 0.019 0.047	0 21 30 43 21 30 43	BASF method No L0076/08 LOQ = 0.005 mg/kg
390016 2014/1010807 41727 Maribañez Spain (L130084)	SO 0702 Safira	1. 26.04.2013 2. 22.06.-01.07.2013 3. 05.09.2013	Foliar spray	0.05	200	0.100	1 10.07.2013	75	Plants* Seed Seed Seed Rest of plants* Rest of plants* Rest of plants*	1.1 0.012 0.013 <0.005 0.94 1.1 4.9	<0.005 <0.005 <0.005 <0.005 0.021 0.028 0.17	0 21 30 51 21 30 51	
390016 2014/1010807 59032 Plantanos Greece (L130085)	SO 0702 Sikklos	1. 02.05.2013 2. 20.06.-30.06.2013 3. 20.08.-30.08.2013	Foliar spray	0.05	200	0.100	1 26.07.2013	75	Plants* Seed Seed Rest of plants* Rest of plants*	1.1 0.022 0.018 1.5 0.78	<0.005 <0.005 <0.005 0.045 0.045	0 20 31 20 31	

0 Actual application rates varied by 10% at most except where noted otherwise
1 Days after last application
2 At last treatment
n.r. Not reported
* Without roots

Dimoxystrobin

Document M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

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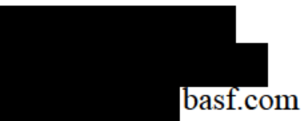
E-mail basf.com

Version history[1]

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

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

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CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Dimoxystrobin (BAS 505 F), a fungicide for use in rape and sunflower, is registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive No 2006/75/EC. Inclusion entered into force on 11 September 2006. The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011. Approval extension was granted until 31 January 2018 by Implementing Regulation (EU) No 1136/2012.

All relevant information on the first Annex I review and the endpoints used in environmental risk assessments can be found in the monograph of dimoxystrobin, in the EFSA conclusion on dimoxystrobin (2005) and in SANCO/10531/05-Final document (EU Review Report of April 2006).

For the current registration renewal under Regulation 1107/2009, a data gap analysis according to new guidelines, new guidance documents and new procedures in kinetic evaluations and exposure assessments was performed and new studies or kinetic evaluations were initiated where considered necessary. All new data are provided in this section.

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. Although title and abstract of several publications indicated a potential connection to respective environmental fate chapters of this dossier, the detailed evaluation of these publications showed no endpoint of sufficient reliability which could be used for the required risk assessments. Consequently, for environmental fate no summaries of public literature data on dimoxystrobin are provided in this section. Further information on the literature assessment and respective justifications can be found in M-CA 9.

Studies on the route of degradation were usually performed using the benzyl-^{14}C or phenyl-^{14}C labeled dimoxystrobin.

An overview of metabolites discussed in this section is given below. The table is including the different code numbers that are available for each metabolite. In the following chapters and study summaries synonym metabolite codes are given in brackets where deemed to be helpful.

Substance/ Metabolite Code	Reference code (Reg.No.)	Synonyms	Structure
Dimoxystrobin BAS 505 F	285028	-	
505M01	358104	BF 505-4	

Substance/ Metabolite Code	Reference code (Reg.No.)	Synonyms	Structure
505M03	4035810	BF 505-13 M505F003	
505M04	4035807	BF 505-12 M505F004	
505M08	354562	BF 505-7 M505F008	
505M09	354563	BF 505-8 M505F009	
505M96	4091091	M505F096	
505M97		M505F097	
505M98	360056	M505F098 Z-isomer of dimoxystrobin	

Substance/ Metabolite Code	Reference code (Reg.No.)	Synonyms	Structure
505M100		M505F100	
505M101		M505F101	
505M102		M505F102	
505M103	6001767	M505F103	
505M104	6001768	M505F104	
505M106		M505F106	

CA 7.1 Fate and behaviour in soil

CA 7.1.1 Route of degradation in soil

The route of degradation in soil of dimoxystrobin was overall investigated with two different radio-labels (^{14}C-benzyl- and ^{14}C-phenyl-label). Dimoxystrobin appears in two isomer forms (E/Z-isomers). Dimoxystrobin consists by definition of the E-isomer, whereas the Z-isomer represents only about 2 - 3% of the applied substance and can thus be considered as an impurity of the applied substance. Since the Z-isomer remained at low levels (max. 6.8 %) during all environmental fate studies, the significance of this isomer for the overall environmental behaviour can be neglected. For risk assessments, always the sum of both isomers was considered.

Most of the information on the route of degradation is derived from studies already peer-reviewed during the previous Annex I inclusion process. Nevertheless, some new studies are described below, which were conducted in order to confirm or complement the knowledge of dimoxystrobin degradation in soil.

CA 7.1.1.1 Aerobic degradation

No new aerobic soil metabolism study with dimoxystrobin was performed. The studies already peer-reviewed during the previous Annex I inclusion process are considered still valid.

The degradation of dimoxystrobin in aerobic soil studies is characterized by a moderate mineralization rate (about 15 - 25% of applied radioactivity (AR) within 120 days) and a moderate formation of bound residues (about 24% AR within 120 days).

Two metabolites, 505M08 (BF 505-7) and 505M09 (BF 505-8), were found in all soil types. They were formed by oxidation of either one of the two methyl groups at the phenyl ring, resulting in the respective carboxylic acids. Metabolite 505M09 reached a maximum of 13% AR in dark aerobic soil, whereas metabolite 505M08 never exceeded 3.3% AR. Bound residues increased with time and the major portion of radioactivity was associated with insoluble humins and high-molecular humic acids. A release of dimoxystrobin or metabolites could not be observed.

The overall route of degradation in soil (including old and new information) is shown in Figure 7.1.1.3-6.

CA 7.1.1.2 Anaerobic degradation

No new anaerobic soil metabolism study with dimoxystrobin was performed. The studies already peer-reviewed during the previous Annex I inclusion process are considered still valid.

Under anaerobic conditions, no relevant degradation took place. After 120 days of incubation, 93% AR of unchanged dimoxystrobin remained in soil.

CA 7.1.1.3 Soil photolysis

No new soil photolysis study was performed with dimoxystrobin. The studies already peer-reviewed during the previous Annex I inclusion process are considered still valid.

Major metabolite during soil photolysis was the cleavage product 505M01 (BF 505-4) which appeared at 11% of applied radioactivity (AR) with the benzyl-labelled test item. Two further metabolites appeared in the soil extracts (with both labels), reaching maximum amounts of 2.9 and 3.3% AR in the dark control and 5.2 and 7.1% AR in the irradiated samples, respectively. They were designated in the reports as "unknown a" and "unknown b". Structure proposals were given, however, they were not further considered during evaluation for Annex I approval.

Since they exceed 5% AR at the end of incubation, those metabolites have now to be assessed according to the new EU Regulation 1107/2009. Therefore, the proposed structures given in the old reports as well as the GLP raw data of the structure elucidation part of these studies were re-examined. It became obvious that the structures shown in the report were (1) not clear (i.e. unknown location of hydroxy group) and (2) could partly not be scientifically correct (methylation at the phenyl-ring considered not possible). By re-evaluation of the MS/MS spectra, the structures could be revised and clarified.

Report: CA 7.1.1.3/1
Willmann J., 2013a
Statement - Reevaluation of MS data for photolysis of 14C-BAS 505 F (phenyl label) on soil
2013/1256897

Guidelines: none

GLP: no

Reference (already peer-reviewed during previous Annex I listing):

BASF DocID 1997/5335	Jewel Trollinger Photolysis of ^{14}C-BAS 505 F (Phenyl Label) on Soil	GLP yes Agricultural Products Center; Research Triangle Park, NC 27709-3528; United States of America

In the dimoxystrobin (BAS 505 F) soil photolysis study, two metabolites with HPLC retention times of 19 and 21 minutes were detected, reaching amounts of max. 5.2 and 7.1% of the total applied radioactivity (TAR) after 15 days of continuous irradiation, respectively.

Although at time of study performance the formal trigger for starting metabolite structure elucidation was still at 10% TAR, attempts were made to further characterize these two photoproducts. Structure proposals and mass spectra are shown in the respective report (see pages 50 and 142 ff).

One structure was proposed as a hydroxylated derivative of the parent with a mass of 342u (peak 1, R_t 19 min, "unknown a") and the other one as a methylated derivative with a mass of 340u (peak 2, R_t 21 min, "unknown b"). Both structures are shown in Figure 7.1.1.3-1 below.

Figure 7.1.1.3-1: Structure proposals for metabolites as shown in the original BAS 505 F-soil photolysis report

HO O H N N O O

Hydroxylated BAS 505 F

H_3C O H N N O O

Methylated BAS 505 F

When evaluating the dimoxystrobin data package for Renewal of Approval in the EU according to the new Regulation 1107/2009, questions were raised on the correctness of the proposed chemical structures. Therefore, the available MS spectra for this study were reviewed again and re-evaluated.

II. RESULTS AND DISCUSSION

Peak 1, R_t 19 min, "unknown a"

For the peak proposed as hydroxylated BAS 505 F it is stated on page 117 in the soil photolysis report that the dominant peak in the MS spectrum is at m/z 325 with the molecular ion at m/z 343 being very small. The mass of the hydroxylated BAS 505 F is 342 with a molecular ion $[M+H]^+$ of 343. The dominant peak at m/z 325 can be explained by an in-source or transfer fragmentation of m/z 343 in the mass spectrometer and a resulting neutral loss of water (M = 18u).

In the soil photolysis report it is stated further that it is unusual with the ESI conditions on the Finnigan LCQ to have a product ion dominate the MS spectrum, which is correct in many cases. ESI is known as a soft ionization technique, which will form cluster ions, but will not form fragment ions during the ionization process. In the skimmer region of a mass spectrometer independently from the ionization technique (ESI or APCI) it is possible to have a non-targeted fragmentation of ionized molecules due to collisions with the background gas in the low vacuum region of the mass spectrometer.

In case of the hydroxylated BAS 505 F, it can be concluded that the hydroxylation took place on one of the methyl groups, because after the neutral loss of water a benzyl cation which will rearrange to the extremely stable aromatic tropylium cation is formed in the gas phase. Figure 7.1.1.3-2 will illustrate the fragmentation process and the formation of the tropylium cation.

Figure 7.1.1.3-2: Neutral loss and formation of the tropylium ion of the hydroxylated BAS 505 F (mass 340u).

The position of the hydroxylation on the dimethyl phenyl moiety is also underlined by the fragment of m/z 205 which corresponds to an unchanged pharmacophore. In case of a core hydroxylation on the dimethyl phenyl ring (phenol derivative formation), the loss of water by in-source/ transfer fragmentation can be excluded due to the high stability of aromatic hydroxyl groups in mass spectrometry.

Taking this argumentation into account, the location of hydroxylation shown in the soil photolysis report can now be specified, i.e. hydroxylation took place most probably at one of the methyl groups (see Figure 7.1.1.3-3).

Figure 7.1.1.3-3: New structure proposal for the dimoxystrobin metabolite with the mass 342 ("unknown a")

Peak 2, R_t 21 min, "unknown b"

For the second peak in the soil photolysis report, originally a methylation was suggested (Figure 7.1.1.3-1). This structure proposal had to be revised for several reasons. To the best of our knowledge, a methylation at a ring carbon atom due to photolysis can be excluded. And in the MS/MS spectrum on page 142, a characteristic fragmentation pattern for aldehyde functionalities can be observed. Aldehyde functions in a molecule show usually a neutral loss of carbon monoxide with a mass of 28u under MS/MS or EI conditions. In Figure 7.1.1.3-4, all fragments are assigned with their proposed gas phase structure. The ion with m/z 252 shows exactly this neutral loss of 28u which leads then to the mass 224. Therefore, it is justified to propose a new structure for the compound with the mass 340 (see Figure 7.1.1.3-5). Instead of a ring-methylation, it is more plausible that oxidation of one of the methyl groups to an aldehyde occurred. However, by means of MS/MS it is not possible to distinguish between the two possible aldehydes.

Figure 7.1.1.3-4: MS/MS Spectrum of the dimoxystrobin metabolite with mass 340 (R_t 21 min, "unknown b")

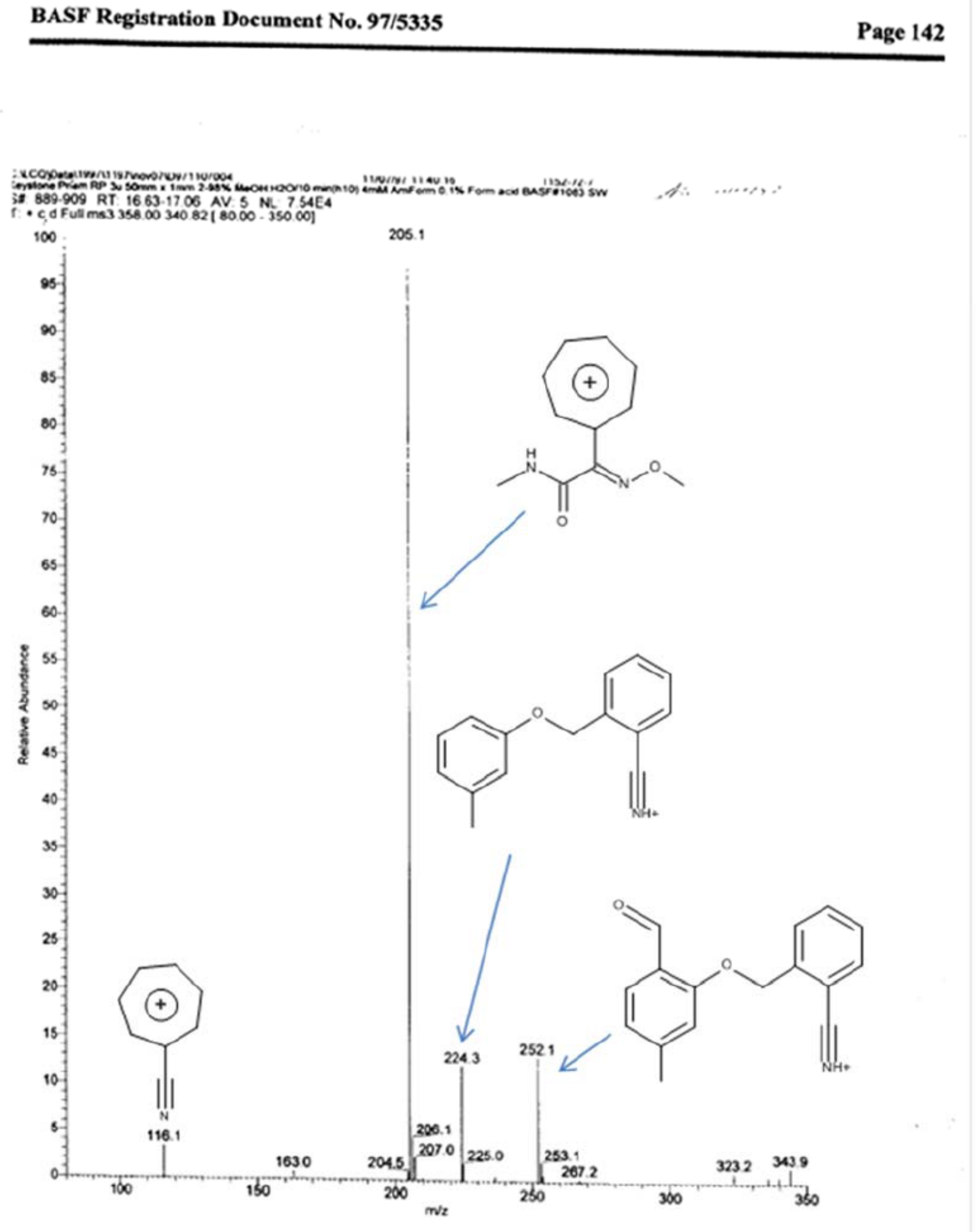

Figure 7.1.1.3-5: New structure proposal for the BAS 505 F metabolite with the mass 340 ("unknown b")

or

III. CONCLUSION

After re-evaluation of the MS spectra in the dimoxystrobin soil photolysis study, the proposed structures for the two detected metabolites had to be revised.

For the hydroxy-metabolite (peak 1, "*unknown a*"), the location of the hydroxy group could be specified. For the second metabolite (peak 2, "*unknown b*"), the structure had to be completely revised. Instead of the originally proposed methylation, which would be a very unusual reaction under the applied soil incubation conditions, the MS spectra rather suggest a further oxidation forming an aldehyde.

Summary Route of Degradation in Soil

Figure 7.1.1.3-6: Proposed route of degradation of dimoxystrobin in soil

BAS 505 F

505M03

505M04

505M01
(BF 505-4)

505M103

505M104

505M08
(BF 505-7)

505M09
(BF 505-8)

CO_2 + bound residues

The re-evaluation of metabolite structures of the soil photolysis study did not change the known scheme of route of degradation of dimoxystrobin in soil. The new structure proposals complement the understanding of formation of metabolites 505M08 and 505M09, since the carboxylic acids are formed from the methyl groups of the parent by a 2-step-oxidation via the alcohol and aldehyde intermediates.

Both intermediates are expected to be transient and very short-lived under realistic outdoor conditions. Therefore, the soil exposure and risk assessments are focused on the two frequently in laboratory studies detected carboxylic acids 505M08 and 505M09 (as the final products of the stepwise oxidation reaction) as well as on the photo-degradate 505M01.

Major sink of dimoxystrobin degradation in soil is the formation of bound residues, which amounted to about 24% TAR after 122 days. Mineralization to CO_2 was observed with both labels (up to 15 - 25% TAR after 119/120 days), indicating that dimoxystrobin can be completely degraded in soil.

CA 7.1.2 Rate of degradation in soil

CA 7.1.2.1 Laboratory studies

For parent, no new laboratory soil studies to determine the rate of degradation were performed. The already peer-reviewed laboratory aerobic soil studies are considered still valid. However, dimoxystrobin degradation rates were re-calculated using the experimental data of these studies and analysing the kinetic parameters according to the current FOCUS guidance.

Summary tables of all obtained laboratory soil degradation values for dimoxystrobin can be found at the end of this chapter.

CA 7.1.2.1.1 Aerobic degradation of the active substance

Report: CA 7.1.2.1.1/1
Eickler B., 2015a
Kinetic evaluation of aerobic soil degradation of BAS 505 F - Dimoxystrobin: Determination of modeling endpoints according to FOCUS Degradation Kinetics
2014/1175670

Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 1.1 of Dec. 2014

GLP: no

Executive Summary

The degradation of BAS 505 F - dimoxystrobin in soil has been investigated in four aerobic laboratory soil degradation studies in a range of soils. Additionally, non-standard incubations (20% MWHC, 5°C, 30°C) were performed with one soil. The purpose of this evaluation was to analyze the degradation kinetics of dimoxystrobin and its metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) observed in the studies according to current guidance of the FOCUS workgroup on degradation kinetics.

Modeling endpoints for dimoxystrobin were obtained from standard incubations (20°C, 40% MWHC), using SFO and DFOP kinetics in four and three soils, respectively. The calculated normalized modeling endpoints (20°C, pF2) ranged between 124.9 and 468.3 days.

Results for metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) are presented in CA 7.1.2.1.2/1.

I. MATERIAL AND METHODS

The degradation of dimoxystrobin and its soil metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) in six different soils in ten experiments [*Stephan A. – BASF DocID 1999/10087, Ebert D. – BASF DocID 1999/10077, Staudenmaier H., Schaefer C. – BASF DocID 1999/11656, Kurth H.-H. – BASF DocID 1998/1000808*] was analyzed taking into account the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 (November 2011), 436 pp*]. Soil characteristics are summarized in Table 7.1.2.1.1-1.

Table 7.1.2.1.1-1: Characteristics of the four aerobic soil degradation studies

Study	DocID 1999/10087; DocID 1999/10077	DocID 1998/1000808
Soil Designation	**Bruch West, Germany**	**Borstel, Germany**
Soil texture (USDA) sand [%] (0.050 – 2 mm) silt [%] (0.002 – 0.050 mm) clay [%] (< 0.002 mm)	sandy loam 69 18 13	loamy sand 77.7 17.3 5.0
Organic C [%]	2.0	1.2
Microbial biomass [mg C 100 g^{-1} dry soil]	45.7	23.7
CEC [meq 100 g^{-1}]	15.6	7.0
pH ($CaCl_2$) [-]	7.5	4.6
MWHC [g H_2O 100 g^{-1} dry soil]	44.0	5.6
FC [g H_2O/100 g dry soil] at 0.33 bar	19.0	28.8

Study	DocID 1999/11656				
Soil Designation	**Lufa 2.2, Germany**		**Minto, Canada**	**Dinuba, USA**	**Li 35 b, Germany**
	97/736/02[a]	**97/736/03[b]**			**98/145/02**
Soil texture (USDA) sand [%] (0.050 – 2 mm) silt [%] (0.002 – 0.050 mm) clay [%] (< 0.002 mm)	sand / loamy sand 86 9 5	loamy sand 76.0 15.3 8.7	loam 49 36 15	sandy loam 72 14 14	sandy loam 76 12 12
Organic C [%]	2.5	1.9	3.0	0.6	1.1
Microbial biomass [mg C 100 g^{-1} dry soil]	55.9	46.2	51.0	70.3	27.2
CEC [meq 100 g^{-1}]	11.2	10.0	33.0	11.0	10.0
pH ($CaCl_2$) [-]	5.8	5.8	7.7	7.0	6.8
MWHC [g H_2O/100 g dry soil]	43	50.3	43	29	34
FC [g H_2O/100 g dry soil] at 0.33 bar	14.7	16.3	33.0	n.a.	11.7

CEC cation exchange capacity
MWHC maximum water holding capacity
FC field capacity
[a] LUFA 2.2, 20°C, 40% and 20% MWHC
[b] LUFA 2.2, 30°C and 5°C, 40% MWHC
n.a. not available

An overview of the studies is given in Table 7.1.2.1.1-2.

Table 7.1.2.1.1-2: Overview on aerobic soil degradation studies with dimoxystrobin

Soil	Soil type	Incubation			Application rate [g ha^{-1}]	Analyte	Sampling days [DAT]	Study (BASF DocID)
		Moisture [MWHC]	Temp.	Time [d]				
Bruch West	sandy loam	40%	20°C	364	250	[benzyl-^{14}C]-dimoxystrobin	0, 7, 14, 31, 57, 87, 119, 183, 269, 364	1999/10087
Bruch West	sandy loam	40%	20°C	360	250	[phenyl-^{14}C]-dimoxystrobin	0, 3, 7, 14, 28, 63, 90, 122, 180, 270, 360	1999/10077
Lufa 2.2, 97/736/02	sand / loamy sand	40%	20°C	120	250	[benzyl-^{14}C]-dimoxystrobin	0, 3, 7, 14, 34, 63, 90, 120	1999/11656
		20%	20°C	120			0, 3, 7, 14, 34, 63, 90, 120	
Lufa 2.2, 97/736/03	loamy sand	40%	5°C	120			0, 3, 7, 14, 30 ,63, 90, 120	
		40%	30°C	120			0, 3, 7, 14, 30, 70, 90, 120	
Minto	loam	40%	20°C	125			0, 3, 7, 14, 30, 62, 90, 125	
Dinuba	sandy loam			125			0, 3, 7, 14, 34, 62, 90, 125	
Li 35 b	sandy loam			120			0, 3, 7, 14, 30, 63, 90, 120	
Borstel	Loamy sand	40%	20°C	99	250	[benzyl-^{14}C]-dimoxystrobin	0, 1, 3, 7, 14, 30, 47, 61, 79, 99	1998/1000808

Kinetic modeling strategy

Kinetic evaluation was performed in order to derive degradation parameters for the parent compound dimoxystrobin as well as the metabolite 505M08 and 505M09 observed in the studies. Appropriate $DegT_{50}$ for use in environmental fate models were derived depending on the kinetic model.

Kinetic evaluation of the formation and degradation of dimoxystrobin metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) was included in the assessment. The metabolites were added in a linked model to the appropriate kinetic model for the parent. It was assumed that both metabolites may be formed directly from the parent and may further degrade (sink).

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation reports.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the appropriate model. The recommended kinetic models, i.e. the single first order kinetics (SFO) and the double-first-order in parallel model (DFOP) were applied.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling and software for kinetic evaluation

For all samplings, single measurements were available. The experimental data were derived from the study report and were adjusted according to FOCUS. If results were reported for dimoxystrobin and its z-isomer, the sum of both measurements was used as input for the parent. The initial concentration of the parent substance was set to the material balance recovered at day 0 and the initial concentration of the degradation products was set to zero. Where necessary, values <LOD were set to ½ LOD as recommended by the FOCUS Kinetics guidance document. As no separate values for LOQ or LOD were reported, the LOD was defined as the lowest reported significant value (i.e. 0.1% TAR).

The software package KinGUI version 2.2014.224.1704 was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to $1x10^{-6}$ and 100, respectively.

Normalization to reference conditions

According to FOCUS [*FOCUS (2006)*] the DT_{50} values obtained from laboratory studies should be normalized for modeling purposes to reference conditions at a temperature of 20°C and soil moisture at pF2 to account for the different soil temperature and moisture conditions during incubation. The temperature normalization was performed using a Q_{10} value of 2.58. The actual soil moisture was used as specified in the study report. The corresponding water content at field capacity was estimated according to FOCUS [*FOCUS (2000): "FOCUS groundwater scenarios in the EU review of active substances" Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev. 2*].

II. RESULTS AND DISCUSSION

Modeling endpoints for dimoxystrobin were obtained using SFO and DFOP kinetics in seven and three soils, respectively. The $DegT_{50}$ values suitable for modeling obtained under different incubation conditions were normalized to reference moisture of pF2 and a temperature of 20°C. Results of non-standard incubation conditions were not considered for derivation of modeling endpoints.

Parameters included in the normalization procedure are shown in Table 7.1.2.1.1-3, while derived modeling endpoints are summarized in Table 7.1.2.1.1-4.

Table 7.1.2.1.1-3: Calculated factors for normalization to reference conditions (20°C, pF2)

Study (DocID)	Soil & incubation conditions	θ_{act}	θ_{ref} [a]	f_{moist}	$Temp_{act}$ [°C]	$Temp_{ref}$ [°C]	f_{temp}
1999/10087	Bruch West sandy loam 20°C, 40% MWHC	17.6	19	0.95	20	20	1
1999/10077	Bruch West sandy loam 20°C, 40% MWHC	17.6	19	0.95	20	20	1
1999/11656	Lufa 2.2 sand/loamy sand 20°C, 40% MWHC	17.2	13[c]	1.00	20	20	1
	Minto (Canada) loam 20°C, 40% MWHC	17.6	25	0.77	20	20	1
	Dinuba (USA) sandy loam 20°C, 40% MWHC	11.6	19	0.71	20	20	1
	Li 35 b sandy loam 20°C, 40% MWHC	13.6	19	0.79	20	20	1
1998/1000808	Borstel loamy sand (DIN)/sandy loam (extrapolated to USDA)[b] 20°C, 40% MWHC	11.5	19[b]	0.70	20	20	1

θ_{act} actual soil moisture [g/100 g dry soil]
θ_{ref} reference soil moisture at field capacity (pF 2, 10 kPa) according to FOCUS [g/100 g dry soil]
f_{moist} moisture correction factor [-]
f_{temp} temperature correction factor [-]
[a] estimated according to FOCUS [2000]
[b] soil classification was extrapolated from the German system to the USDA classification by addition of 5% to the sand fraction [*VAN VLAARDINGEN, P. L. A., SMIT, C., E. (2009): Verification of moisture content in test soils. National Institute for Public Health and the Environment (rivm), Letter Report 601516016/2009*]
[c] average of default gravimetric water content at field capacity for sand (12%) and loamy sand (14%) derived from FOCUS (2000)

Table 7.1.2.1.1-4: Degradation endpoints for dimoxystrobin (laboratory studies)

Study (DocID)	Soil	Kinetic model	χ^2 error level	Best-fit		Modeling endpoints	
				$DegT_{50}$ [d]	$DegT_{90}$ [d]	$DegT_{50}$ [d]	Normalized $DegT_{50}$ [d] [a]
1999/10087	Bruch West sandy loam 20°C, 40% MWHC	DFOP	2.1	76.1	381.3	145.4[b]	137.8
1999/10077	Bruch West sandy loam 20°C, 40% MWHC	DFOP	2.4	68.6	368.9	131.8[b]	124.9
1999/11656	Lufa 2.2 sand/loamy sand 20°C, 40% MWHC	DFOP	1.2	419.9	>1000	468.3[b]	468.3
	Minto (Canada) loam 20°C, 40% MWHC	SFO	2.4	357.2	>1000	357.2	274.9
	Dinuba (USA) sandy loam 20°C, 40% MWHC	SFO	1.5	258.9	860.1	258.9	183.3
	Li 35 b sandy loam 20°C, 40% MWHC	SFO	1.4	396.4	>1000	396.4	313.7
	Lufa 2.2 sand/loamy sand 20°C, 20% MWHC	SFO	1.6	294.1	976.8	294.1	n.r.
	Lufa 2.2 sand/loamy sand 5°C, 40% MWHC	SFO	1.4	>1000	>1000	1184[c]	n.r.
	Lufa 2.2 sand/loamy sand 30°C, 40% MWHC	SFO	1.9	198.3	658.7	198.3	n.r.
1998/1000808	Borstel loamy sand (DIN) 20°C, 40% MWHC	SFO	1.0	305.6	1015	305.6	215.3
Geometric mean							**243.6 [d]**

[a] Reference conditions: 20°C, pF 2
[b] derived from slow phase of biphasic model (DT_{50slow}=ln(2)/k2)
[c] calculated from k (DT_{50}=ln(2)/k)
[d] Results from Bruch West soil with two labels were averaged before calculating the overall geometric mean
n.r. non-standard incubation conditions not considered for derivation of modeling endpoints

Results for metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) are presented in CA 7.1.2.1.2/1.

III. CONCLUSION

Modeling endpoints were derived for dimoxystrobin in four laboratory degradation studies with ten soils.

Modeling endpoints for dimoxystrobin were obtained from standard incubations (20°C, 40% MWHC), using SFO and DFOP kinetics in four and three soils, respectively. The calculated normalized modeling endpoints (20°C, pF2) ranged between 124.9 and 468.3 days.

Summary of degradation endpoints for dimoxystrobin in different soils under aerobic conditions

The derived degradation endpoints (best-fit) for dimoxystrobin are summarized in Table 7.1.2.1.1-5. The degradation endpoints suitable for modelling and normalized to reference conditions (20°C, pF2.0) are summarized in Table 7.1.2.1.1-6.

Table 7.1.2.1.1-5: Summary table on degradation endpoints of dimoxystrobin obtained in laboratory soil studies

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	Best-fit $DegT_{50}$ / $DegT_{90}$ [d]	Kinetic model	χ^2 error level
1999/10087 [1] 2014/1175670	Bruch West sandy loam (b)	7.5	2.0	20	40	76.1 / 381.3	DFOP	2.1
1999/10077 [1] 2014/1175670	Bruch West sandy loam (p)	7.5	2.0	20	40	68.6 / 368.9	DFOP	2.4
1999/11656 [1] 2014/1175670	Lufa 2.2 sand/loamy sand (b)	5.8	2.5	20	40	419.9 / >1000	DFOP	1.2
	Minto loam (b)	7.7	3.0	20	40	357.2 / >1000	SFO	2.4
	Dinuba sandy loam (b)	7.0	0.6	20	40	258.9 / 860.1	SFO	1.5
	Li 35b sandy loam (b)	6.8	1.1	20	40	396.4 / >1000	SFO	1.4
	Lufa 2.2 sand/loamy sand (b)	5.8	2.5	20	20	294.1 / 976.8	SFO	1.6
	Lufa 2.2 sand/loamy sand (b)	5.8	2.5	5	40	>1000 / >1000	SFO	1.4
	Lufa 2.2 sand/loamy sand (b)	5.8	2.5	30	40	198.3 / 658.7	SFO	1.9
1998/1000808 [1] 2014/1175670	Borstel loamy sand (b)	4.6	1.2	20	40	305.6 / >1000	SFO	1.0

(b) (p) - benzyl- or phenyl-labeled test item used
MWHC maximum water holding capacity
[1] already peer-reviewed during former Annex I listing

Table 7.1.2.1.1-6: Summary table on degradation endpoints for modeling of dimoxystrobin obtained in laboratory soil studies (20°C, pF2)

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	$DegT_{50}$ normalized to 20°C, pF2 [d]	Kinetic model	χ^2 error level
1999/10087 [1] 2014/1175670	Bruch West sandy loam (b)	7.5	2.0	20	40	137.8	DFOP	2.1
1999/10077 [1] 2014/1175670	Bruch West sandy loam (p)	7.5	2.0	20	40	124.9	DFOP	2.4
1999/11656 [1] 2014/1175670	Lufa 2.2 sand/loamy sand (b)	5.8	2.5	20	40	468.3	DFOP	1.2
	Minto loam (b)	7.7	3.0	20	40	274.9	SFO	2.4
	Dinuba sandy loam (b)	7.0	0.6	20	40	183.3	SFO	1.5
	Li 35b sandy loam (b)	6.8	1.1	20	40	313.7	SFO	1.4
1998/1000808 [1] 2014/1175670	Borstel	4.6	1.2	20	40	215.3	SFO	1.0

(b) (p) - benzyl- or phenyl-labeled test item used
MWHC maximum water holding capacity
[1] already peer-reviewed during former Annex I listing

Calculation of degradation rates at 10°C

$DegT_{50}$ values at a temperature of 10°C were calculated by multiplying the normalized $DegT_{50}$ values at 20°C and pF 2 with a default Q_{10} value of 2.58 [*EFSA (2007): "Opinion on a request from EFSA related to the default Q_{10} value used to describe the temperature effect on transformation rates of pesticides in soil. Scientific Opinion of the Panel on Plant Protection Products and their Residues (PPR-Panel). Question No EFSA-Q-2007-048. The EFSA Journal (2007) 622, 1-32*]. The calculated half-lives of dimoxystrobin at 10°C are given in the table below.

Table 7.1.2.1.1-7: Summary table on degradation endpoints for modeling of dimoxystrobin obtained in laboratory soil studies, re-calculated to 10°C and pF 2

BASF DocID	Soil / Soil type	pH ($CaCl_2$)	Org. C [%]	Temp. [°C]	Moisture [% MWHC]	$DegT_{50}$ at 20°C, pF2 [d]	$DegT_{50}$ at 10°C, pF2 [d]	Method of calculation
1999/10087 [1] 2014/1175670	Bruch West sandy loam (b)	7.5	2.0	20	40	137.8	355.5	Q_{10} = 2.58
1999/10077 [1] 2014/1175670	Bruch West sandy loam (p)	7.5	2.0	20	40	124.9	322.2	Q_{10} = 2.58
1999/11656 [1] 2014/1175670	Lufa 2.2 sand/loamy sand (b)	5.8	2.5	20	40	468.3	1208.2	Q_{10} = 2.58
	Minto loam (b)	7.7	3.0	20	40	274.9	709.2	Q_{10} = 2.58
	Dinuba sandy loam (b)	7.0	0.6	20	40	183.3	472.9	Q_{10} = 2.58
	Li 35b sandy loam (b)	6.8	1.1	20	40	313.7	809.3	Q_{10} = 2.58
1998/1000808 [1] 2014/1175670	Borstel	4.6	1.2	20	40	215.3	555.5	Q_{10} = 2.58

(b) (p) - benzyl- or phenyl-labeled test item used
MWHC maximum water holding capacity
[1] already peer-reviewed during former Annex I listing

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

Degradation rates in three soils could not be reliably obtained for all metabolites from the parent studies. Therefore, in addition to the re-calculation of the degradation rates from the old parent studies, separate degradation rate studies were performed using the respective metabolites as test items.

Summary tables on maximum occurrence and degradation rates of metabolites obtained in different parent and metabolite studies are provided at the end of this chapter.

Report:	CA 7.1.2.1.2/1 Eickler B., 2015a Kinetic evaluation of aerobic soil degradation of BAS 505 F - Dimoxystrobin: Determination of modeling endpoints according to FOCUS Degradation Kinetics 2014/1175670
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 1.1 of Dec. 2014
GLP:	no

Executive Summary

The degradation of BAS 505 F - dimoxystrobin in soil has been investigated in four aerobic laboratory soil degradation studies in a range of soils. Additionally, non-standard incubations (20% MWHC, 5°C, 30°C) were performed with one soil. The purpose of this evaluation was to analyze the degradation kinetics of dimoxystrobin and its metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) observed in the studies according to current guidance of the FOCUS workgroup on degradation kinetics.

Modeling-DegT_{50} and corresponding formation fractions could be derived for each metabolite in five out of six soils where the metabolites were observed in sufficient amounts. Normalized modeling-DegT_{50} ranged between 12.2 and 68.1 days for metabolite 505M08 (BF 505-7) and between 29.1 and 76.7 days for metabolite 505M09 (BF 505-8). Reliable formation fractions were between 0.041 and 0.361 for metabolite 505M08 (BF 505-7), and between 0.070 and 0.411 for metabolite 505M09 (BF 505-8).

I. MATERIAL AND METHODS

Please refer to CA 7.1.2.1.1/1 for a description of the kinetic evaluation and normalization of modeling endpoints.

II. RESULTS AND DISCUSSION

For details of the parent kinetics, please refer to CA 7.1.2.1.1/1.

For the metabolites 505M08 (BF-505-7) and 505M09 (BF-505-8), the derived SFO-$DegT_{50}$ values are suitable as modeling endpoints after normalization to reference conditions. Calculated modeling endpoints are summarized in Table 7.1.2.1.2-1 and Table 7.1.2.1.2-2.

Table 7.1.2.1.2-1: Degradation endpoints for metabolite 505M08

Study (DocID)	Soil	Kinetic model	χ^2 error level	Modeling endpoints		
				$DegT_{50}$ [d]	Normalized $DegT_{50}$ [d][a]	FF [-]
1999/10087	Bruch West sandy loam 20°C, 40% MWHC	SFO	16.7	12.9	12.2	0.254
1999/10077	Bruch West sandy loam 20°C, 40% MWHC	SFO	8.5	57.3	54.3	0.110
1999/11656	Lufa 2.2 sand/loamy sand 20°C, 40% MWHC	SFO	9.1	68.1	68.1	0.223
	Minto (Canada) loam 20°C, 40% MWHC	SFO	26.6	No reliable degradation endpoints derived		0.041
	Dinuba (USA) sandy loam 20°C, 40% MWHC	SFO	14.8	27.9	19.8	0.361
	Li 35 b sandy loam 20°C, 40% MWHC	SFO	12.4	61.3	48.5	0.219

[a] Reference conditions: 20°C, pF 2

Table 7.1.2.1.2-2: Degradation endpoints for metabolite 505M09

Study (DocID)	Soil	Kinetic model	χ^2 error level	Modeling endpoints		
				$DegT_{50}$ [d]	Normalized $DegT_{50}$ [d][a]	FF [-]
1999/10087	Bruch West sandy loam 20°C, 40% MWHC	SFO	4.6	56.5	53.6	0.411
1999/10077	Bruch West sandy loam 20°C, 40% MWHC	SFO	3.4	80.9	76.7	0.344
1999/11656	Lufa 2.2 sand/loamy sand 20°C, 40% MWHC	SFO	12.8	29.1	29.1	0.323
	Minto (Canada) loam 20°C, 40% MWHC	SFO	11.6	No reliable degradation endpoints derived		0.070
	Dinuba (USA) sandy loam 20°C, 40% MWHC	SFO	9.1	69.6	49.3	0.358
	Li 35 b sandy loam 20°C, 40% MWHC	SFO	11.1	62.5	49.5	0.291

[a] Reference conditions: 20°C, pF 2

III. CONCLUSION

Modeling endpoints were derived for dimoxystrobin and its metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) in four laboratory degradation studies with ten soils.

For modeling endpoints for dimoxystrobin, please refer to CA 7.1.2.1.1/1.

For metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) modeling-$DegT_{50}$ and corresponding formation fractions could be derived for each metabolite in five out of six soils where the metabolites were observed in sufficient amounts. Normalized modeling-$DegT_{50}$ ranged between 12.2 and 68.1 days for metabolite 505M08 (BF 505-7) and between 29.1 and 76.7 days for metabolite 505M09 (BF 505-8). Reliable formation fractions were between 0.041 and 0.361 for metabolite 505M08 (BF 505-7), and between 0.070 and 0.411 for metabolite 505M09 (BF 505-8).

Report: CA 7.1.2.1.2/2
Sacchi R., 2015a
Rate of degradation of 505M08 (Reg. No. 354562) on European Soils under aerobic conditions
2013/3009622

Guidelines: OECD 307 (2002)

GLP: yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

The objective of the present study was to investigate the rate of degradation of 505M08 in three European soil types at a temperature of 20 ± 2°C.

The soils were treated with a nominal rate of 0.133 mg ^{14}C-labelled 505M08 per kg of dry soil which corresponds to a field application rate of 50 g a.i. ha^{-1}. The incubation was carried out in the dark in the laboratory under aerobic conditions at a soil moisture of 40% of the maximum water holding capacity. A closed incubation system with continuous aeration (moistened air) was used with an attached trapping system for the determination of volatile compounds. Samples were taken at 0, 2, 7, 14, 30, 62, 90, and 119 days after treatment (DAT).

The soil samples were extracted once with methanol, twice with methanol/water (1:1, v/v), and subsequently rinsed with acetone. The extracts were analyzed by means of LSC and HPLC. The amount of non-extractable residues was determined by combustion and subsequent LSC measurements.

The mass balance throughout the study ranged from 90.0 to 102.9% of total applied radioactivity (TAR). The extractable radioactivity decreased from > 95.6% TAR at day 0 to 61.5 – 68.4% after 119 days. The majority of radioactivity in the extracts was always unchanged test item. At the end of incubation, 505M08 was detected in relative amounts of 34.5 – 61.5% TAR.

In two of the soils (Li 10 and Lufa 5M), no metabolite exceeded 5% TAR at any sampling time. In soil Lufa 2.2, one metabolite reached amounts of 30.0% TAR after 119 days. This metabolite was identified as the amide of the test item and designated as 505M100. Formation of CO_2 was observed in all three soils reaching 3.6 – 5.4% TAR after 119 days. No other volatile compounds were detected. Non-extractable residues were formed in amounts reaching a maximum of 21.9 to 27.6% TAR at the end of the study.

Degradation times of 505M08 were calculated using KinGUI (version 2) applying first order kinetics (DFOP, FOMC). $DegT_{50}$ values ranged between 60.7 and 255.7 days.

I. MATERIAL AND METHODS

A. MATERIALS

BAS code:	505M08 / BF505-7
Reg.No.:	354562
Chemical name:	(E)-o-[(2-hydroxycarbonyl-5-methyl)phenoxymethyl]-2-methoxyimino-N-methylphenylacetamide
Molecular weight:	356.38 g mol^{-1}
Position of radiolabel:	Benzyl-ring-U-C^{14}
Batch No.:	695-2038
Specific radioactivity:	7.43 MBq mg^{-1}
Radiochemical purity:	99.3% (according to certificate) 97.5% (determined by radio-HPLC prior to soil treatment in this study – average of 3 analyses)

2. Soils

Three different soils were used for treatment and incubation. The soils were sieved through a 2 mm sieve before use. The soil characteristics are summarized in Table 7.1.2.1.2-3.

Table 7.1.2.1.2-3: Properties of soils used to investigate degradation rate of 505M08

Soil designation	Li 10 (12/1680/05)	LUFA 2.2 (12/736/05)	LUFA 5M (12/1651/05)
Origin	Limburgerhof, RP, Germany	Hanhofen, RP, Germany	Mechtersheim, RP, Germany
DIN Particle size distribution [%]			
Sand 0.063 – 2 mm	82.8	68.5	54.1
Silt 0.002 – 0.063 mm	11.7	20.0	31.7
Clay < 0.002 mm	5.5	11.5	14.2
Textural class	Loamy sand	Loamy sand	Loamy sand
USDA Particle size distribution [%]			
Sand 0.050 – 2 mm	83.5	69.9	58.4
Silt 0.002 – 0.050 mm	11.0	18.6	27.4
Clay < 0.002 mm	5.5	11.5	14.2
Textural class	Loamy sand	Sandy loam	Sandy loam
Organic C [%]	0.81	1.6	2.18
pH (H_2O)	7.1	6.4	8.0
pH ($CaCl_2$)	6.3	5.4	7.4
Cation exchange capacity [$cmol^+ kg^{-1}$]	5.0	8.6	8.8
Maximum water holding capacity [g/100g dry soil]	23.1	31.0	26.2
Microbial biomass* [mg C/100g dry soil]	33.6	42.1	31.1
Microbial biomass at 62 DAT [mg C/100g dry soil]	36	44	32
Microbial biomass at 119 DAT [mg C/100g dry soil]	40	40	35

*data obtained before starting the study
DAT = days after treatment

The soils were transported from Germany to Brazil in a temperature range of 4 - 20°C and were stored in a refrigerator at 4 ± 2°C before use.

B. STUDY DESIGN

1. Experimental conditions

The soils were adjusted to about 40% MWHC and treated at a nominal concentration of 0.133 mg ^{14}C-505M08 per kg dry soil, which corresponds to a field application rate of 50 g a.i. ha^{-1} (assuming an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}).

For the soil treatment, one kg of each soil was treated with 1 mL of the treatment solution. The treated soils were homogenized and soil portions of 50 g dry soil equivalents were filled into suitable test vessels. The number of vessels was sufficient to allow duplicate sampling at each sampling time and to keep a limited number of reserve vessels.

The vessels were incubated in the dark for 119 days at a temperature of 20 ± 2°C. Throughout the incubation period, the test vessels were continuously aerated with a slight stream of moistened and CO_2-free air. The outgoing air was led through a series of gas-washing bottles containing trapping solutions for potential volatiles (ethylene glycol, 0.5M H_2SO_4, 0.5M NaOH). The water content of the soils was monitored throughout the incubation period by weighing representative vessels at each sampling time. If necessary, the soil moisture was readjusted (to 40% MWHC).

For determination of the microbial biomass during (62 days) and at the end of incubation (119 days), additional soil samples treated with methanol but without test item were incubated under the same conditions.

2. Sampling

Sampling times were 0 (no sampling of volatiles), 2, 7, 14, 30, 62, 90 and 119 days after treatment (DAT).

At 0 DAT as well as at 62 DAT and 119 DAT two replicate samples were worked up. At the other sampling times only one replicate was worked up, the second one was stored in a freezer.

3. Description of the analytical procedures

The soil was extracted consecutively one time with 100 mL methanol and two times with 100 mL methanol/water (1:1, v:v) by shaking for about 30 minutes. After each extraction step, the suspension was centrifuged and two aliquots of the supernatant were measured by LSC.

After the last extraction, the soil was rinsed with 100 mL acetone. The acetone was also checked for radioactivity, but no HPLC analysis was performed since the amounts of radioactive residues never exceeded 2.9% of the total applied radioactivity (TAR).

All the extracts were combined and concentrated to a small volume. Prior to HPLC analysis, the concentrated extracts were centrifuged. Then the supernatant was radio-assayed and analyzed by HPLC to obtain the metabolite pattern.

The extracted soil was dried at room temperature and homogenized with a small mill. At least three aliquot parts of soil per sampling date were combusted to determine the amount of the non-extractable radioactive residues (NER).

All liquid samples were measured for radioactivity by LSC. The radioactivity in the extracted and dried soil samples was determined by combustion of at least three aliquot parts. Combustion products were absorbed in the Oxysolve C-400 scintillation cocktail.

4. Kinetic modeling

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] in order to derive degradation kinetics and trigger endpoints. The analysis was conducted by non-linear regression methods using the software tool KinGUI (version 2).

For each data set, the kinetic models proposed by the FOCUS Kinetic guidance document were tested in order to identify the best-fit model. The recommended kinetic models, i.e. the single first order kinetics (SFO), the Gustafson-Holden model (FOMC) and the bi-exponential kinetic model (DFOP) are already implemented in KinGUI.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures recommended by the FOCUS guidance. The goodness-of-fit measure used for the identification of the best-fit kinetic model is the χ^2 minimum error level. The kinetic endpoints (DT_{50} and DT_{90} values) are reported for the kinetic models, which were selected based on statistical and visual assessment. The reliability of individual parameters was judged by means of a single-sided t-test. When available, replicate measurements were used for the parameter estimation.

In all soils, the initial concentration of the applied test item was set to the material balance recovered at day 0. At later sampling time points (2, 7, 14, 30, 62, 90 and 119 DAT), the measured residues of the applied substance were used for modeling.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The detailed results on the extractability of radioactive residues in the soils are presented in Table 7.1.2.1.2-4 to Table 7.1.2.1.2-6. The material balance ranged from 90.0 to 102.9% TAR for all soils.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (ERR), non-extractable residues (NER), CO_2 and other volatiles for each soil is shown in Table 7.1.2.1.2-4 to Table 7.1.2.1.2-6. The amount of extractable radioactivity moderately decreased from more than 95.6% TAR at 0 DAT to 61.5 – 68.4% TAR after 119 days of incubation.

Non-extractable radioactive residues (NER) reached maximum values of 21.9 to 27.6% TAR at the end of the study after 119 days of incubation.

Table 7.1.2.1.2-4: Distribution of radioactivity and mass balance in Li 10 soil after treatment with 505M08 [%TAR]

DAT	extractable				NER	Volatiles*				Mass Balance
	MeOH	MeOH + water	Acetone	Total		CO_2 trap	H_2SO_4 trap	Ethylene Glycol trap	Total	
0 (rep 1)	52.2	43.6	1.2	97.0	1.6	n.d.	n.d.	n.d.	n.d.	98.6
0 (rep 2)	52.1	46.4	1.3	99.8	1.6	n.d.	n.d.	n.d.	n.d.	101.4
0 (mean)	**52.1**	**45.0**	**1.3**	**98.4**	**1.6**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**100.0**
2	47.8	42.4	1.4	91.7	5.7	0.2	0.0	0.0	0.2	97.6
7	49.6	39.5	1.0	90.1	9.2	0.7	0.0	0.0	0.7	100.1
14	43.5	38.2	1.0	82.7	10.2	1.1	0.0	0.0	1.1	94.0
30	44.6	36.4	1.5	82.5	16.1	1.9	0.0	0.0	1.9	100.5
62 (rep 1)	39.6	32.3	1.2	73.0	18.2	2.7	0.0	0.0	2.7	93.8
62 (rep 2)	39.7	33.7	1.2	74.6	19.5	2.7	0.0	0.0	2.7	96.8
62 (mean)	**39.6**	**33.0**	**1.2**	**73.8**	**18.8**	**2.7**	**0.0**	**0.0**	**2.7**	**95.3**
90	35.9	33.6	1.6	71.1	21.4	4.2	0.0	0.0	4.2	96.7
119 (rep 1)	34.8	32.1	1.6	68.4	23.7	5.3	0.0	0.0	5.4	97.4
119 (rep 2)	34.7	31.1	1.5	67.2	24.3	5.4	0.0	0.0	5.4	97.0
119 (mean)	**34.7**	**31.6**	**1.5**	**67.8**	**24.0**	**5.4**	**0.0**	**0.0**	**5.4**	**97.2**
									mean:	**97.6**

TAR total applied radioactivity (100% = 0.127 mg/kg)
DAT days after treatment
NER non-extractable radioactive residues
MeOH methanol
n.d. not determinated
rep replicate
* cumulated values

Table 7.1.2.1.2-5: Distribution of radioactivity and mass balance in Lufa 2.2 soil after treatment with 505M08 [%TAR]

DAT	extractable				NER	Volatiles*				Mass Balance
	MeOH	MeOH + water	Aceton e	Total		CO_2 trap	H_2SO_4 trap	Ethylene Glycol trap	Total	
0 (rep 1)	35.7	57.1	2.8	95.6	1.5	n.d.	n.d.	n.d.	n.d.	97.1
0 (rep 2)	38.6	58.7	2.9	100.2	2.7	n.d.	n.d.	n.d.	n.d.	102.9
0 (mean)	**37.1**	**57.9**	**2.9**	**97.9**	**2.1**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**100.0**
2	32.2	54.2	2.5	88.8	9.1	0.2	0.0	0.0	0.2	98.1
7	31.6	49.7	2.3	83.6	13.6	0.8	0.0	0.0	0.8	98.0
14	31.9	48.3	1.9	82.0	14.7	1.3	0.0	0.0	1.3	98.0
30	29.9	43.5	2.1	75.5	19.9	2.5	0.0	0.0	2.5	98.0
62 (rep 1)	30.0	36.1	1.7	67.8	22.7	2.5	0.0	0.0	2.5	93.0
62 (rep 2)	30.0	33.5	1.6	65.1	23.6	2.5	0.0	0.0	2.5	91.3
62 (mean)	**30.0**	**34.8**	**1.7**	**66.4**	**23.2**	**2.5**	**0.0**	**0.0**	**2.5**	**92.1**
90	30.8	33.5	2.2	66.6	22.4	3.4	0.0	0.0	3.5	92.4
119 (rep 1)	34.1	30.6	1.6	66.3	22.8	4.0	0.0	0.0	4.0	93.1
119 (rep 2)	35.0	30.1	1.5	66.7	21.9	4.0	0.0	0.0	4.0	92.6
119 (mean)	**34.5**	**30.4**	**1.6**	**66.5**	**22.4**	**4.0**	**0.0**	**0.0**	**4.0**	**92.9**
									mean:	**95.7**

TAR total applied radioactivity (100% = 0.139 mg/kg)
DAT days after treatment
NER non-extractable radioactive residues
MeOH methanol
n.d. not determinated
rep replicate
* cumulated values
mean mean value of replicates

Table 7.1.2.1.2-6: Distribution of radioactivity and mass balance in Lufa 5M soil after treatment with 505M08 [%TAR]

DAT	ERR				NER	Volatiles*				Mass Balance
	MeOH	MeOH + water	Acetone	Total		CO_2 trap	H_2SO_4 trap	Ethylene Glycol trap	Total	
0 (rep 1)	38.7	58.5	1.9	99.1	1.0	n.d.	n.d.	n.d.	n.d.	100.1
0 (rep 2)	38.5	58.5	2.0	99.0	0.9	n.d.	n.d.	n.d.	n.d.	99.9
0 (mean)	**38.6**	**58.5**	**2.0**	**99.1**	**0.9**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**100.0**
2	31.7	57.7	2.0	91.5	5.3	0.2	0.0	0.0	0.2	96.9
7	35.0	53.7	1.2	90.0	10.5	0.6	0.0	0.0	0.6	101.1
14	34.2	54.2	1.4	89.8	10.8	0.9	0.0	0.0	0.9	101.5
30	32.3	48.5	1.6	82.4	16.1	1.5	0.0	0.0	1.6	100.0
62 (rep 1)	28.5	41.1	1.3	70.8	22.9	1.6	0.0	0.0	1.6	95.3
62 (rep 2)	27.5	37.3	1.2	66.0	22.5	1.6	0.0	0.0	1.6	90.0
62 (mean)	**28.0**	**39.2**	**1.2**	**68.4**	**22.7**	**1.6**	**0.0**	**0.0**	**1.6**	**92.7**
90	24.5	38.4	1.8	64.6	24.4	2.6	0.0	0.0	2.7	91.7
119 (rep 1)	24.6	35.7	1.5	61.7	27.6	3.6	0.0	0.0	3.6	93.0
119 (rep 2)	24.5	35.3	1.6	61.5	27.4	3.6	0.0	0.0	3.7	92.5
119 (mean)	**24.5**	**35.5**	**1.6**	**61.6**	**27.5**	**3.6**	**0.0**	**0.0**	**3.7**	**92.8**
									mean:	**96.2**

TAR total applied radioactivity (100% = 0.132 mg/kg)
DAT days after treatment
ERR extractable radioactive residues
NER non-extractable radioactive residues
MeOH methanol
n.d. not determinated
rep replicate
* cumulated values

C. VOLATILIZATION

Formation of CO_2 was negligible in all three soils reaching in total only 3.6 to 5.4% TAR after 119 days. No other volatile compounds were detected.

D. TRANSFORMATION OF 505M08

All combined soil extracts were analyzed by radio-HPLC. The results obtained with this system are summarized in Table 7.1.2.1.2-7 to Table 7.1.2.1.2-9.

The concentration of 505M08 decreased continuously from 94.4 – 98.6% TAR at the beginning to 34.5 – 61.5% TAR at the end of the study. The identification of 505M08 was achieved by chromatographic comparison with the retention time of the ^{14}C-test item.

In two soils (Li 10 and Lufa 5M), none of the observed metabolites exceeded 5% TAR at any sampling time. In soil Lufa 2.2, one metabolite was formed in significant amounts and reached 30.0% TAR after 119 days. It was identified as the amide of the test item by LC-MS/MS investigations (non-GLP) and designated as 505M100.

Table 7.1.2.1.2-7: Summary results of radio-HPLC analysis of total soil extracts after treatment of soil Li 10 with ^{14}C-505M08

DAT	%TAR											
	Total extract	UK t_R~13.8	UK 17.6	UK 21.3	UK 27.7	505M100 28.6	UK 30.2	505M08 31.8	UK 32.8	UK 35.0	UK 38.8	UK 40.6
0 (rep 1)	97.0	-	-	-	-	-	-	95.0	2.0	-	-	-
0 (rep 2)	99.8	-	-	-	-	-	-	98.6	1.2	-	-	-
0 (mean)	**98.4**	-	-	-	-	-	-	**96.8**	**1.6**	-	-	-
2	91.7	-	-	-	-	-	-	90.1	1.6	-	-	-
7	90.1	-	-	-	0.6	0.9	-	85.2	2.6	-	0.9	-
14	82.7	-	-	-	0.8	1.3	-	79.0	0.9	-	0.8	-
30	82.5	-	3.4	-	1.0	2.7	-	73.0	1.1	1.2	-	-
62 (rep 1)	73.0	-	-	-	3.6	3.2	1.5	63.9	0.9	-	-	-
62 (rep 2)	74.6	-	-	-	3.2	4.0	0.0	65.9	1.5	-	-	-
62 (mean)	**73.8**	-	-	-	**3.4**	**3.6**	**0.7**	**64.9**	**1.2**	-	-	-
90	71.1	-	-	1.8	2.0	3.6	-	62.1	0.6	-	1.0	-
119 (rep 1)	68.4	-	-	-	5.2	3.5	1.6	56.9	1.2	-	0.0	-
119 (rep 2)	67.2	-	-	-	4.6	3.4	1.4	56.7	0.0	-	1.2	-
119 (mean)	**67.8**	-	-	-	**4.9**	**3.4**	**1.5**	**56.8**	**0.6**	-	**0.6**	-

TAR total applied radioactivity (0.127 mg/kg)
DAT days after treatment
UK unknown compound
t_R approximate retention time [min]
rep replicate
mean mean value of replicates
- means: no peak detected

Table 7.1.2.1.2-8: Summary results of radio-HPLC analysis of total soil extracts after treatment of soil Lufa 2.2 with ^{14}C-505M08

DAT	%TAR										
	Total	**UK t_R~13.8**	**UK 17.6**	**UK 21.3**	**UK 27.7**	**505M100 28.6**	**UK 30.2**	**505M08 31.8**	**UK 32.8**	**UK 35.0**	**UK 38.8**	**UK 40.6**
0 (rep 1)	95.6	-	-	-	-	-	0.0	94.4	1.2	-	-	-
0 (rep 2)	100.2	-	-	-	-	-	2.4	95.9	1.9	-	-	-
0 (mean)	**97.9**	-	-	-	-	-	**1.2**	**95.2**	**1.5**	-	-	-
2	88.8	-	-	-	-	-	-	87.1	1.7	-	-	-
7	83.6	-	1.4	-	-	4.2	-	75.5	1.3	-	1.1	-
14	82.0	-	-	-	-	8.1	-	71.2	1.3	-	1.4	-
30	75.5	-	-	-	-	10.3	-	63.4	1.1	-	0.6	-
62 (rep 1)	67.8	-	-	-	-	17.4	0.4	46.7	1.2	-	2.1	-
62 (rep 2)	65.1	-	-	-	-	16.1	0.7	45.8	0.9	-	1.5	-
62 (mean)	**66.4**	-	-	-	-	**16.8**	**0.6**	**46.2**	**1.1**	-	**1.8**	-
90	66.6	-	-	-	-	22.5	-	40.5	1.9	-	1.7	-
119 (rep 1)	66.3	-	-	-	-	30.0	-	34.5	-	-	1.8	-
119 (rep 2)	66.7	-	-	-	-	29.2	-	35.8	-	-	1.7	-
119 (mean)	**66.5**	-	-	-	-	**29.6**	-	**35.1**	-	-	**1.7**	-

Total methanol, methanol+water and acetone extracts
TAR total applied radioactivity (0.139 mg/kg)
DAT days after treatment
UK unknown compound
t_R approximate retention time [min]
rep replicate
mean mean value of replicates
\- means: no peak detected

Table 7.1.2.1.2-9: Summary results of radio-HPLC analysis of soil extracts after treatment of soil Lufa 5M with ^{14}C-505M08

DAT	%TAR											
	Total	UK t_R~13.8	UK 17.6	UK 21.3	UK 27.7	505M100 28.6	UK 30.2	505M08 31 8	UK 32.8	UK 35.0	UK 38.8	UK 40.6
0 (rep 1)	99.1	-	-	-	-	-	0.2	97.5	1.4	-	-	-
0 (rep 2)	99.0	-	-	-	-	-	0.0	97.4	1.6	-	-	-
0 (mean)	**99.1**	-	-	-	-	-	**0.1**	**97.4**	**1.5**	-	-	-
2	91.5	-	-	-	-	-	-	90.6	0.8	-	-	-
7	90.0	-	-	-	-	-	-	88.1	1.8	-	-	-
14	89.8	-	-	-	0.8	-	-	87.6	1.4	-	-	-
30	82.4	2.0	-	-	-	1.3	-	76.4	2.0	-	-	0.7
62 (rep 1)	70.8	-	-	-	0.0	-	1.1	68.6	1.1	-	-	-
62 (rep 2)	66.0	-	-	-	1.0	-	0.0	63.6	1.4	-	-	-
62 (mean)	**68.4**	-	-	-	**0.5**	-	**0.5**	**66.1**	**1.3**	-	-	-
90	64.6	3.3	-	-	-	-	-	60.0	1.4	-	-	-
119 (rep 1)	61.7	3.9	-	-	-	1.2	-	56.6	-	-	-	-
119 (rep 2)	61.5	0.0	-	-	-	0.0	-	61.5	-	-	-	-
119 (mean)	**61.6**	**2.0**	-	-	-	**0.6**	-	**59.0**	-	-	-	-

Total methanol, methanol+water and acetone extracts
TAR total applied radioactivity (0.132 mg/kg)
DAT days after treatment
UK unknown compound
t_R approximate retention time [min]
rep replicate
mean mean value of replicates
\- means: no peak detected

Calculation of the degradation rates

All visual fits of the chosen kinetic models (DFOP and FOMC) were good or excellent and the residuals were evenly distributed. The χ^2 errors were below 8% for all models. The kinetic evaluation showed that the DFOP model is appropriate to derive trigger endpoints for the 505M08 from the experimental data obtained in the present laboratory study using Li 10 soil and Lufa 2.2 soil, and that the FOMC model is appropriate to derive trigger endpoints for the 505M08 from the experimental data obtained using Lufa 5M soil.

The most appropriate kinetic models and the derived degradation parameters are summarized in the table below:

Table 7.1.2.1.2-10: Best-fit kinetic $DegT_{50}$ and $DegT_{90}$ values of metabolite 505M08

Soil	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Kinetic model	χ^2 error level
Li 10	165.2	737.8	DFOP	1.7
LUFA 2.2	60.7	288.2	DFOP	2.2
Lufa 5M	255.7	>1000	FOMC	2.6

III. CONCLUSION

The results of the present study show that 505M08 was degraded in three different soils with half-lives of 165.2 days (Li 10), 60.7 days (Lufa 2.2) and 255.7 days (Lufa 5M) when incubated under aerobic conditions at 20 ± 2°C and a soil moisture of 40% of the maximum water holding capacity.

In one of the soils, one major metabolite appeared in amounts up to 30.0% TAR. It was identified as the amide of the test item and designated as 505M100. In the other two soils, no metabolite exceeded 5% TAR at any sampling time. After 119 days of incubation, the mineralization rate had reached 3.6 to 5.4% TAR and the non-extractable residues amounted to 21.9 – 27.6% TAR.

Report:	CA 7.1.2.1.2/3 Sacchi R.R., 2015b Rate of degradation of 505M09 (Reg.No. 354563) on European soils at 20+/-2°C under aerobic conditions 2013/3009623
Guidelines:	OECD 307 (2002), POP-PA.1006, EPA 835.4100
GLP:	yes (certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

The objective of the present study was to investigate the rate of degradation of 505M09 in three European soil types at a temperature of 20 ± 2°C.

The soils were treated with a nominal rate of 0.133 mg ^{14}C-labeled 505M09 per kg of dry soil which corresponds to a field application rate of 50 g a.i. ha^{-1}. The incubation was carried out in the dark in the laboratory under aerobic conditions at a soil moisture of 40% of the maximum water holding capacity. A closed incubation system with continuous aeration (moistened air) was used with an attached trapping system for the determination of volatile compounds. Samples were taken at 0, 2, 7, 14, 30, 63, 92, and 120 days after treatment (DAT).

The soil samples were extracted once with methanol, twice with methanol/water (1:1, v/v) and rinsed once with acetone. The extracts were analyzed by means of LSC and HPLC. The amount of non-extractable residues was determined by combustion and subsequent LSC measurements.

The mass balance throughout the study ranged from 90.5 to 103.5% of total applied radioactivity (TAR). The extractable radioactivity decreased from > 95.7% TAR at day 0 to 61.0 – 62.1% after 120 days. At the end of incubation, 505M09 was detected in amounts of 35.5 – 58.5% TAR.

In soil Lufa 5M, no metabolite exceeded 4.5% TAR at any sampling time. In soils Li 10 and Lufa 2.2, one metabolite appeared in maximum amounts of 6.7% TAR (after 92 days) and 24.6% TAR (after 120 days), respectively. This metabolite was identified as the amide of the test item and was designated as 505M101. Formation of CO_2 was observed in the three soils reaching 3.9 – 6.8% TAR after 120 days. No other volatile compounds were detected. Non-extractable residues were formed in amounts with a maximum of 22.1 to 31.6% TAR at the end of the study.

Degradation times of 505M09 were calculated with the computer program KinGUI (version 2) applying first order kinetics (DFOP). $DegT_{50}$ values were between 62.6 and 153.8 days.

I. MATERIAL AND METHODS

A. MATERIALS

BASF code:	505M09 / BF505-8
Reg.No.:	354563
Chemical name:	(E)-o-[(5-hydroxycarbonyl-2-methyl)phenoxymethyl]-2-methoxyimino-N-methylphenylacetamide
Molecular weight:	356.38 g mol^{-1}
Position of radiolabel:	Benzyl-ring-U-C^{14}
Batch No.:	664-2005
Specific radioactivity:	7.55 MBq mg^{-1}
Radiochemical purity:	99.2% (according to certificate) 98.1% (determined by radio-HPLC prior to soil treatment in this study – average of 3 analyses)

2. Soils

Three different soils were used for treatment and incubation. The soils were sieved through a 2 mm sieve before use. The soil characteristics are summarized in Table 7.1.2.1.2-11.

Table 7.1.2.1.2-11: Properties of soils used to investigate degradation rate of 505M09

Soil designation	Li 10 (12/1680/05)	LUFA 2.2 (12/736/05)	LUFA 5M (12/1651/05)
Origin	Limburgerhof, RP, Germany	Hanhofen, RP, Germany	Mechtersheim, RP, Germany
DIN Particle size distribution [%]			
Sand 0.063 – 2 mm	82.8	68.5	54.1
Silt 0.002 – 0.063 mm	11.7	20.0	31.7
Clay < 0.002 mm	5.5	11.5	14.2
Textural class	Loamy sand	Loamy sand	Loamy sand
USDA Particle size distribution [%]			
Sand 0.050 – 2 mm	83.5	69.9	58.4
Silt 0.002 – 0.050 mm	11.0	18.6	27.4
Clay < 0.002 mm	5.5	11.5	14.2
Textural class	Loamy sand	Sandy loam	Sandy loam
Organic C [%]	0.81	1.6	2.18
pH (H_2O)	7.1	6.4	8.0
pH ($CaCl_2$)	6.3	5.4	7.4
Cation exchange capacity [$cmol^+ kg^{-1}$]	5.0	8.6	8.8
Maximum water holding capacity [g/100g dry soil]	23.1	31.0	26.2
Microbial biomass* [mg C/100g dry soil]	33.6	42.1	31.1
Microbial biomass at 63 DAT [mg C/100g dry soil]	38	44	32
Microbial biomass at 120 DAT [mg C/100g dry soil]	39	46	19

*data obtained before starting the study
DAT = days after treatment

The soils were transported from Germany to Brazil in a temperature range of 4 - 20°C and were stored in a refrigerator at 4 ± 2°C before use.

B. STUDY DESIGN

1. Experimental conditions

The soils were adjusted to about 40% MWHC and treated at a nominal concentration of 0.133 mg ^{14}C-505M09 per kg dry soil, which corresponds to a field application rate of 50 g a.i. ha^{-1} (assuming an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}).

For the soil treatment, one kg of each soil was treated with 1 mL of the treatment solution. The treated soils were homogenized and soil portions of 50 g dry soil equivalents were filled into suitable test vessels. The number of vessels was sufficient to allow duplicate sampling at each sampling time and to keep a limited number of reserve vessels.

The vessels were incubated in the dark for 120 days at a temperature of 20 ± 2°C. Throughout the incubation period, the test vessels were continuously aerated with a slight stream of moistened and CO_2-free air. The outgoing air was led through a series of gas-washing bottles containing trapping solutions for potential volatiles (ethylene glycol, 0.5M H_2SO_4, 0.5M NaOH). The water content of the soils was monitored throughout the incubation period by weighing representative vessels at each sampling time. If necessary, the soil moisture was readjusted.

For determination of the microbial biomass during (63 days) and at the end of incubation (120 days), additional soil samples treated with methanol but without test item were incubated under the same conditions.

2. Sampling

Sampling times were 0 (no sampling of volatiles), 2, 7, 14, 30, 63, 92 and 120 days after treatment (DAT).

At 0 DAT as well as at 63 DAT and 120 DAT two replicate samples were worked up. At the other sampling times only one replicate was worked up, the second one was stored in a freezer.

3. Description of the analytical procedures

The soil was extracted consecutively one time with 100 mL methanol and two times with 100 mL methanol/water (1:1) by shaking for about 30 minutes. After each extraction step, the suspension was centrifuged and two aliquots of the supernatant were measured by LSC.

After the last extraction, the soil was rinsed with 100 mL acetone. The acetone was also checked for radioactivity but no HPLC analysis was performed since the amounts of radioactive residues never exceeded 3.3% of the total applied radioactivity (TAR).

All the extracts were combined and concentrated to a small volume. Prior to HPLC analysis, the concentrated extracts were centrifuged. Then the supernatant was radio-assayed and analyzed by HPLC to obtain the metabolite pattern.

The extracted soil was dried at room temperature and homogenized with a small mill. At least three aliquot parts of soil per sampling date were combusted to determine the amount of the non-extractable radioactive residues (NER).

All liquid samples were measured for radioactivity by LSC. The radioactivity in the extracted and dried soil samples was determined by combustion of at least three aliquot parts. Combustion products were absorbed in the Oxysolve C-400 scintillation cocktail.

4. Kinetic modeling

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] in order to derive degradation kinetics and trigger endpoints. The analysis was conducted by non-linear regression methods using the software tool KinGUI (version 2).

For each data set, the kinetic models proposed by the FOCUS Kinetic guidance document were tested in order to identify the best-fit model. The recommended kinetic models, i.e. the single first order kinetics (SFO), the Gustafson-Holden model (FOMC) and the bi-exponential kinetic model (DFOP) are already implemented in KinGUI.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures recommended by the FOCUS guidance. The goodness-of-fit measure used for the identification of the best-fit kinetic model is the χ^2 minimum error level. The kinetic endpoints (DT_{50} and DT_{90} values) are reported for the kinetic models, which were selected based on statistical and visual assessment. The reliability of individual parameters was judged by means of a single-sided t-test. When available, replicate measurements were used for the parameter estimation.

In all soils, the initial concentration of the applied test item was set to the material balance recovered at day 0. At later sampling time points (2, 7, 14, 30, 63, 92 and 120 DAT), the measured residues of the applied substance were used for modeling.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The detailed results on the extractability of radioactive residues in the soils are presented in Table 7.1.2.1.2-12 to Table 7.1.2.1.2-14. The material balance ranged from 90.5 to 103.5% TAR for all soils.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (ERR), non-extractable residues (NER), CO_2 and other volatiles for each soil is shown in Table 7.1.2.1.2-12 to Table 7.1.2.1.2-14. The amount of extractable radioactivity moderately decreased from more than 95.0% TAR at 0 DAT to 61.0 – 62.1% TAR after 120 days of incubation.

Non-extractable radioactive residues reached maximum values of 22.1 to 31.6% TAR at the end of the study after 120 days of incubation.

Table 7.1.2.1.2-12: Distribution of radioactivity and mass balance in Li 10 soil after treatment with 505M09 [%TAR]

DAT	extractable				NER	Volatiles*				Mass Balance
	MeOH	MeOH + water	Acetone	Total		CO_2 trap	H_2SO_4 trap	Ethylene Glycol trap	Total	
0 (rep 1)	36.9	57.9	1.1	95.9	1.4	n.d.	n.d.	n.d.	n.d.	97.4
0 (rep 2)	37.8	61.4	1.3	100.4	2.2	n.d.	n.d.	n.d.	n.d.	102.6
0 (mean)	**37.3**	**59.6**	**1.2**	**98.2**	**1.8**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**100.0**
2	35.1	53.4	1.4	89.9	8.0	0.2	0.0	0.0	0.2	98.1
7	35.1	50.2	1.6	86.9	11.4	0.9	0.0	0.0	0.9	99.2
14	32.6	47.2	1.6	81.3	12.8	1.2	0.0	0.0	1.3	95.4
30	31.6	45.8	1.4	78.8	15.0	2.0	0.0	0.0	2.0	95.8
63 (rep 1)	30.1	38.5	1.4	70.0	20.9	2.7	0.0	0.0	2.7	93.6
63 (rep 2)	24.0	44.8	1.7	70.5	22.0	2.7	0.0	0.0	2.7	95.2
63 (mean)	**27.0**	**41.7**	**1.5**	**70.2**	**21.5**	**2.7**	**0.0**	**0.0**	**2.7**	**94.4**
92	32.1	33.8	2.5	68.4	22.9	5.8	0.0	0.0	5.8	97.0
120 (rep 1)	26.9	33.0	1.8	61.7	22.1	6.8	0.0	0.0	6.8	90.5
120 (rep 2)	25.5	34.2	1.9	61.6	24.9	6.8	0.0	0.0	6.8	93.3
120 (mean)	**26.2**	**33.6**	**1.9**	**61.6**	**23.5**	**6.8**	**0.0**	**0.0**	**6.8**	**91.9**
									mean	**96.0**

TAR total applied radioactivity (100% = 0.123 mg/kg)
DAT days after treatment
NER non-extractable radioactive residues
MeOH methanol
n.d. not determinated
rep replicate
* cumulated values
mean mean value of replicates

Table 7.1.2.1.2-13: Distribution of radioactivity and mass balance in Lufa 2.2 soil after treatment with 505M09 [%TAR]

DAT	extractable				NER	Volatiles*				Mass Balance
	MeOH	MeOH + water	Acetone	Total		CO_2 trap	H_2SO_4 trap	Ethylene Glycol trap	Total	
0 (rep 1)	20.1	73.1	2.7	95.8	4.0	n.d.	n.d.	n.d.	n.d.	99.9
0 (rep 2)	20.5	72.7	2.5	95.7	4.4	n.d.	n.d.	n.d.	n.d.	100.1
0 (mean)	**20.3**	**72.9**	**2.6**	**95.8**	**4.2**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**100.0**
2	18.5	66.0	3.3	87.8	14.6	0.2	0.0	0.0	0.2	102.6
7	19.1	60.2	2.9	82.1	19.3	0.8	0.0	0.0	0.8	102.2
14	20.4	55.0	2.2	77.5	21.4	1.3	0.0	0.0	1.3	100.2
30	21.1	49.6	2.4	73.2	25.9	2.2	0.0	0.0	2.2	101.4
63 (rep 1)	21.9	40.6	1.9	64.4	27.7	3.2	0.0	0.0	3.2	95.3
63 (rep 2)	21.7	40.5	1.7	63.9	30.1	3.2	0.0	0.0	3.2	97.2
63 (mean)	**21.8**	**40.6**	**1.8**	**64.2**	**28.9**	**3.2**	**0.0**	**0.0**	**3.2**	**96.2**
92	24.2	35.8	3.1	63.0	28.9	3.2	0.0	0.0	3.2	95.1
120 (rep 1)	25.0	34.4	2.4	61.8	29.4	3.9	0.0	0.0	3.9	95.1
120 (rep 2)	24.0	35.4	2.7	62.1	29.2	3.9	0.0	0.0	3.9	95.2
120 (mean)	**24.5**	**34.9**	**2.5**	**62.0**	**29.3**	**3.9**	**0.0**	**0.0**	**3.9**	**95.2**
									mean	**98.3**

TAR total applied radioactivity (100% = 0.121 mg/kg)
DAT days after treatment
NER non-extractable radioactive residues
MeOH methanol
n.d. not determinated
rep replicate
* cumulated values

Table 7.1.2.1.2-14: Distribution of radioactivity and mass balance in Lufa 5M soil after treatment with 505M09 [%TAR]

DAT	extractable				NER	Volatiles*				Mass Balance
	MeOH	MeOH + water	Acetone	Total		CO_2 trap	H_2SO_4 trap	Ethylene Glycol trap	Total	
0 (rep 1)	29.6	67.3	1.5	98.4	2.1	n.d.	n.d.	n.d.	n.d.	100.5
0 (rep 2)	29.6	66.8	1.5	97.9	1.7	n.d.	n.d.	n.d.	n.d.	99.5
0 (mean)	**29.6**	**67.1**	**1.5**	**98.2**	**1.9**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**100.0**
2	27.0	64.1	1.3	92.4	7.4	0.2	0.0	0.0	0.2	100.0
7	25.5	62.8	1.4	89.7	11.2	0.7	0.0	0.0	0.7	101.6
14	24.3	59.6	1.5	85.4	13.5	0.9	0.0	0.0	0.9	99.9
30	23.2	59.2	1.6	84.1	18.1	1.4	0.0	0.0	1.4	103.5
63 (rep 1)	22.6	44.7	1.2	68.4	26.7	2.7	0.0	0.0	2.7	97.9
63 (rep 2)	21.4	48.2	1.3	70.9	25.3	2.6	0.0	0.0	2.6	98.8
63 (mean)	**22.0**	**46.4**	**1.2**	**69.7**	**26.0**	**2.7**	**0.0**	**0.0**	**2.7**	**98.3**
92	20.9	43.7	2.2	66.9	29.4	3.7	0.0	0.0	3.7	100.0
120 (rep 1)	19.6	40.1	1.5	61.2	31.6	4.8	0.0	0.0	4.8	97.7
120 (rep 2)	18.8	40.4	1.8	61.0	30.6	4.8	0.0	0.0	4.8	96.4
120 (mean)	**19.2**	**40.2**	**1.7**	**61.1**	**31.1**	**4.8**	**0.0**	**0.0**	**4.8**	**97.0**
									mean:	**99.4**

TAR total applied radioactivity (100% = 0.123 mg/kg)
DAT days after treatment
ERR extractable radioactive residues
NER non-extractable radioactive residues
MeOH methanol
n.d. not determinated
rep replicate
* cumulated values
mean mean value of replicates

C. VOLATILIZATION

Formation of CO_2 was negligible in all three soils reaching in total only 3.9 to 6.8% TAR after 120 days. No other volatile compounds were detected.

D. TRANSFORMATION OF 505M09

All combined soil extracts were analyzed by radio-HPLC. The results obtained with this system are summarized in Table 7.1.2.1.2-15 to Table 7.1.2.1.2-17.

The concentration of 505M09 decreased continuously from 93.9 – 98.1% TAR at the beginning to 35.5 – 58.5% TAR at the end of the study. The identification of 505M09 was achieved by chromatographic comparison with the retention time of the ^{14}C-test item.

In soil Lufa 5M, none of the metabolites observed exceeded 4.5% TAR at any sampling time. In soils Li 10 and Lufa 2.2, one metabolite appeared in maximum amounts of 6.7% TAR and 24.6% TAR, respectively. In soil Li 10, this metabolite degraded again reaching about 4% TAR after 120 days. In soil Lufa 2.2, no degradation could be observed. This metabolite was identified as the amide of the test item and designated as 505M101.

Table 7.1.2.1.2-15: Summary results of radio-HPLC analysis of total soil extracts after treatment of soil Li 10 with ^{14}C-505M09

DAT	%TAR							
	Total	UK t_R~27.5	505M101 29.2	UK 31.3	UK 32.0	505M09 33.0	UK 35.0	UK 40.7
0 (rep 1)	95.9	-	-	-	-	94.0	1.9	-
0 (rep 2)	100.4	-	-	-	-	98.1	2.4	-
0 (mean)	**98.2**	-	-	-	-	**96.0**	**2.1**	-
2	89.9	-	-	-	-	88.4	1.5	-
7	86.9	1.1	2.1	-	-	82.2	1.4	-
14	81.3	1.3	3.1	-	-	75.1	1.4	0.4
30	78.8	1.0	4.6	-	-	71.8	1.3	-
63 (rep 1)	70.0	3.4	5.3	-	-	59.5	1.0	0.7
63 (rep 2)	70.5	4.1	6.0	-	-	60.4	0.0	0.0
63 (mean)	**70.2**	**3.7**	**5.7**	-	-	**60.0**	**0.5**	**0.4**
90	68.4	3.8	6.7	-	-	56.6	1.3	-
120 (rep 1)	61.7	4.0	3.7	1.5	1.2	50.0	1.3	-
120 (rep 2)	61.6	4.8	4.1	0.9	0.7	49.6	1.4	-
120 (mean)	**61.6**	**4.4**	**3.9**	**1.2**	**1.0**	**49.8**	**1.4**	-

Total methanol, methanol+water and acetone extracts
TAR total applied radioactivity (0.123 mg/kg
DAT days after treatment
UK unknown compound
t_R approximate retention time [min]
rep replicate
mean mean value of replicates
\- means: no peak detected

Table 7.1.2.1.2-16: Summary results of radio-HPLC analysis of total soil extracts after treatment of soil Lufa 2.2 with ^{14}C-505M09

DAT	%TAR							
	Total	**UK t_R~27.5**	**505M101 29.2**	**UK 31.3**	**UK 32.0**	**505M09 33.0**	**UK 35.0**	**UK 40.7**
0 (rep 1)	95.8	-	-	-	-	93.9	2.0	-
0 (rep 2)	95.7	-	-	-	-	94.1	1.7	-
0 (mean)	**95.8**	-	-	-	-	**94.0**	**1.8**	-
2	87.8	-	2.0	-	-	83.8	2.0	-
7	82.1	-	5.3	-	-	75.3	1.6	-
14	77.5	-	8.6	-	-	67.9	1.1	-
30	73.2	0.3	12.9	-	-	58.6	1.4	-
63 (rep 1)	64.4	2.1	15.3	-	-	46.3	0.7	-
63 (rep 2)	63.9	1.1	14.2	-	-	48.2	0.5	-
63 (mean)	**64.2**	**1.6**	**14.7**	-	-	**47.3**	**0.6**	-
90	63.0	0.0	20.1	-	-	42.9	-	-
120 (rep 1)	61.8	1.1	24.6	0.0	0.5	35.5	0.0	-
120 (rep 2)	62.1	0.0	21.7	0.9	0.0	38.6	0.9	-
120 (mean)	**62.0**	**0.6**	**23.1**	**0.5**	**0.2**	**37.1**	**0.5**	-

Total methanol, methanol+water and acetone extracts
TAR total applied radioactivity (0.121 mg/kg)
DAT days after treatment
UK unknown compound
t_R approximate retention time [min]
rep replicate
mean mean value of replicates
\- means: no peak detected

Table 7.1.2.1.2-17: Summary results of radio-HPLC analysis of total soil extracts after treatment of soil Lufa 5M with ^{14}C-505M09

DAT	%TAR							
	Total	UK t_R~27.5	505M101 29.2	UK 31.3	UK 32.0	505M09 33.0	UK 35.0	UK 40.7
0 (rep 1)	98.4	-	-	-	-	97.0	1.5	-
0 (rep 2)	97.9	-	-	-	-	96.5	1.4	-
0 (mean)	**98.2**	-	-	-	-	**96.7**	**1.4**	-
2	92.4	-	-	-	-	91.0	1.4	-
7	89.7	-	-	-	-	88.5	1.2	-
14	85.4	0.5	-	0.2	0.4	82.7	1.6	-
30	84.1	-	-	-	-	82.9	1.2	-
63 (rep 1)	68.4	-	0.8	-	0.7	65.9	1.1	-
63 (rep 2)	70.9	-	1.7	-	0.3	68.0	0.8	-
63 (mean)	**69.7**	-	**1.3**	-	**0.5**	**67.0**	**0.9**	-
90	66.9	4.5	-	-	-	62.4	-	-
120 (rep 1)	61.2	0.0	0.8	0.7	-	58.5	1.2	-
120 (rep 2)	61.0	0.4	0.6	0.0	-	58.5	1.6	-
120 (mean)	**61.1**	**0.2**	**0.7**	**0.4**	-	**58.5**	**1.4**	-

Total methanol, methanol+water and acetone extracts
TAR total applied radioactivity (0.123 mg/kg)
DAT days after treatment
UK unknown compound
t_R approximate retention time [min]
rep replicate
mean mean value of replicates
- means: no peak detected

Calculation of the degradation rates

All visual fits were good or excellent for the selected kinetic model (DFOP) and the residuals were evenly distributed. The χ^2 errors were below 10 % for all models. The kinetics evaluation showed that the DFOP model is appropriate to derive trigger endpoints for the 505M09 from the experimental data obtained in the present laboratory study using Li 10 soil, Lufa 2.2 soil and Lufa 5M soil.

The most appropriate kinetic model and the derived degradation parameters are summarized in the table below:

Table 7.1.2.1.2-18: Best-fit kinetic $DegT_{50}$ and $DegT_{90}$ values of metabolite 505M09

Soil	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Kinetic model	χ^2 error level
Li 10	117.2	518.2	DFOP	1.8
LUFA 2.2	62.6	343.7	DFOP	2.7
Lufa 5M	153.8	584.6	DFOP	2.3

III. CONCLUSION

The results of the present study show that 505M09 was degraded in three different soils with half-lives of 117.2 days (Li 10), 62.6 days (Lufa 2.2) and 153.8 days (Lufa 5M) when incubated under aerobic conditions at 20 ± 2°C and a soil moisture of 40% of the maximum water holding capacity.

One metabolite reached a maximum amount of 24.6% TAR. It was identified as the amide of the test item and designated as 505M101. All other metabolites never exceeded 4.5% TAR at any sampling time. After 120 days of incubation, the mineralization rate had reached 3.9 to 6.8% TAR and the non-extractable residues amounted to 22.1 – 31.6% TAR.

Report: CA 7.1.2.1.2/4
Sacchi R.R., 2015c
Rate of degradation of M505F01 (Reg.No. 358104) on European soils at 20 ± 2ºC under aerobic conditions
2013/3009624
Guidelines: OECD 307 (2002), POP-PA.1006, EPA 835.4100
GLP: yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

The objective of the present study was to investigate the rate of degradation of 505M01 in three European soil types at a temperature of 20 ± 2°C.

The soils were treated with a nominal rate of 0.133 mg ^{14}C-labelled 505M01 per kg of dry soil which corresponds to a field application rate of 50 g a.i. ha^{-1}. The incubation was carried out in the dark in the laboratory under aerobic conditions at a soil moisture of 40 % of the maximum water holding capacity. A closed incubation system with continuous aeration (moistened air) was used with an attached trapping system for the determination of volatile compounds. Samples were taken at 0, 2, 7, 15, 28, 62, 93, and 121 days after treatment (DAT).

The soil samples were extracted once with methanol, twice with methanol/water (1:1, v/v), and subsequently rinsed with acetone. The extracts were analyzed by means of LSC and HPLC. The amount of non-extractable residues was determined by combustion and subsequent LSC measurements.

The mass balance throughout the study ranged from 89.1 to 104.4 % of total applied radioactivity (TAR). The extractable radioactivity decreased from > 97.0 % TAR at day 0 to 15.3 – 41.5 % after 121 days. 505M01 decreased continuously from 87.9 – 91.9 %TAR at the beginning to “no peak detected” – 1.3 % TAR at the end.

Beside the parent compound, several metabolites were observed. One metabolite, observed as two peaks on the chromatogram (isomers) and designated as 505M106 reached a maximum of 30.6 %TAR after 15 days. One peak which reached 25.8 %TAR after 2 days was found to consist of two metabolites: 505M96 and 505M97. One further metabolite, designated as 505M102, also occurred in two isomer forms, reaching in sum up to 49.5 %TAR after 7 days. Further metabolites were present in low amounts only (below 4 %TAR at any sampling time).

Formation of CO_2 was observed in all the three soils reaching in total 9.7 to 39.2 % TAR after 121 days. No other volatile compounds were detected. Non-extractable residues were formed to a maximum of 36.1 to 51.2 % TAR at the end of the study.

Degradation times of 505M01 were calculated using KinGUI (version 2) applying first order kinetics (SFO, DFOP). $DegT_{50}$ values ranged from 0.2 to 11.7 days.

I. MATERIAL AND METHODS

A. MATERIALS

BAS code:	505M01 / BF505-4
Reg.No.:	358104
Chemical name:	(E)-2-(2-hydroxymethylphenyl)-2-methoxyimino-N-methyl-acetamide
Molecular weight:	222.24 g mol^{-1}
Position of radiolabel:	benzyl-[ring-U-C^{14}]
Batch No.:	665-2003
Specific radioactivity:	12.3 MBq mg^{-1}
Radiochemical purity:	99.2 % (according to certificate) 93.0 % (determined by radio-HPLC prior to soil treatment in this study – average of 3 analyses)

2. Soils

Three different soils were used for treatment and incubation. The soils were sieved through a 2 mm sieve before use. The soil characteristics are summarized in Table 7.1.2.1.2-19.

Table 7.1.2.1.2-19: Properties of soils used to investigate degradation rate of 505M01

Soil designation	Li 10 (12/1680/05)	LUFA 2.2 (12/736/05)	LUFA 5M (12/1651/05)
Origin	Limburgerhof, RP, Germany	Hanhofen, RP, Germany	Mechtersheim, RP, Germany
DIN Particle size distribution [%]			
Sand 0.063 – 2 mm	82.8	68.5	54.1
Silt 0.002 – 0.063 mm	11.7	20.0	31.7
Clay < 0.002 mm	5.5	11.5	14.2
Textural class	Loamy sand	Loamy sand	Loamy sand
USDA Particle size distribution [%]			
Sand 0.050 – 2 mm	83.5	69.9	58.4
Silt 0.002 – 0.050 mm	11.0	18.6	27.4
Clay < 0.002 mm	5.5	11.5	14.2
Textural class	Loamy sand	Sandy loam	Sandy loam
Organic C [%]	0.81	1.6	2.18
pH (H_2O)	7.1	6.4	8.0
pH ($CaCl_2$)	6.3	5.4	7.4
Cation exchange capacity [$cmol^+ kg^{-1}$]	5.0	8.6	8.8
Maximum water holding capacity [g/100g dry soil]	23.1	31.0	26.2
Microbial biomass* [mg C/100g dry soil]	33.6	42.1	31.1
Microbial biomass at 62 DAT [mg C/100g dry soil]	38	44	31
Microbial biomass at 121 DAT [mg C/100g dry soil]	45	53	38

*data obtained before starting the study
DAT = days after treatment

The soils were transported from Germany to Brazil in a temperature range of 4 - 20°C and were stored in a refrigerator at 4 ± 2°C before use.

B. STUDY DESIGN

1. Experimental conditions

The soils were adjusted to about 40 % MWHC and treated at a nominal concentration of 0.133 mg ^{14}C-505M01 per kg dry soil, which corresponds to a field application rate of 50 g a.i. ha^{-1} (assuming an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}).

For the soil treatment, one kg of each soil was treated with 1 mL of the treatment solution. The treated soils were homogenized and soil portions of 50 g dry soil equivalents were filled into suitable test vessels. The number of vessels was sufficient to allow duplicate sampling at each sampling time and to keep a limited number of reserve vessels.

The vessels were incubated in the dark for 121 days at a temperature of 20 ± 2°C. Throughout the incubation period, the test vessels were continuously aerated with a slight stream of moistened and CO_2-free air. The outgoing air was led through a series of gas-washing bottles containing trapping solutions for potential volatiles (ethylene glycol, 0.5M H_2SO_4, 0.5M NaOH). The water content of the soils was monitored throughout the incubation period by weighing representative vessels at each sampling time. If necessary, the soil moisture was readjusted (to 40 % MWHC).

For determination of the microbial biomass during (62 days) and at the end of incubation (121 days), additional soil samples treated with methanol but without test item were incubated under the same conditions.

2. Sampling

Sampling times were 0 (no sampling of volatiles), 2, 7, 15, 28, 62, 93 and 121 days after treatment (DAT).

At 0 DAT as well as at 62 DAT and 121 DAT two replicate samples were worked up. At the other sampling times only one replicate was worked up, the second one was stored in a freezer.

3. Description of the analytical procedures

The soil was extracted consecutively one time with 100 mL methanol and two times with 100 mL methanol/water (1:1, v:v) by shaking for about 30 minutes. After each extraction step, the suspension was centrifuged and two aliquots of the supernatant were measured by LSC. After the last extraction, the soil was rinsed with 100 mL acetone and the supernatant was also measured by LSC.

All the extracts were combined and concentrated to a small volume. Prior to LSC and HPLC analysis, the concentrated extracts were centrifuged. Then the supernatant was analyzed by HPLC to obtain the metabolite pattern.

The extracted soil was dried at room temperature and homogenized with a small mill. At least three aliquot parts of soil per sampling date were combusted to determine the amount of the non-extractable radioactive residues (NER).

All liquid samples were measured for radioactivity by LSC. The radioactivity in the extracted and dried soil samples was determined by combustion of at least three aliquot parts. Combustion products were absorbed in the Oxysolve C-400 scintillation cocktail.

In order to aid in the identification of major metabolites, a composite sample named Pool 6 combining all the remaining soils extracts was prepared and concentrated by means of a rotary evaporator at 36 ºC. The concentrated and homogenized material was divided in three aliquots and stored frozen. The sample was submitted to mass spectrometric investigation in order to identify the major metabolites.

4. Kinetic modeling

The kinetic analysis was carried out following the recommendations of the FOCUS work group on degradation kinetics [*FOCUS (2006)*] in order to derive degradation kinetics and trigger endpoints. The analysis was conducted by non-linear regression methods using the software tool KinGUI (version 2).

For each data set, the kinetic models proposed by the FOCUS Kinetic guidance document were tested in order to identify the best-fit model. The recommended kinetic models, i.e. the single first order kinetics (SFO), the Gustafson-Holden model (FOMC) and the bi-exponential kinetic model (DFOP) are already implemented in KinGUI.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures recommended by the FOCUS guidance. The goodness-of-fit measure used for the identification of the best-fit kinetic model is the χ^2 minimum error level. The kinetic endpoints (DT_{50} and DT_{90} values) are reported for the kinetic models, which were selected based on statistical and visual assessment. The reliability of individual parameters was judged by means of a single-sided t-test. When available, replicate measurements were used for the parameter estimation.

In all soils, the initial concentration of the applied test item was set to the material balance recovered at day 0. At later sampling time points (2, 7, 15, 28, 62, 93 and 121 DAT), the measured residues of the applied substance were used for modeling.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The detailed results on the extractability of radioactive residues in the soils are presented in Table 7.1.2.1.2-20 to Table 7.1.2.1.2-22. The material balance ranged from 89.1 to 104.4 % TAR for all soil samples with average values of 94.4, 94.6 %, and 97.0 % TAR for the soils Li10, Lufa 2.2 and Lufa 5M respectively, throughout the incubation period of 121 days.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of radioactivity in extractable residues (ERR), non-extractable residues (NER), CO_2 and other volatiles for each soil is shown in Table 7.1.2.1.2-20 to Table 7.1.2.1.2-22. The extractability of the radioactive residues decreased from >97.0 % TAR at 0 DAT to about 15.3 - 41.5 % TAR after 121 days of incubation. The predominant part of the radioactivity could be extracted with methanol (76.1 to 8.3 % TAR). Between 6.4 % and 56.6 % TAR were extracted by the second extraction with methanol/H_2O. The acetone fractions obtained by rinsing the soil with acetone after the last extraction step contained only low amounts of radioactivity (≤ 2.2 % TAR).

Non-extractable radioactive residues in all three soils were formed in amounts reaching their maximum of 36.1 to 51.2 % TAR at the end of the study after 121 days of incubation.

Table 7.1.2.1.2-20: Distribution of radioactivity and mass balance in Li 10 soil after treatment with 505M01 [%TAR]

DAT	extractable				NER	Volatiles*				Mass Balance
	MeOH	MeOH + water	Acetone	Total		CO_2 trap	H_2SO_4 trap	Ethylene Glycol trap	Total	
0 (rep 1)	76.1	22.7	0.6	99.4	1.0	n.d.	n.d.	n.d.	n.d.	100.4
0 (rep 2)	73.8	24.4	0.5	98.7	0.9	n.d.	n.d.	n.d.	n.d.	99.6
0 (mean)	**74.9**	**23.5**	**0.6**	**99.1**	**0.9**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**100.0**
2	53.2	34.4	1.3	88.8	9.7	0.4	0.0	0.0	0.4	98.8
7	45.8	26.2	1.6	73.6	16.6	2.4	0.0	0.0	2.4	92.6
15	39.4	21.6	1.7	62.6	26.8	4.2	0.0	0.0	4.2	93.7
28	35.2	16.1	1.2	52.5	33.8	6.0	0.0	0.0	6.0	92.3
62 (rep 1)	30.3	14.8	1.3	46.4	39.1	7.8	0.0	0.0	7.8	93.3
62 (rep 2)	29.6	14.7	1.3	45.6	39.1	7.9	0.0	0.0	7.9	92.5
62 (mean)	**30.0**	**14.7**	**1.3**	**46.0**	**39.1**	**7.9**	**0.0**	**0.0**	**7.9**	**92.9**
93	27.7	13.8	1.3	42.8	42.0	9.1	0.0	0.0	9.1	93.9
121 (rep 1)	25.6	12.9	1.7	40.2	39.1	9.7	0.0	0.0	9.7	89.1
121 (rep 2)	26.4	13.6	1.5	41.5	40.6	9.7	0.0	0.0	9.7	91.9
121 (mean)	**26.0**	**13.2**	**1.6**	**40.9**	**39.9**	**9.7**	**0.0**	**0.0**	**9.7**	**90.5**
									mean:	**94.4**

TAR total applied radioactivity (100% = 0.113 mg/kg)
DAT days after treatment
NER non-extractable radioactive residues
MeOH methanol
n.d. not determinated
rep replicate
* cumulated values

Table 7.1.2.1.2-21: Distribution of radioactivity and mass balance in Lufa 2.2 soil after treatment with 505M01 [%TAR]

DAT	extractable				NER	Volatiles*				Mass Balance
	MeOH	MeOH + water	Acetone	Total		CO_2 trap	H_2SO_4 trap	Ethylene Glycol trap	Total	
0 (rep 1)	64.6	31.5	1.2	97.2	1.3	n.d.	n.d.	n.d.	n.d.	98.5
0 (rep 2)	67.8	31.5	1.0	100.3	1.2	n.d.	n.d.	n.d.	n.d.	101.5
0 (mean)	**66.2**	**31.5**	**1.1**	**98.8**	**1.2**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**100.0**
2	26.7	56.6	1.8	85.1	10.3	0.3	0.0	0.0	0.3	95.7
7	24.2	50.4	2.0	76.6	17.3	3.1	0.0	0.0	3.1	97.0
15	20.4	40.0	1.6	62.0	23.2	8.8	0.0	0.0	8.8	94.0
28	17.4	23.4	1.1	41.8	32.7	18.1	0.0	0.0	18.2	92.7
62 (rep 1)	11.6	11.1	1.3	24.0	34.6	33.5	0.0	0.0	33.6	92.1
62 (rep 2)	11.6	11.5	1.3	24.3	35.2	33.5	0.0	0.0	33.6	93.1
62 (mean)	**11.6**	**11.3**	**1.3**	**24.2**	**34.9**	**33.5**	**0.0**	**0.0**	**33.6**	**92.6**
93	9.8	7.4	0.6	17.7	38.0	37.9	0.0	0.0	37.9	93.7
121 (rep 1)	8.3	6.5	0.6	15.4	36.1	39.2	0.0	0.0	39.2	90.7
121 (rep 2)	8.3	6.4	0.6	15.3	37.3	39.2	0.0	0.0	39.2	91.8
121 (mean)	**8.3**	**6.5**	**0.6**	**15.3**	**36.7**	**39.2**	**0.0**	**0.0**	**39.2**	**91.3**
									mean:	**94.6**

TAR total applied radioactivity (100% = 0.113 mg/kg)
DAT days after treatment
NER non-extractable radioactive residues
MeOH methanol
n.d. not determinated
rep replicate
* cumulated values

Table 7.1.2.1.2-22: Distribution of radioactivity and mass balance in Lufa 5M soil after treatment with 505M01 [%TAR]

DAT	extractable				NER	Volatiles*				Mass Balance
	MeOH	MeOH + water	Acetone	Total		CO_2 trap	H_2SO_4 trap	Ethylene Glycol trap	Total	
0 (rep 1)	75.7	24.6	0.7	100.9	0.8	n.d.	n.d.	n.d.	n.d.	101.8
0 (rep 2)	72.0	24.8	0.6	97.4	0.9	n.d.	n.d.	n.d.	n.d.	98.3
0 (mean)	**73.9**	**24.7**	**0.6**	**99.2**	**0.9**	**n.d.**	**n.d.**	**n.d.**	**n.d.**	**100.0**
2	55.7	33.9	1.4	91.1	11.3	0.6	0.0	0.0	0.6	103.0
7	47.0	32.5	2.2	81.7	19.6	3.1	0.0	0.0	3.1	104.4
15	39.2	26.4	1.9	67.5	27.8	3.1	0.0	0.0	3.1	98.4
28	30.0	22.9	1.6	54.4	33.6	8.0	0.0	0.0	8.0	96.1
62 (rep 1)	25.1	15.8	1.5	42.4	40.4	12.2	0.0	0.0	12.2	95.0
62 (rep 2)	25.1	15.2	1.4	41.7	40.9	12.1	0.0	0.0	12.1	94.7
62 (mean)	**25.1**	**15.5**	**1.5**	**42.0**	**40.6**	**12.1**	**0.0**	**0.0**	**12.2**	**94.8**
93	20.6	12.7	1.4	34.7	45.3	13.0	0.0	0.0	13.1	93.0
121 (rep 1)	18.3	12.3	1.6	32.3	51.2	13.2	0.0	0.1	13.2	96.7
121 (rep 2)	12.5	12.2	1.7	26.3	49.7	13.2	0.0	0.1	13.2	89.2
121 (mean)	**15.4**	**12.3**	**1.6**	**29.3**	**50.4**	**13.2**	**0.0**	**0.1**	**13.2**	**93.0**
									mean:	**97.0**

TAR total applied radioactivity (100% = 0.113 mg/kg)
DAT days after treatment
NER non-extractable radioactive residues
MeOH methanol
n.d. not determinated
rep replicate
* cumulated values

C. VOLATILIZATION

Formation of CO_2 in all three soils reaching in total 9.7 to 39.2 % TAR after 121 days. No other volatile compounds were detected.

D. TRANSFORMATION OF 505M01

All combined soil extracts were analyzed by radio-HPLC. One HPLC-system was applied (run time 60 min). The results obtained with this system are summarized in Table 7.1.2.1.2-23 to Table 7.1.2.1.2-25.

505M01 decreased continuously from 87.9 – 91.9 % TAR at the beginning of the study to 1.3 % TAR or no peak detected at the end of the study.

Beside the parent compounds, several metabolites were observed. The peaks observed on the radio-HPLC chromatograms at 11.0 min and 13.0 min were identified as two isomer forms of 505M106. LC-MS/MS analysis also revealed the presence of a third isomer of 505M106, present only in trace amounts and not observable on the radio-HPLC chromatograms.
505M106 only appeared in significant amounts in soil Lufa 5M where it reached a maximum of 30.6 % TAR after 15 days (sum of two isomer forms). In the soils Li10 and Lufa 2.2, 505M106 stayed below 3 % TAR.

The peak observed at 20.3 min on the radio-HPLC chromatograms was found to consist of two metabolites: 505M96 and 505M97. The peak at 20.3 min was observed in all three soils. It reached a maximum of 25.8 % TAR on soil Lufa 5M after 2 days. The maximum concentrations on soils Li10 and Lufa 2.2 were observed on day 0 with 9.0 % TAR and 8.7 % TAR respectively.

The peaks observed on the radio-HPLC chromatogram at 28.1 min and 32.2 min were identified as two isomer forms of 505M102. It reached maximum concentrations of 49.5 % TAR on soil Lufa 5M after 7 days, 52.3 % TAR on soil Li10 after 28 days, and 17.1 % TAR on soil Lufa 2.2 after 7 days.

Further metabolites were present only in low amounts (below 4 % TAR at any sampling time).

The 3.3 minutes peak could not be identified. Since this "peak" elutes almost with the void volume, it might consist of more than one clearly defined compound. And since this peak represents metabolite(s) of a metabolite (505M01), which occurs only under photolytic conditions during soil photolysis of BAS 505 F, its environmental significance is considered very low.

Table 7.1.2.1.2-23: Summary results of radio-HPLC analysis of total soil extracts after treatment of soil Li 10 with ^{14}C-505M01

DAT	%TAR																
	Total	UK t_R~3.3	UK 5.6	UK 10.3	505M106 11.0	505M106 13.0	UK 16.0	505M01 17.0	505M96 and 505M97 20.3	UK 22.9	UK 24.5	505M102 28.1	505M102 32.2	UK 33.8	UK 40.7	UK 41.6	UK 52.5
0 (rep 1)	99.4	0.0	-	-	-	-	-	90.6	8.8	-	-	-	-	-	-	-	-
0 (rep 2)	98.7	0.4	-	-	-	-	-	89.0	9.3	-	-	-	-	-	-	-	-
0 (mean)	**99.1**	**0.2**	-	-	-	-	-	**89.8**	**9.0**	-	-	-	-	-	-	-	-
2	88.8	-	-	-	-	-	1.6	57.5	-	-	-	24.3	5.3	-	-	-	-
7	73.6	-	-	-	-	-	-	25.7	-	-	-	39.9	8.0	-	-	-	-
15	62.6	-	-	-	-	-	-	10.3	-	-	-	40.9	11.4	-	-	-	-
28	52.5	-	-	-	-	-	-	1.0	-	-	-	44.8	6.7	-	-	-	-
62 (rep 1)	46.4	1.5	1.0	-	-	2.6	-	1.0	-	2.4	-	31.5	6.5	-	-	-	-
62 (rep 2)	45.6	3.1	0.5	-	-	1.9	-	0.4	-	0.8	-	31.3	7.5	-	-	-	-
62 (mean)	**46.0**	**2.3**	**0.7**	**-**	**-**	**2.3**	**-**	**0.7**	**-**	**1.6**	**-**	**31.4**	**7.0**	**-**	**-**	**-**	**-**
93	42.8	13.7	-	-	-	-	-	-	-	-	-	25.7	3.5	-	-	-	-
121 (rep 1)	40.2	3.0	-	-	-	-	-	1.3	-	-	-	29.9	6.1	-	-	-	-
121 (rep 2)	41.5	0.0	-	-	-	-	-	0.0	-	-	-	33.1	8.4	-	-	-	-
121 (mean)	**40.9**	**1.5**	-	-	-	-	-	**0.6**	-	-	-	**31.5**	**7.2**	-	-	-	-

Total methanol, methanol+water and acetone extracts
TAR total applied radioactivity (0.116 mg/kg)
DAT days after treatment
UK unknown compound
t_R approximate retention time [min]
rep replicate
mean mean value of replicates
\- means: no peak detected

Table 7.1.2.1.2-24: Summary results of radio-HPLC analysis of total soil extracts after treatment of soil Lufa 2.2 with ^{14}C-505M01

DAT	%TAR																
	Total	**UK t_R~3.3**	**UK 5.6**	**UK 10.3**	**505M106 11.0**	**505M106 13.0**	**UK 16.0**	**505M01 17.0**	**505M96 and 505M97 20.3**	**UK 22.9**	**UK 24.5**	**505M102 28.1**	**505M102 32.2**	**UK 33.8**	**UK 40.7**	**UK 41.6**	**UK 52.5**
0 (rep 1)	97.2	0.8	-	-	-	-	-	87.9	8.6	-	-	-	-	-	-	-	-
0 (rep 2)	100.3	0.2	-	-	-	-	-	91.2	8.9	-	-	-	-	-	-	-	-
0 (mean)	**98.8**	**0.5**	-	-	-	-	-	**89.5**	**8.7**	-	-	-	-	-	-	-	-
2	85.1	-	-	-	-	-	2.0	71.0	3.6	-	1.3	6.6	0.4	-	-	-	-
7	76.6	1.1	-	-	-	-	-	58.4	-	-	-	14.7	2.4	-	-	-	-
15	62.0	2.4	-	-	-	-	-	42.8	-	-	-	13.5	3.3	-	-	-	-
28	41.8	2.7	-	-	-	-	0.7	22.1	-	-	-	13.6	2.8	-	-	-	-
62 (rep 1)	24.0	5.3	-	-	-	-	0.8	0.1	3.3	-	-	12.3	2.3	-	-	-	-
62 (rep 2)	24.3	5.6	-	-	-	-	0.9	0.3	3.9	-	-	10.7	3.0	-	-	-	-
62 (mean)	**24.2**	**5.4**	**-**	**-**	**-**	**-**	**0.8**	**0.2**	**3.6**	**-**	**-**	**11.5**	**2.6**	**-**	**-**	**-**	**-**
93	17.7	5.1	-	-	0.8	-	-	0.6	1.1	-	-	8.1	2.0	-	-	-	-
121 (rep 1)	15.4	4.1	-	-	-	-	-	0.6	-	-	-	8.7	2.0	-	-	-	-
121 (rep 2)	15.3	4.2	-	-	-	-	-	-	-	-	-	8.7	2.4	-	-	-	-
121 (mean)	**15.3**	**4.2**	-	-	-	-	-	**0.3**	-	-	-	**8.7**	**2.2**	-	-	-	-

Total methanol, methanol+water and acetone extracts
TAR total applied radioactivity (0.115 mg/kg)
DAT days after treatment
UK unknown compound
t_R approximate retention time [min]
rep replicate
mean mean value of replicates
\- means: no peak detected

Table 7.1.2.1.2-25: Summary results of radio-HPLC analysis of total soil extracts after treatment of soil Lufa 5M with ^{14}C-505M01

DAT	%TAR																
	Total	**UK t_R~3.3**	**UK 5.6**	**UK 10.3**	**505M106 11.0**	**505M106 13.0**	**UK 16.0**	**505M01 17.0**	**505M96 and 505M97 20.3**	**UK 22.9**	**UK 24.5**	**505M102 28.1**	**505M102 32.2**	**UK 33.8**	**UK 40.7**	**UK 41.6**	**UK 52.5**
0 (rep 1)	100.9	-	-	-	-	-	-	91.9	9.0	-	-	-	-	-	-	-	-
0 (rep 2)	97.4	-	-	-	-	-	-	88.7	8.7	-	-	-	-	-	-	-	-
0 (mean)	**99.2**	**-**	-	-	-	-	-	**90.3**	**8.8**	-	-	-	-	-	-	-	-
2	91.1	-	-	-	-	-	1.3	27.4	25.8	-	-	32.0	4.4	-	-	-	-
7	81.7	1.0	-	-	-	-	-	27.1	3.4	-	0.6	38.9	10.6	-	-	-	-
15	67.5	3.9	-	0.9	1.0	29.6	-	23.0	-	-	-	6.6	2.6	-	-	-	-
28	54.4	2.7	-	0.7	-	16.3	-	13.6	1.1	-	-	12.0	2.9	1.8	1.3	0.9	1.1
62 (rep 1)	42.4	5.7	2.7	-	22.8	1.5	-	-	6.7	-	-	-	2.2	1.0	-	-	-
62 (rep 2)	41.7	7.5	2.9	-	20.0	3.7	-	-	4.9	-	-	-	1.6	1.0	-	-	-
62 (mean)	**42.0**	**6.6**	**2.8**	**-**	**21.4**	**2.6**	**-**	**-**	**5.8**	**-**	**-**	**-**	**1.9**	**1.0**	**-**	**-**	**-**
93	34.7	7.3	-	2.8	-	14.9	-	-	2.3	-	-	3.5	-	3.8	-	-	-
121 (rep 1)	32.3	7.4	-	-	3.8	14.3	-	-	2.4	-	-	1.2	0.8	2.4	-	-	-
121 (rep 2)	26.3	5.1	-	-	3.8	11.0	-	-	2.6	-	-	1.2	0.4	2.2	-	-	-
121 (mean)	**29.3**	**6.2**	-	-	3.9	12.6	-	**-**	2.5	-	-	**1.2**	**0.6**	2.3	-	-	-

Total methanol, methanol+water and acetone extracts
TAR total applied radioactivity (0.113 mg/kg)
DAT days after treatment
UK unknown compound
t_R approximate retention time [min]
rep replicate
mean mean value of replicates
\- means: no peak detected

Calculation of the degradation rates

All visual fits of the chosen kinetic models (DFOP and SFO) were good or moderate and the residuals were evenly distributed. The χ^2 errors were below 12.5 % for all models. The kinetics evaluation showed that the DFOP model is appropriate to derive trigger endpoints for 505M01 from the experimental data obtained in the present laboratory study using Li 10 soil and Lufa 5M soil and that the SFO model is appropriate to derive trigger endpoints for 505M01 from the experimental data obtained using Lufa 2.2 soil.

The most appropriate kinetic models and the derived degradation parameters are summarized in the table below.

Table 7.1.2.1.2-26: Best-fit kinetic $DegT_{50}$ and $DegT_{90}$ values of metabolite 505M01

Soil	$DegT_{50}$ [d]	$DegT_{90}$ [d]	Kinetic model	χ^2 error
Li 10	2.7	14.8	DFOP	2.0
Lufa 2.2	11.7	38.9	SFO	12.0
Lufa 5M	0.2	33.5	DFOP	8.2

III. CONCLUSION

The results of the present study show that 505M01 was degraded in three different soils with half-lives of 2.7 days (Li 10), 11.7 days (Lufa 2.2) and 0.2 day (Lufa 5M) when incubated under aerobic conditions at 20 ±2°C and a soil moisture of 40 % of the maximum water holding capacity.

Beside the parent compound, several metabolites were observed. One metabolite, observed as two peaks on the chromatogram (isomers) and designated as 505M106 reached a maximum of 30.6 %TAR after 15 days. One peak which reached 25.8 %TAR after 2 days was found to consist of two metabolites: 505M96 and 505M97. One further metabolite, designated as 505M102, also occurred in two isomer forms, reaching in sum up to 49.5 %TAR after 7 days. Further metabolites were present only in low amounts (below 4 %TAR at any sampling time). After 121 days of incubation, the mineralization rate had reached 9.7 to 39.2 % TAR and the non-extractable residues amounted to 36.1 – 51.2 % TAR.

Report: CA 7.1.2.1.2/5
Budde E., 2015a
Kinetic evaluation of a laboratory soil degradation study with 505M08, metabolite of Dimoxystrobin, according to Focus degradation kinetics
2014/1113059
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP: no

Executive Summary

The degradation of 505M08, metabolite of BAS 505 F - dimoxystrobin in soil, has been investigated in three soils under aerobic conditions in the laboratory. The purpose of the evaluation was to analyze the degradation kinetics observed in the study, taking into account the current guidance of the FOCUS workgroup on degradation kinetics, in order to derive degradation parameters as triggers for additional work (trigger endpoints) and degradation parameters for environmental fate models (modeling endpoints).

The best-fit $DegT_{50}$ were between 60.7 and 255.7 days, with $DegT_{90}$ between 288.2 and >1000 days, following DFOP or FOMC kinetics. Modeling endpoints could be derived from DFOP or HS kinetics. Normalized modeling $DegT_{50}$ were between 72.7 and 259.5 days.

I. MATERIAL AND METHODS

The degradation of the metabolite of dimoxystrobin 505M08 was investigated in three European soils under aerobic conditions in the laboratory [see CA 7.1.2.1.2/2, *BASF DocID 2013/3009622*]. The test substance was applied at a nominal concentration of 0.133 mg kg^{-1} dry soil. The treated soil was incubated at 40% maximum water holding capacity (MWHC) at 20°C in the dark.

The measured data as well as resulting datasets submitted to kinetic analysis are given in Table 7.1.2.1.2-27.

Table 7.1.2.1.2-27 Data used for kinetic evaluation of 505M08

DAT	Li 10 soil		Lufa 2.2 soil		Lufa 5M soil	
	Residue data [%TAR]	Input data according to FOCUS [%TAR]	Residue data [%TAR]	Input data according to FOCUS [%TAR]	Residue data [%TAR]	Input data according to FOCUS [%TAR]
0	95.0	98.6 [a]	94.4	97.1 [a]	97.5	100.1 [a]
0	98.6	101.4 [a]	95.9	102.9 [a]	97.4	99.9 [a]
2	90.1	90.1	87.1	87.1	90.6	90.6
7	85.2	85.2	75.5	75.5	88.1	88.1
14	79.0	79.0	71.2	71.2	87.6	87.6
30	73.0	73.0	63.4	63.4	76.4	76.4
62	63.9	63.9	46.7	46.7	68.6	68.6
62	65.9	65.9	45.8	45.8	63.6	63.6
90	62.1	62.1	40.5	40.5	60.0	60.0
119	56.9	56.9	34.5	34.5	56.6	56.6
119	56.7	56.7	35.8	35.8	61.5	61.5

[a] set to material balance
TAR = total applied radioactivity

Kinetic modeling strategy

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) as well as modeling endpoints. The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 (November 2011), 436 pp*]. The best-fit model was selected based on visual and statistical assessment and the corresponding $DegT_{50}$ and $DegT_{90}$ values are reported as trigger endpoints. Appropriate $DegT_{50}$ values for use in environmental fate models were derived depending on the kinetic model.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the best-fit model, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC), the Hockey-stick (HS) kinetics, and the bi-exponential (DFOP) kinetics. The respective model descriptions and corresponding equations for calculating endpoints ($DegT_{50}$, $DegT_{90}$) are shown in the FOCUS Kinetics guidance [*FOCUS (2006)*], Box 5-1, Box 5-2, Box 5-3, and Box 5-4.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling and software for kinetic evaluation

Where available, replicate measurements were used in the parameter estimation. The experimental data were derived from the study reports and adjusted according to FOCUS [*FOCUS (2006)*]. The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting [*SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. BASF DocID 2007/1062781; Schmitt, W., Gao, Z., Meyer, H. (2011) KinGUII, Version 2.2012.320.1629 Bayer CropScience AG*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to $1x10^{-6}$ and 100, respectively.

Normalization of degradation rates to reference conditions

According to FOCUS [*FOCUS (2006)*] the DT_{50} values obtained from laboratory studies should be normalized for modeling purposes to reference conditions at a temperature of 20°C and soil moisture at pF2 to account for the different soil temperature and moisture conditions during incubation. The laboratory trials were conducted at 20°C, therefore temperature correction was not necessary. The soil moisture normalization was conducted according to FOCUS [*FOCUS (2006)*]. The actual soil moisture and the water content at pF2 for each soil were taken from the study report.

II. RESULTS AND DISCUSSION

The derived trigger endpoints for 505M08 are summarized in Table 7.1.2.1.2-28. The kinetic evaluation showed that the DFOP model is appropriate to derive trigger endpoints for additional work in two soils, while FOMC was selected as best-fit model in one soil.

Table 7.1.2.1.2-28 Trigger endpoints for 505M08 based on best-fit models

Soil	Best-fit model	χ^2 error level	Trigger endpoints	
			$DegT_{50}$ [d]	$DegT_{90}$ [d]
Li 10	DFOP	1.7	165.2	737.8
Lufa 2.2	DFOP	2.2	60.7	288.2
Lufa 5M	FOMC	2.6	255.7	>1000

Modeling endpoints for 505M08 could be derived from DFOP or HS kinetics. Prior to deriving modeling endpoints for 505M08, the $DegT_{50}$ values suitable for modeling obtained under different incubation conditions were normalized to reference conditions (20 °C, pF2). The resulting $DegT_{50}$ values for modeling are summarized in Table 7.1.2.1.2-29.

Table 7.1.2.1.2-29 Modeling endpoints for 505M08

Soil	Best-fit model	χ^2 error [%]	Modeling endpoints	
			$DegT_{50}$ [d]	Normalized $DegT_{50}$ [d] [a]
Li 10	DFOP	1.7	246.6 [b]	231.7
Lufa 2.2	DFOP	2.2	98.0 [b]	72.7
Lufa 5M	HS	2.9	345.9 [b]	259.5

[a] Reference conditions: 20°C, pF 2
[b] Derived from slow rate of the respective biphasic model (ln2/k2)

III. CONCLUSION

Trigger and modeling endpoints were derived for the metabolite of dimoxystrobin 505M08 in three soils. The best-fit $DegT_{50}$ were between 60.7 and 255.7 days, with $DegT_{90}$ between 288.2 and >1000 days, following DFOP or FOMC kinetics.
Modeling endpoints could be derived from DFOP or HS kinetics. Normalized modeling $DegT_{50}$ were between 72.7 and 259.5 days.

Report: CA 7.1.2.1.2/6
Budde E., 2015b
Kinetic evaluation of a laboratory soil degradation study with 505M09, metabolite of Dimoxystrobin, according to Focus degradation kinetics
2014/1113060
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP: no

Executive Summary

The degradation of 505M09, metabolite of BAS 505 F - dimoxystrobin in soil, has been investigated in three soils under aerobic conditions in the laboratory. The purpose of the evaluation was to analyze the degradation kinetics observed in the study, taking into account the current guidance of the FOCUS workgroup on degradation kinetics, in order to derive degradation parameters as triggers for additional work (trigger endpoints) and degradation parameters for environmental fate models (modeling endpoints).

The best-fit $DegT_{50}$ were between 62.6 and 153.8 days, with $DegT_{90}$ between 343.7 and 584.6 days, following DFOP kinetics. Modeling endpoints could be derived from DFOP or HS kinetics. Normalized modeling $DegT_{50}$ were between 89.8 and 162.3 days.

I. MATERIAL AND METHODS

The degradation of the metabolite of dimoxystrobin 505M09 was investigated in three European soils under aerobic conditions in the laboratory [see CA 7.1.2.1.2/3, *BASF DocID 2013/3009623*]. The test substance was applied at a nominal concentration of 0.133 mg kg^{-1} dry soil, corresponding to an application rate of 50 g ha^{-1} on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}. The treated soil was incubated at 40% maximum water holding capacity (MWC) at 20°C in the dark.

The measured data as well as resulting datasets submitted to kinetic analysis are given in Table 7.1.2.1.2-30.

Table 7.1.2.1.2-30 Data used for kinetic evaluation of 505M09

DAT	Li 10 soil		Lufa 2.2 soil		Lufa 5M soil	
	Residue data [%TAR]	Input data according to FOCUS [%TAR]	Residue data [%TAR]	Input data according to FOCUS [%TAR]	Residue data [%TAR]	Input data according to FOCUS [%TAR]
0	94.0	97.4 [a]	93.9	99.9 [a]	97.0	100.5 [a]
0	98.1	102.6 [a]	94.1	100.1 [a]	96.5	99.5 [a]
2	88.4	88.4	83.8	83.8	91.0	91.0
7	82.2	82.2	75.3	75.3	88.5	88.5
14	75.1	75.1	67.9	67.9	82.7	82.7
30	71.8	71.8	58.6	58.6	82.9	82.9
63	59.5	59.5	46.3	46.3	65.9	65.9
63	60.4	60.4	48.2	48.2	68.0	68.0
92	56.6	56.6	42.9	42.9	62.4	62.4
120	50.0	50.0	35.5	35.5	58.5	58.5
120	49.6	49.6	38.6	38.6	58.5	58.5

[a] set to material balance

TAR = total applied radioactivity

Kinetic modeling strategy

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) as well as modeling endpoints. The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 (November 2011), 436 pp*]. The best-fit model was selected based on visual and statistical assessment and the corresponding $DegT_{50}$ and $DegT_{90}$ values are reported as trigger endpoints. Appropriate $DegT_{50}$ values for use in environmental fate models were derived depending on the kinetic model.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the best-fit model, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC), the Hockey-stick (HS) kinetics, and the bi-exponential (DFOP) kinetics. The respective model descriptions and corresponding equations for calculating endpoints ($DegT_{50}$, $DegT_{90}$) are shown in the FOCUS Kinetics guidance [*FOCUS (2006)*], Box 5-1, Box 5-2, Box 5-3, and Box 5-4.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling and software for kinetic evaluation

Where available, replicate measurements were used in the parameter estimation. The experimental data were derived from the study reports and adjusted according to FOCUS [*FOCUS (2006)*]. The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting [*SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. BASF DocID 2007/1062781; Schmitt, W., Gao, Z., Meyer, H. (2011) KinGUII, Version 2.2012.320.1629 Bayer CropScience AG*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to $1x10^{-6}$ and 100, respectively.

Normalization of degradation rates to reference conditions

According to FOCUS [*FOCUS (2006)*] the DT_{50} values obtained from laboratory studies should be normalized for modeling purposes to reference conditions at a temperature of 20°C and soil moisture at pF2 to account for the different soil temperature and moisture conditions during incubation. The laboratory trials were conducted at 20°C, therefore temperature correction was not necessary. The soil moisture normalization was conducted according to FOCUS [*FOCUS (2006)*]. The actual soil moisture and the water content at pF2 for each soil were taken from the study report.

II. RESULTS AND DISCUSSION

The derived trigger endpoints for 505M09 are summarized in Table 7.1.2.1.2-31. The kinetic evaluation showed that the DFOP model is appropriate to derive trigger endpoints for all three soils.

Table 7.1.2.1.2-31 Trigger endpoints for 505M09 based on best-fit models

Soil	Best-fit model	χ^2 error level	Trigger endpoints	
			$DegT_{50}$ [d]	$DegT_{90}$ [d]
Li 10	DFOP	1.8	117.2	518.2
Lufa 2.2	DFOP	2.7	62.6	343.7
Lufa 5M	DFOP	2.3	153.8	584.6

Modeling endpoints for 505M09 could be derived from DFOP or HS kinetics. Prior to deriving modeling endpoints for 505M09, the $DegT_{50}$ values suitable for modeling obtained under different incubation conditions were normalized to reference conditions (20 °C, pF2). The resulting $DegT_{50}$ values for modeling are summarized in Table 7.1.2.1.2-32.

Table 7.1.2.1.2-32 Modeling endpoints for 505M09

Soil	Kinetic model	χ^2 error level	Modeling endpoints	
			$DegT_{50}$ [d]	Normalized $DegT_{50}$ [d] [a]
Li 10	DFOP	1.8	172.7 [b]	162.3
Lufa 2.2	DFOP	2.7	121.1 [b]	89.8
Lufa 5M	HS	2.3	185.4 [b]	139.1

[a] Reference conditions: 20°C, pF 2
[b] Derived from slow rate of the respective biphasic model (ln2/k2)

III. CONCLUSION

Trigger and modeling endpoints were derived for the metabolite of dimoxystrobin 505M09 in one laboratory degradation study. The best-fit $DegT_{50}$ were between 62.6 and 153.8 days, with $DegT_{90}$ between 343.7 and 584.6 days, following DFOP kinetics.

Modeling endpoints could be derived from DFOP or HS kinetics. Normalized modeling $DegT_{50}$ were between 89.8 and 162.3 days.

Report: CA 7.1.2.1.2/7
Budde E., 2015c
Kinetic evaluation of a laboratory soil degradation study with 505M01, metabolite of Dimoxystrobin, according to Focus degradation kinetics
2014/1113526
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP: no

Executive Summary

The degradation of 505M01, metabolite of BAS 505 F - dimoxystrobin in soil, has been investigated in three soils under aerobic conditions in the laboratory. The purpose of the evaluation was to analyze the degradation kinetics observed in the study, taking into account the current guidance of the FOCUS workgroup on degradation kinetics, in order to derive degradation parameters as triggers for additional work (trigger endpoints) and degradation parameters for environmental fate models (modeling endpoints).

The best-fit $DegT_{50}$ were between 0.2 and 11.7 days, with $DegT_{90}$ between 14.8 and 38.9 days, following DFOP or SFO kinetics. Normalized modeling $DegT_{50}$ resulted in values between 3.1 and 8.7 days.

I. MATERIAL AND METHODS

The degradation of the metabolite of dimoxystrobin 505M01 was investigated in three European soils under aerobic conditions in the laboratory [see CA 7.1.2.1.2/4, *BASF DocID 2013/3009624*]. The test substance was applied at a nominal concentration of 0.133 mg kg^{-1} dry soil, corresponding to an application rate of 50 g ha^{-1} on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g cm^{-3}. The treated soil was incubated at 40% maximum water holding capacity (MWHC) at 20°C in the dark.

The measured data as well as resulting datasets submitted to kinetic analysis are given in Table 7.1.2.1.2-33.

Table 7.1.2.1.2-33 Data used for kinetic evaluation of 505M01

DAT	Li 10 soil		Lufa 2.2 soil		Lufa 5M soil	
	Residue data [%TAR]	Input data according to FOCUS [%TAR]	Residue data [%TAR]	Input data according to FOCUS [%TAR]	Residue data [%TAR]	Input data according to FOCUS [%TAR]
0	90.6	100.4 [a]	87.9	98.5 [a]	91.9	101.8 [a]
0	89.0	99.6 [a]	91.2	101.5 [a]	88.7	98.3 [a]
2	57.5	57.5	71.0	71.0	27.4	27.4
7	25.7	25.7	58.4	58.4	27.1	27.1
15	10.3	10.3	42.8	42.8	23.0	23.0
28	1.0	1.0	22.1	22.1	13.6	13.6
62	1.0	1.0	0.1	0.1	<LOD	0.05 [b]
62	0.4	0.4	0.3	0.3	<LOD	0.05 [b]
93	<LOD	0.05 [b]	0.6	0.6	<LOD	
121	1.3	1.3	0.6	0.6	<LOD	
121	0.0	0.05 [b]	<LOD	0.05 [b]	<LOD	

[a] set to material balance
[b] set to ½ LOD (LOD not reported; pragmatically assumed to be 0.1% TAR)
TAR = total applied radioactivity

Kinetic modeling strategy

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints) as well as modeling endpoints. The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 (November 2011), 436 pp*]. The best-fit model was selected based on visual and statistical assessment and the corresponding $DegT_{50}$ and $DegT_{90}$ values are reported as trigger endpoints. Appropriate $DegT_{50}$ values for use in environmental fate models were derived depending on the kinetic model.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the best-fit model, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC), the Hockey-stick (HS) kinetics, and the bi-exponential (DFOP) kinetics. The respective model descriptions and corresponding equations for calculating endpoints ($DegT_{50}$, $DegT_{90}$) are shown in the FOCUS Kinetics guidance [*FOCUS (2006)*], Box 5-1, Box 5-2, Box 5-3, and Box 5-4.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling and software for kinetic evaluation

Where available, replicate measurements were used in the parameter estimation. The experimental data were derived from the study reports and adjusted according to FOCUS [*FOCUS (2006)*]. The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting [*SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. BASF DocID 2007/1062781; Schmitt, W., Gao, Z., Meyer, H. (2011) KinGUII, Version 2.2012.320.1629 Bayer CropScience AG*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to $1x10^{-6}$ and 100, respectively.

Normalization of degradation rates to reference conditions

According to FOCUS [*FOCUS (2006)*] the DT_{50} values obtained from laboratory studies should be normalized for modeling purposes to reference conditions at a temperature of 20°C and soil moisture at pF2 to account for the different soil temperature and moisture conditions during incubation. The laboratory trials were conducted at 20°C, therefore temperature correction was not necessary. The soil moisture normalization was conducted according to FOCUS [*FOCUS (2006)*]. The actual soil moisture and the water content at pF2 for each soil were taken from the study report.

II. RESULTS AND DISCUSSION

The derived trigger endpoints for 505M01 are summarized in Table 7.1.2.1.2-34. The kinetic evaluation showed that the DFOP model is appropriate to derive trigger endpoints for additional work in two soils, while SFO was selected as best-fit model in one soil.

Table 7.1.2.1.2-34 Trigger endpoints for 505M01 based on best-fit models

Soil	Best-fit model	χ^2 error level	Trigger endpoints	
			$DegT_{50}$ [d]	$DegT_{90}$ [d]
Li 10	DFOP	2.0	2.7	14.8
Lufa 2.2	SFO	12.0	11.7	38.9
Lufa 5M	DFOP	8.2	0.2	33.5

Modeling endpoints for 505M01 could be derived from SFO or DFOP kinetics. Prior to deriving modeling endpoints for 505M01, the $DegT_{50}$ values suitable for modeling obtained under different incubation conditions were normalized to reference conditions (20 °C, pF2). The resulting $DegT_{50}$ values for modeling are summarized in Table 7.1.2.1.2-35.

Table 7.1.2.1.2-35 Modeling endpoints for 505M01

Soil	Best-fit model	χ^2 error level	Modeling endpoints	
			$DegT_{50}$ [d]	Normalized $DegT_{50}$ [d] [a]
Li 10	SFO	11.6	3.3	3.1
Lufa 2.2	SFO	12.0	11.7	8.7
Lufa 5M	DFOP	8.2	10.1 [b]	7.6

[a] Reference conditions: 20°C, pF 2
[b] Derived from DFOP fit as $DegT_{50} = DegT_{90}/3.32$ (since 10% of initially measured concentration was reached within the experimental period)

III. CONCLUSION

Trigger and modeling endpoints were derived for the metabolite of dimoxystrobin 505M01 in three soils. The best-fit $DegT_{50}$ were between 0.2 and 11.7 days, and $DegT_{90}$ were between 14.8 and 38.9 days, following DFOP or SFO kinetics. Normalized modeling $DegT_{50}$ resulted in values between 3.1 and 8.7 days.

Summary tables on occurrence and degradation rate of dimoxystrobin metabolites

Table 7.1.2.1.2-36: Maximum occurrence of dimoxystrobin metabolites in laboratory soil studies

Metabolite	BASF DocID	Study	Parent label	Soil	Maximum % AR
505M08	1999/10087 [1]	aerobic soil	benzyl	Bruch West	3.0
	1999/10077 [1]	aerobic soil	phenyl	Bruch West	3.3
	1999/11656 [1]	aerobic soil	benzyl	LUFA 2.2 Minto Dinuba Li35b	2.0 1.0 3.1 2.1
	1997/5030 [1]	soil photolysis	benzyl	Bruch West	0
	1997/5335 [1]	soil photolysis	phenyl	Bruch West	0
	1998/10890 [1]	anaerobic soil	phenyl	Bruch West	0
505M09	1999/10087 [1]	aerobic soil	benzyl	Bruch West	12.2
	1999/10077 [1]	aerobic soil	phenyl	Bruch West	13.0
	1999/11656 [1]	aerobic soil	benzyl	LUFA 2.2 Minto Dinuba Li35b	2.4 1.5 5.3 2.8
	1997/5030 [1]	soil photolysis	benzyl	Bruch West	0
	1997/5335 [1]	soil photolysis	phenyl	Bruch West	0
	1998/10890 [1]	anaerobic soil	phenyl	Bruch West	0
505M01	1997/5030 [1]	soil photolysis	benzyl	Bruch West	10.8
	1997/5335 [1]	soil photolysis	phenyl	Bruch West	5.2
505M03/04	1997/5030 [1]	soil photolysis	benzyl	Bruch West	3.8
(former unknown a)	1997/5335 [1]	soil photolysis	phenyl	Bruch West	5.2
505M103/104	1997/5030 [1]	soil photolysis	benzyl	Bruch West	4.3
(former unknown b)	1997/5335 [1]	soil photolysis	phenyl	Bruch West	7.1

[1] already peer-reviewed during former Annex I listing

Table 7.1.2.1.2-37: Persistence endpoints of dimoxystrobin metabolites in aerobic soil studies (laboratory, 20°C, 40-50% MWHC)

Metabolite	Data source BASF DocID	Test item (label)	Soil	Persistence $DegT_{50}$/$DegT_{90}$ [d]	Kinetic model	χ^2 error [%]
505M08	1999/10087 [1] 2014/1175670	parent (benzyl)	Bruch West	12.9 / 42.8	SFO [b]	16.7
	1999/10077 [1] 2014/1175670	parent (phenyl)	Bruch West	57.3 / 190.3	SFO [b]	8.5
	1999/11656 [1] 2014/1175670	parent (benzyl)	LUFA 2.2	68.1 / 226.1	SFO	9.1
			Minto	- [2]	SFO	26.6
			Dinuba	27.9 / 92.6	SFO	14.8
			Li35b	61.3 / 203.7	SFO	12.4
	2013/3009622 2014/1113059	505M08 (benzyl)	Li10	165.2 / 737.8	DFOP	1.7
			Lufa 2.2	60.7 / 288.2	DFOP	2.2
			Lufa 5M	255.7 / >1000	FOMC	2.6
505M09	1999/10087 [1] 2014/1175670	parent (benzyl)	Bruch West	56.5 / 187.7	SFO [b]	4.6
	1999/10077 [1] 2014/1175670	parent (phenyl)	Bruch West	80.9 / 268.6	SFO [b]	3.4
	1999/11656 [1] 2014/1175670	parent (benzyl)	LUFA 2.2	29.1 / 96.6	SFO	12.8
			Minto	- [a]	SFO	11.6
			Dinuba	69.6 / 231.3	SFO	9.1
			Li35b	62.5 / 207.5	SFO	11.1
	2013/3009623 2014/1113060	505M09 (benzyl)	Li10	117.2 / 518.2	DFOP	1.8
			Lufa 2.2	62.6 / 343.7	DFOP	2.7
			Lufa 5M	153.8 / 584.6	DFOP	2.3
505M01	2013/3009624	505M01 (benzyl)	Li10	2.7 / 14.8	DFOP	2.0
			Lufa 2.2	11.7 / 38.9	SFO	12.0
			Lufa 5M	0.2 / 33.5	DFOP	8.2

MWHC maximum water holding capacity
[1] already peer-reviewed during former Annex I listing
[a] no reliable endpoints derived in kinetic evaluation
[b] DFOP kinetics for parent

Table 7.1.2.1.2-38: Modeling endpoints for dimoxystrobin metabolites in aerobic soil studies (laboratory, 20°C, 40-50% MWHC)

Metabolite	Data source BASF DocID	Test item (label)	Soil	$DegT_{50}$ at study conditions [d]	Kinetic model	$DegT_{50}$ 20°C / pF2 [d]	Formation fraction
505M08	1999/10087 [1] 2014/1175670	parent (benzyl)	Bruch West	12.9	SFO [b]	12.2	0.254
	1999/10077 [1] 2014/1175670	parent (phenyl)	Bruch West	57.3	SFO [b]	54.3	0.110
	1999/11656 [1] 2014/1175670	parent (benzyl)	LUFA 2.2	68.1	SFO	68.1	0.223
			Minto	- [a]	SFO	- [a]	0.041 [f]
			Dinuba	27.9	SFO	19.8	0.361
			Li35b	61.3	SFO	48.5	0.219
	2013/3009622 2014/1113059	505M08 (benzyl)	Li10	246.6 [c]	DFOP	231.7	-
			Lufa 2.2	98.0 [c]	DFOP	72.7	-
			Lufa 5M	345.9 [c]	HS	259.5	-
	Geometric mean					**68.9 [e]**	
	Arithmetic mean						**0.246 [e]**
505M09	1999/10087 [1] 2014/1175670	parent (benzyl)	Bruch West	56.5	SFO [b]	53.6	0.411
	1999/10077 [1] 2014/1175670	parent (phenyl)	Bruch West	80.9	SFO [b]	76.7	0.344
	1999/11656 [1] 2014/1175670	parent (benzyl)	LUFA 2.2	29.1	SFO	29.1	0.323
			Minto	- [a]	SFO	- [a]	0.070 [f]
			Dinuba	69.6	SFO	49.3	0.358
			Li35b	62.5	SFO	49.5	0.291
	2013/3009623 2014/1113060	505M09 (benzyl)	Li10	172.7 [c]	DFOP	162.3	-
			Lufa 2.2	121.1 [c]	DFOP	89.8	-
			Lufa 5M	185.4 [c]	HS	139.1	-
	Geometric mean					**71.1 [e]**	
	Arithmetic mean						**0.337 [e]**
505M01	2013/3009624 2014/1113526	505M01 (benzyl)	Li10	3.3	SFO	3.1	-
			Lufa 2.2	11.7	SFO	8.7	-
			Lufa 5M	10.1 [d]	DFOP	7.6	-
	Geometric mean					**5.9**	
	Arithmetic mean						**-**

MWHC maximum water holding capacity
[1] already peer-reviewed during former Annex I listing
[a] no reliable endpoints derived in kinetic evaluation
[b] SFO kinetics for parent
[c] Derived from slow rate of the respective bi-phasic model (ln2/k2)
[d] Derived from DFOP fit as $DegT_{50} = DegT_{90}/3.32$
[e] Results from Bruch West soil with two labels were averaged before calculating the overall mean
[f] Not taken into account for averaging of formation fractions because outside of range

CA 7.1.2.1.3 Anaerobic degradation of the active substance

No new experimental data were generated.

Although the available study was done according to the previous guideline on anaerobic soil metabolism (without an aerobic pre-incubation phase), the results are still considered valid. Dimoxystrobin showed no significant degradation under anaerobic conditions. Since major route of degradation of dimoxystrobin in soil are oxidative reactions (oxidation of the methyl-groups at the phenyl-ring or formation of bound residues by oxidative coupling), it can be expected that those reactions simply stop when anaerobic conditions in soil will occur.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

No new experimental data were generated.

In the field soil dissipation studies, the (aerobic) metabolites were detected if at all only in concentrations close to LOQ and dimoxystrobin itself proved to be stable under anaerobic conditions. Therefore, no metabolite needs to be considered for anaerobic degradation rate in soil.

CA 7.1.2.2 Field studies

The two for the previous Annex I listing submitted and peer-reviewed field soil dissipation studies are considered still valid. Overall, 6 field trials (bare soil) distributed over Europe were performed (3 in Germany, 2 in Spain and 1 in Sweden) at an application rate of nominal 250 g/ha. The kinetic evaluation of these trials were updated according to the newest guidelines and guidance documents.

Since these already peer-reviewed studies were performed on bare soil without exclusion of surface processes like photolysis and/or volatilization, a new study according to EFSA 2010 was initiated in 2012 in order to be able to use the degradation rates for leaching assessment. A storage stability study for the field soils was initiated in parallel.

Since several DT_{90} values of dimoxystrobin of the old field soil dissipation studies (running between 1997 and 2000) exceeded 1 year, an accumulation study was initiated already back in 1998. At submission of the EU dossier in 2001, only an interim report covering the first two years was available and submitted. The study was at that time ongoing and ran in total for 4 experimental years. It was finalized in the year 2004. Since the final report was not peer-reviewed yet, it is included into this supplemental dossier.

CA 7.1.2.2.1 Soil dissipation studies

Report:	CA 7.1.2.2.1/1 Budde E., 2015a Kinetic evaluation of two field dissipation studies with BAS 505 F - Dimoxystrobin conducted between 1997 and 2000 in Europe: Determination of trigger and modeling endpoints according to FOCUS Degradation Kinetics and EFSA 2013/1335916
Guidelines:	FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0, EFSA Guidance to obtain DegT50 values in soil (2010)
GLP:	no

Executive Summary

The dissipation behavior of dimoxystrobin in soil has been investigated in two field dissipation studies [*BASF DocID 2000/1000122; BASF DocID 1999/11287*]. The purpose of this evaluation was to analyze the degradation kinetics of dimoxystrobin observed in six soils under different climatic conditions and to derive non-normalized trigger and normalized modeling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA guidance on estimating $DegT_{50}$ values in soil for modeling purposes.

The field trials were situated in different regions of Europe (Germany, Spain and Sweden), considering a range of different soils and climatic conditions. The two field dissipation trials in Spain were rejected in the evaluation process because of the long drying period after the second sampling point. However, best-fit endpoints were estimated as field trials can be assumed to represent worst-case conditions. For the remaining four trials (Germany and Sweden), the time-step normalization approach was applied as recommended by the EFSA Guidance Document for evaluating laboratory and field dissipation studies.

The non-normalized field half-lives (DT_{50}, trigger endpoints) for dimoxystrobin ranged from 1.7 to 104.4 days, with corresponding DT_{90} values between 526.7 and >1000 days.

Normalized (pF2, 20°C) field half-lives ($DegT_{50}$, modeling endpoints) for dimoxystrobin ranged from 62.0 to 346.6 days.

I. MATERIAL AND METHODS

Field trials

The kinetic evaluation was conducted for six field trials with dimoxystrobin from the data of two field dissipation studies [*KELLNER, O., KELLER, W. – BASF DocID 2000/1000122; KELLNER, O., KELLER, W. – BASF DocID 1999/11287*]. The trials were situated in typical agricultural regions in Germany (three trials), Spain (two trials), and Sweden (one trial) considering a range of different soils and climatic conditions. Dimoxystrobin was applied as SC formulation to bare soil using a knapsack sprayer. The nominal application rate was 250 g a.s. ha^{-1} at all trial sites. Soil samples were collected at seven sampling times up to one year after application and down to a depth of 50 cm and analyzed for dimoxystrobin.

Characteristics of the soils from the trial sites were determined from soil samples taken before application to a depth of 25 cm. Soil characteristics are summarized in Table 7.1.2.1.1-1.

Table 7.1.2.2.1-1: Characteristics of the field dissipation studies

	Trial					
	ALO/03/98, Spain	**ALO/04/98, Spain**	**HUS/09/98, Sweden**	**D05/03/97, Germany**	**DU3/04/97, Germany**	**DU2/03/97, Germany**
Soil texture (German classification)	Loamy sand	Sandy loam	Loamy sand	Loamy sand	Loamy sand	Silty clayey loam
sand [%] (63 – 200 µm)	62	41	78	50.9	77.6	2.3
silt [%] (2 – 63 µm)	24	38	13	33	16	65
clay [%] (< 2 µm)	14	21	9	16.1	6.4	32.7
Organic C [%]	0.7	0.9	1.3	1.08	0.63	1.29
Organic matter [%] [a]	1.2	1.6	2.2	1.86	1.09	2.22
pH ($CaCl_2$)	7.5	7.6	5.8	6.3	5.3	6.4
CEC [meq 100 g^{-1} dry weight]	15	18	13	14	8	21
MWHC [g 100 g^{-1} dry weight]	36	44	32	43.4	26.7	36.8

[a] organic matter = organic carbon x 1.724
CEC = cation exchange capacity
MWHC = maximum water holding capacity

Kinetic modeling

The software package KinGUI version 2.2012.320.1629 was used for parameter fitting [*SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. BASF DocID 2007/1062781; Schmitt, W., Gao, Z., Meyer, H. (2011) KinGUII, Version 2.2012.320.1629 Bayer CropScience AG*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

Datasets were prepared for kinetic evaluation as follows:

- Values below the quantification or detection limit were treated as recommended by the FOCUS workgroup [*see FOCUS (2006), chapter 6.1.4*]. The limit of quantification (LOQ) for dimoxystrobin reported in the two studies was 0.01 mg kg^{-1}. A limit of detection (LOD) was not provided in the study reports and was therefore set equal to LOQ. According to FOCUS, values below LOD were set to $0.5 \times$ LOD=0.005 mg kg^{-1}.

- For each sampling point, the residues of the single core segments given in mg kg^{-1} were transformed to residues given in g ha^{-1} considering the thickness of the respective segment and a bulk density of 1.5 g cm^{-3} for each soil layer. The total residues in the sampled soil core were calculated as the sum of residues of the single soil core segments.

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation report.

Kinetic models included in the evaluations

The kinetic models which can be employed for these evaluations were described by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]:

- Single-First-Order (SFO); Box 5-1 in *FOCUS (2006)*
- Gustafson and Holden (FOMC); Box 5-2 in *FOCUS (2006)*
- Double first-order in parallel (DFOP); Box 5-4 in *FOCUS (2006)*
- Hockey-stick (HS); Box 5-3 in *FOCUS (2006)*

According to the EFSA guidance [*EFSA (2014): EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain $DegT_{50}$ values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662*], the kinetic models named above with exception of the FOMC model are proposed for the calculation of the DT_{50} for normalized decline curves.

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Normalization procedure

Evaluation of the suitability of field dissipation data for normalization was performed according to the evaluation criteria for normalization compiled by the Dutch regulatory authority (CTB criteria).

The two field dissipation trials in Spain (ALO/03/98 and ALO/04/98) were rejected in the evaluation process because of the long drying period after the second sampling point. For the remaining four trials, the time-step normalization approach was applied as recommended by the EFSA Guidance Document for evaluating laboratory and field dissipation studies [*EFSA (2014)*].

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily soil temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20°C using the Q_{10} approach as described in the report of the FOCUS soil modeling working group [*FOCUS (1997): Soil persistence models and EU registration. The final report of the work of the Soil Modeling Work group of FOCUS*]. The Q_{10} response function was applied for temperatures above 0°C and below field temperatures of 0°C it was assumed that no degradation occurs (Equation 7.1.2.2.1-1c). A Q_{10} value of 2.58 was included in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture at field capacity (pF 2) (Equation 7.1.2.2.1-1d).

Equation 7.1.2.2.1-1:Calculation of normalized day length based on combination of soil moisture and soil temperature correction factors

a) $D_{norm} = D * f_{temp} * f_{moisture}$

b) $t_i = \sum_{t=1}^{i-1} D_{norm}$

with:
t_i = Time from application till sampling at day i [d]
D_{norm} = Normalized day length (20°C, pF2) [d]
i = Time span between application and sampling [d]

c) $$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact-Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

d) $$f_{moist} = \begin{cases} (\frac{\theta_{act}}{\theta_{ref}})^B & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:
D_{norm} = normalized day length (temperature and moisture)
f_{temp} = temperature correction factor (-)
f_{moist} = moisture correction factor (-)
D = 1 d (days)
T_{act} = actual soil temperature (°C)
T_{ref} = reference temperature (20 °C)
Q_{10} = factor of increase of degradation rate with an increase in temperature of 10°C (Q_{10} = 2.58) (-)
θ_{act} = actual soil moisture (vol. water content) (m^3 m^{-3})
θ_{ref} = reference soil moisture at pF2 (m^3 m^{-3})
B = exponent of the moisture response function, B = 0.7 (-)

The trial data were normalized by time-step correction (varying the ‘day length’ virtually by applying the correction factors to the time given in days). The corrected day lengths for the time-step normalization are shown in Table 7.1.2.2.1-2.

Table 7.1.2.2.1-2: Time-step normalized sampling days

HUS/09/98		D05/03/97		DU3/04/97		DU2/03/97	
DAT	D_{norm}	DAT	D_{norm}	DAT	D_{norm}	DAT	D_{norm}
0	0	0	0	0	0	0	0
16	8.3	14	5.0	12	6.5	13	6.6
31	17.1	29	11.8	29	17.6	30	17.5
59	34.4	63	29.9	57	37.6	57	36.6
100	63.6	106	63.8	96	71.0	93	64.8
177	98.0	183	119.6	173	138.3	175	133.8
352	130.1	360	159.9	350	183.5	349	176.0

To derive appropriate field half-lives for environmental fate modeling, the recommendations provided in the EFSA guidance to obtain DT_{50} values in soil [*EFSA (2014)*] were considered, i.e. splitting of field dissipation trials into two parts, i.e. before and after 10 mm of rain has fallen since application.

The data sets of the four field trials which were considered suitable for normalization and derivation of modeling endpoints were evaluated for the criterion of 10 mm cumulative rainfall. The evaluation showed that it was possible to perform kinetic analysis for all trial locations, because the number of data points in each of the data sets was still appropriate (six sampling days, Table 7.1.2.2.1-3) after excluding sampling dates before 10 mm of cumulative rainfall.

Table 7.1.2.2.1-3: Day at which 10 mm of rainfall was reached

Trial	Day of 10 mm rain (DAT)	Amount of rain until 1st considered data point [mm]	Remaining sampling days
HUS/09/98 (Sweden)	9	12.4	6
D05/03/97 (Germany)	6	10.8	6
DU3/04/97 (Germany)	3	13.9	6
DU2/03/97 (Germany)	3	11.1	6

II. RESULTS AND DISCUSSION

Bi-phasic dissipation behavior of dimoxystrobin could be observed in all field trials: rapid decline between the day of application and the next sampling point, as well as slow decline of the substance at later sampling points. This was corroborated during kinetic evaluation, where FOMC always provided the better visual fit compared to SFO. A clear breakpoint could be determined in the subsequently performed DFOP kinetic fits. However, one or both degradation rates (k_1 or k_2) were not always statistically significant: k_1 was often derived from only 2-3 data points, while the slow rate k_2 was often not significantly different from zero (no significant decline observed). For trigger endpoints, the visual fit and overall model fit as indicated by a low χ^2 error level are rated higher than statistical significance of individual model parameters. Further, later use in environmental fate modeling (PEC_{soil} calculations) might be taken into consideration, since the FOMC model is not suitable for calculation of accumulation in soil. Consequently, the DFOP model was accepted as best-fit kinetic model for all six field trials.

A summary of the adequate $DisT_{50}$ and $DisT_{90}$ values to be used as trigger endpoints is given in Table 7.1.2.2.1-4.

Table 7.1.2.2.1-4: Summary of best-fit endpoints of dimoxystrobin

Field trial	Soil type (DIN)	Best-fit kinetic model	χ^2 error level	$DisT_{50}$ [d]	$DisT_{90}$ [d]
ALO/03/98 (Spain)	Loamy sand	DFOP	5.2	1.7	526.7
ALO/04/98 (Spain)	Sandy loam	DFOP	9.2	2.7	794.1
HUS/09/98 (Sweden)	Loamy sand	DFOP	11.5	104.4	>1000
D05/03/97 (Germany)	Loamy sand	DFOP	1.9	29.3	>1000
DU3/04/97 (Germany)	Loamy sand	DFOP	4.8	63.0	>1000
DU2/03/97 (Germany)	Silty clayey loam	DFOP	6.7	25.1	>1000

The dissipation behavior of dimoxystrobin in the four adequate field trials (excluding the Spanish trials because of long drying periods) was analyzed in a stepwise approach to derive modeling endpoints. The rate constants (*k*) and field half-lives ($DegT_{50}$) adequate to be used in environmental fate modeling are summarized in Table 7.1.2.2.1-5. The visual fits are acceptable for all trials; moreover, the χ^2 error is < 15%.

Table 7.1.2.2.1-5: Summary of endpoints for use in modeling of dimoxystrobin

Field trial	Soil type (DIN)	Kinetic model	χ^2 error level	Rate k [d^{-1}]	$DegT_{50}$ [d]
HUS/09/98	Loamy sand	SFO	12.5	0.008687	79.8
D05/03/97	Loamy sand	HS	5.9	0.002001 [a)]	346.6 [b)]
DU3/04/97	Loamy sand	SFO	7.5	0.01118	62.0
DU2/03/97	Silty clayey loam	DFOP	7.0	0.004363 [a)]	158.9 [b)]
			Geometric mean		**128.5**

a) slow rate of biphasic model (k_2)

b) $DegT_{50}$ calculated from slow rate ($ln2/k_2$)

III. CONCLUSION

Kinetic evaluation of six field trials with dimoxystrobin was conducted in order to derive trigger and modeling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA guidance on estimating $DegT_{50}$ values in soil for modeling purposes. The field trials were situated in different regions of Europe (Germany, Spain and Sweden), considering a range of different soils and climatic conditions. The two field dissipation trials conducted in Spain were found to be not representative and were excluded from the evaluation for derivation of modeling endpoints. However, best-fit endpoints were estimated, as field trials can be assumed to represent worst-case conditions.

The non-normalized field half-lives (DT_{50}, trigger endpoints) for dimoxystrobin ranged from 1.7 to 104.4 days, with corresponding DT_{90} values between 526.7 and >1000 days.

Normalized (pF2, 20°C) field half-lives ($DegT_{50}$, modeling endpoints) for dimoxystrobin ranged from 62.0 to 346.6 days.

Report: CA 7.1.2.2.1/2
Gut T., 2015a
Field soil dissipation study of BAS 505 F (Dimoxystrobin) in the formulation BAS 540 01 F on bare soil at 5 different sites in Northern and Southern Europe, 2012-2014
2014/1289336

Guidelines: NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006), EPA 835.6100, OECD-ENV/JM/MONO(2002)/9, EFSA Guidance to obtain DegT50 values in soil (2010), SANCO/3029/99 rev. 4 (11 July 2000), OECD-DOC ENV/MC/CHEM(98)17 Paris 1998, SETAC Procedures for assessing the environmental fate and ecotoxicity of pesticides - Part 1 Fate and behaviour in the environment - 10 - Aqueous photolysis

GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The dissipation of dimoxystrobin (BAS 505 F) under field conditions was investigated at five sites in Europe representative of Northern and Southern EU conditions. One trial each was performed in Spain (trial 12/02244925-01), Italy (trial 12/02244925-02), United Kingdom (trial 12/02244925-03), Germany (trial 12/02244925-04) and Northern France (trial 12/02244925-05). All sites represent typical regions of agricultural practice representative for growing sunflower (Spain and Italy) or oilseed rape (UK, Germany and France). The trial sites consisted of an untreated and a treated plot, the latter being subdivided into 3 subplots that were assigned for replicates.

The test item BAS 540 01 F (containing dimoxystrobin and boscalid), formulated as a suspension concentrate (SC), was broadcast applied to bare soil in a single application at a nominal rate of 100 g a.s. ha^{-1} using a target water volume of 300-400 L ha^{-1}. Applications were conducted between late May and late July 2012 using a calibrated boom sprayer. The actual application rates for each trial determined by quantifying the amount of spray discharged ranged from 100.1 to 104.0 g a.s. ha^{-1} averaged over the three replicates of each treated plot with an overall average of 101.9 g a.s. ha^{-1}. Results from spray broth analysis for the individual trials revealed averaged concentrations between 93 and 106% of the nominal value with an average of 97.6% across all sites. Dose verification conducted via application monitors (petri dishes) yielded recovery values for the individual trials ranging from 85.4 to 112.2% of the target rate, averaged over all three treated replicates of each treated plot, and an average recovery of 103.8% across all trials.

Immediately after application of the test item, the plots were covered with a layer of sand of approximately 4 mm depth (range 3-7 mm) to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted until complete coverage of the soil surface. The layer of sand remained intact until at least 27 – 56 days at each trial. Within this time period until the sampling event at 27 to 56 days after application, the individual fields received a total precipitation (rain and/or irrigation) of 43.6 mm (Spain), 20.4 mm (Italy), 55.1 mm (UK), 72.4 mm (Germany) and 115.0 mm (France), respectively.

No tillage or fertilization was performed during the course of the study and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate or pendimethalin.

Rainfall was supplemented with irrigation at sites in Spain (308.3 mm), Italy (437.6 mm), UK (388.3 mm), Germany (302 mm) and France (65.2 mm) and the total water input was at least 91.6 % of the historical average rainfall during the study period at the test sites.

Soil specimens were taken at intervals up to 741 days after application and down to a maximum soil depth of 70 cm (except for one sampling event in trial 12/02244925-02, where sampling was done to a depth of 100 cm). Soil cores were cut into 10 cm sections. Soil segments of the same depth and subplot from a defined sampling event were pooled and homogenized and a representative sub-specimen of each depth was taken for residue analysis. All soil specimens were stored at about ≤ -18°C within a maximum of 10 hours and 10 minutes after sampling and remained frozen until analysis.

In order to demonstrate stability of the residues in soil during storage and shipment, shipping verification specimens were prepared at selected sampling occasions by fortifying untreated soil from the test fields with known amounts of dimoxystrobin. These specimens were stored and shipped under the same conditions as the actual residue specimens. Analysis of the shipping verification specimens on dimoxystrobin yielded average recovery values of 95-101% across all trials confirming residue stability during all storage and shipment procedures.

Soil specimens were analyzed for dimoxystrobin (BAS 505 F) and metabolites 505M98, 505M01, 505M08 and 505M09 according to BASF method L0189/01. The analytical method involved extraction with methanol/water (80/20, v/v) on a mechanical shaker and centrifugation. The extract was diluted with the appropriate amount of methanol/water (80/20, v/v) before final determination of the analytes by LC-MS/MS with a limit of quantification (LOQ) of 0.002 mg kg^{-1} for each analyte. Field soil specimens from the treated plot were analyzed down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ). Analysis was performed until a maximum of 741 days after application (DAA). Application monitors (petri dish specimens) and shipping verification specimens were analyzed for parent only using the same analytical method L0189/01.

No residues above the LOQ of any analyte were detected in any of the untreated control specimens proving that there were no interferences of the untreated soil. Procedural recovery experiments performed with untreated soils spiked with the five analytes at concentrations of 0.002, 0.01, 0.02, 0.1 and 0.2 mg kg^{-1} yielded overall mean recovery rates of 91.7 and 97.6 % for the individual analytes, confirming the validity of the analytical method used in this study.

Residue values of dimoxystrobin and metabolites 505M98, 505M01, 505M08 and 505M09 in mg kg^{-1} dry soil were, when detected at residue levels above the LOQ, converted to residue rates in g ha^{-1} taking into account the actual dry soil density of the individual field specimens, and were summed up for all depths between 0 and 40 cm analyzed. Residue values were not corrected for procedural recoveries.

Dimoxystrobin degraded well under field conditions in soil at all five European field sites. The total amount of dimoxystrobin residues detected in the soil profiles decreased from an average across all trials of 96.5 g ha^{-1} at day 0 to an average across all trials of 7.9 g ha^{-1} after 24 months. In one of the five field trials, trial 12/02244925-01 in Spain, no residues above the LOQ (0.002 mg kg^{-1}) were detected after 24 months.

Dimoxystrobin residues were exclusively detected in the upper 20 cm of the soils. No residues above the LOQ were detected below 20 cm in any specimen. Altogether, it can be concluded that dimoxystrobin does not show any significant tendency to move into deeper soil layers indicating low potential for dimoxystrobin residues to leach to groundwater.

No residues of 505M98 and 505M01 above the LOQ were detected in any specimen of any trial.

Residues of 505M08 above the LOQ were exclusively detected in trial 12/02244925-05 (France) in small amounts (maximum 2.6 g ha^{-1}). Detects of 505M08 occurred sporadically and no residues of 505M08 above the LOQ were observed later than 89 DAA (days after application).

Metabolite 505M09 was detected in significant amounts just at the trial sites in UK and France. Residues of 505M09 were detected between 14 and 230 DAA reaching a maximum of 4.3 g ha^{-1}. Residues declined again and were no longer observed after 230 DAA at the latest.

Metabolites 505M08 und 505M09 were exclusively found in the top 0-10 cm soil layer and no residues of 505M08 and 505M09 above the LOQ were observed in deeper soil layers in any specimen at any site.

No calculation of degradation times is provided in the summarized report. A detailed kinetic evaluation of the degradation behavior of dimoxystrobin and its metabolites 505M98, 505M01, 505M08 and 505M09 in the five European field soils is provided in a separate modeling report.

I. MATERIAL AND METHODS

1. Test Material

Test item (formulation): BAS 540 01 F
Active substance (a.s.): Dimoxystrobin (BAS 505 F) and Boscalid (BAS 510 F)
Type of formulation: SC (suspension concentrate)
Batch No.: 0004250565
Content of a.s.: 200.0 g L^{-1} (dimoxystrobin); 200.0 g L^{-1} (boscalid)
Expiration date: 31 Dec 2012

2. Test sites

The dissipation of dimoxystrobin under field conditions was investigated at five sites in Europe representative of Northern and Southern EU conditions. Trials were performed in Spain (12/02244925-01), Italy (12/02244925-02), United Kingdom (12/02244925-03), Germany (12/02244925-04) and Northern France (12/02244925-05). The homogeneity of the upper soil layer was verified prior to the start of the trials. The site characteristics are presented in Table 7.1.2.2.1-6 to Table 7.1.2.2.1-8. Soil parameters were determined from soil specimens taken before application from the boundaries of the treated plot following segmentation to the soil horizons 0-30 and 30-70 cm. Soil taxonomy was determined on the basis of regional soil maps.

Table 7.1.2.2.1-6: Characteristics of the trial sites used to investigate the field dissipation of dimoxystrobin (BAS 505 F) (Spain and Italy)

Trial	**12/02244925-01**		**12/02244925-02**	
Location	**Zafarraya, Spain**		**Dugliolo di Budrio, Italy**	
Soil properties	0 - 30 cm	30 - 70 cm	0 - 30 cm	30 - 70 cm
Soil class (DIN 4220)	Slu Silty loamy sand	Ls3 Medium sandy loam	Sl3 Medium loamy sand	Sl2 Poor loamy sand
sand [%]	42.5	42.6	66.1	73.5
silt [%]	42.7	35.4	25.4	19.3
clay [%]	14.6	22.0	8.5	7.2
Soil class (USDA)	Loam	Loam	Sandy loam	Loamy sand
sand [%]	47.3	43.9	72.4	78.7
silt [%]	36.0	34.9	18.4	14.8
clay [%]	16.7	21.3	9.3	6.5
Total organic C [%]	0.93	0.40	0.45	0.31
Organic matter [%]*	1.60	0.69	0.78	0.53
pH [$CaCl_2$]	7.33	7.42	7.59	7.68
pH [H_2O]	7.96	8.08	8.32	8.53
CEC [mval Ba/100g dry weight]	15.1	15.6	7.0	6.0
MWHC [g/100g dry weight]	47.6	41.9	46.4	40.7
pF 2.0 [g/100g dry weight]**	20.7	26.9	15.9	16.0
pF 2.5 [g/100g dry weight]**	14.5	18.2	12.7	11.3
Dry bulk density [g/cm^3]***	1.23	-	1.45	-
Soil taxonomy	Xerochrept		Calcaric Cambisol	

Table 7.1.2.2.1-7: Characteristics of the trial sites used to investigate the field dissipation of dimoxystrobin (BAS 505 F) (UK and Germany)

Trial	**12/02244925-03**		**12/02244925-04**	
Location	**Ardleigh, Colchester, UK**		**Bakum, Germany**	
Soil properties	0 - 30 cm	30 - 70 cm	0 - 30 cm	30 - 70 cm
Soil class (DIN 4220)	Uls Sandy loamy silt	Sl4 Silty-loamy sand	Su4 High silty sand	Su4 High silty sand
sand [%]	30.9	57.9	49.0	50.1
silt [%]	59.0	28.8	47.9	42.1
clay [%]	10.1	13.2	3.2	7.8
Soil class (USDA)	Silt loam	Sandy loam	Sandy loam	Sandy loam
sand [%]	34.7	61.0	57.1	57.7
silt [%]	54.9	25.5	39.5	34.8
clay [%]	10.4	13.4	3.4	7.5
Total organic C [%]	1.64	0.25	2.07	0.66
Organic matter [%]*	2.83	0.43	3.57	1.14
pH [$CaCl_2$]	6.58	6.90	5.89	5.71
pH [H_2O]	7.22	7.67	6.41	6.43
CEC [mval Ba/100g dry weight]	11.7	8.6	12.6	8.8
MWHC [g/100g dry weight]	54.0	44.6	52.2	39.8
pF 2.0 [g/100g dry weight]**	20.7	20.7	18.7	15.2
pF 2.5 [g/100g dry weight]**	19.3	18.6	16.6	12.9
Dry bulk density [g/cm^3]***	1.20	-	1.46	-
Soil taxonomy	Luvisol		Gleyic Cambisol	

Table 7.1.2.2.1-8: Characteristics of the trial sites used to investigate the field dissipation of dimoxystrobin (BAS 505 F) (France)

Trial	**12/02244925-05**	
Location	**Heining-les-Bouzonville, France**	
Soil properties	0 - 30 cm	30 - 70 cm
Soil class (DIN 4220) sand [%] silt [%] clay [%]	Ut3 Medium clay silt 9.3 78.3 12.4	Ut4 high clay silt 5.9 75.1 19.0
Soil class (USDA) sand [%] silt [%] clay [%]	Silt loam 10.4 76.8 12.8	Loam 7.3 74.5 18.3
Total organic C [%]	0.81	0.25
Organic matter [%]*	1.40	0.43
pH [$CaCl_2$]	4.85	4.47
pH [H_2O]	5.53	5.24
CEC [mval Ba/100g dry weight]	12.8	14.8
MWHC [g/100g dry weight]	66.5	44.2
pF 2.0 [g/100g dry weight]**	30.6	25.8
pF 2.5 [g/100g dry weight]**	22.2	20.6
Dry bulk density [g/cm^3]***	1.38	-
Soil taxonomy	Cambisol	

* organic matter = organic carbon x 1.724
** water retention characteristics, soil moisture at 0.1 or 0.33 bar
*** specimens taken at approx. 10-20 cm depth (mean of 3 replicates)
CEC = cation exchange capacity
MWHC = maximum water holding capacity

The selected fields represented typical regions of agricultural practice with soils representative for growing sunflower (Spain and Italy) or oilseed rape (UK, Germany and France) that had been under cultivation for many years (except trial 12/02244925-03, which had been fallow grassland for 5 years). The sites were flat without any significant slope. Before commencement of the first sampling, the soil at each trial site was prepared as for sowing and was rolled if considered necessary, but then was left fallow.

No product containing the test item a.i. had been used on the test plots in the last three years.

3. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size: 30 m^2) and one treated plot (size: 288 m^2). The treated plot consisted of three equal sized subplots A, B and C that were assigned for replicates.

The product, formulated as an suspension concentrate (SC), was broadcast applied to bare soil in a single application at a nominal rate of 100 g a.s. ha^{-1} using a target water volume of 300 L ha^{-1} in trials 12/02244925-01 (Spain), 12/02244925-03 (UK) and 12/02244925-04 (Germany); 350 L ha^{-1} in trial 12/02244925-05 (France) and 400 L ha^{-1} in trial 12/02244925-02 (Italy). Applications were conducted between late May and late July 2012 using a calibrated boom sprayer. Treated plots were three-fold replicated with subplot sizes of 96 m^2. For each treated replicate, a separate spray mixture was prepared and the test item was applied to each subplot individually. Each spray mixture was visually checked for homogeneity and small aliquots of the spray mixture were taken before and after application of each individual subplot for later analysis.

The actual application rates determined by quantifying the amount of spray discharged ranged from 100.1 to 104.0 g a.s. ha^{-1} averaged over the three replicates of each treated plot with an average of 101.9 g a.s. ha^{-1}. In addition, the dose was verified by means of sampling Petri dishes filled with soil from the respective trial sites (approximately 50 g per dish, sieved to 2 mm). The petri dishes with an inner diameter of 10.8 cm were placed on the treated plot (ten in each subplot) before application. On completion of the application, the petri dishes were closed with a lid, sealed with adhesive tape and frozen on dry ice on-site directly after sampling (generally within 30 minutes) and then placed in freezer storage (≤ -18°C) upon returning to the Test Sites or Test Facility. Further details of application are presented in Table 7.1.2.2.1-9.

Table 7.1.2.2.1-9: Application parameters of field trial sites treated with BAS 540 01 F (SC)

Trial Country	Application Method	No. of application	Subplot (m²)	Application rate per application				Application date
				nominal [g a.s./ha]	actual* [g a.s./ha]	dose verification**		
						[g a.s/ha]	% of nominal	
12/02244925-01 Spain	broadcast spray to bare soil	1	A (96)	100	97.9	70.6	70.6	29-May-2012
			B (96)	100	101.0	92.8	92.8	
			C (96)	100	101.3	92.9	92.9	
			Average	**100**	**100.1**	**85.4**	**85.4**	
12/02244925-02 Italy	broadcast spray to bare soil	1	A (96)	100	104.7	103.6	103.6	31-May-2012
			B (96)	100	106.6	106.6	106.6	
			C (96)	100	100.8	99.6	99.6	
			Average	**100**	**104.0**	**103.3**	**103.3**	
12/02244925-03 UK	broadcast spray to bare soil	1	A (96)	100	106.6	108.7	108.7	20-July-2012
			B (96)	100	102.9	109.9	109.9	
			C (96)	100	97.2	118.1	118.1	
			Average	**100**	**102.2**	**112.2**	**112.2**	
12/02244925-04 Germany	broadcast spray to bare soil	1	A (96)	100	102.4	114.7	114.7	23-May-2012
			B (96)	100	100.7	105.5	105.5	
			C (96)	100	102.1	109.0	109.0	
			Average	**100**	**101.7**	**109.7**	**109.7**	
12/02244925-05 France (North)	broadcast spray to bare soil	1	A (96)	100	100.3	106.9	106.9	31-May-2012
			B (96)	100	103.2	106.0	106.0	
			C (96)	100	100.9	111.6	111.6	
			Average	**100**	**101.4**	**108.2**	**108.2**	

* determined by calculation of spray liquid applied
** determined by means of petri dishes filled with soil

Immediately after application of the test item and sampling of petri dishes, but before subsequent soil sampling, the control plot and the treated replicates were covered with a thin layer of sand to protect the applied product from surface processes like photolysis or volatilization, and to exclude any potential impact on the degradation of the test item caused by any of these processes. The application of sand was conducted manually or using a sand- or fertilizer spreader until complete coverage of the soil surface. Fine to coarse grained sand containing also fine grained material was used. The thickness of the sand layer necessary for complete coverage of the soil was approximately 4 mm (range: 3-7 mm). The layer of sand remained intact until at least 27 to 56 days at all trials. Within this time period of 27 to 35 days, the individual fields received a total precipitation (rain and irrigation) of 43.6 mm (Spain), 20.4 mm (Italy), 55.1 mm (UK), 72.4 mm (Germany) and 115.0 mm (France), respectively.

No tillage or fertilization was performed during the course of the study from first to last sampling and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate and pendimethalin (trial 12/02244925-04 only).

Rainfall was supplemented with irrigation at trials in Spain (308.3 mm), Italy (437.6 mm), UK (388.3 mm), Germany (302 mm) and France (65.2 mm). The trials in Italy, UK, Germany and France were irrigated to adjust the precipitation to historical values in case of dryer than normal conditions. The test plot in Spain was irrigated to compensate for the evaporation at the bare soil plots.

Actual weather data are based on records of appropriate weather stations located on-site. Monthly summary results on temperature, precipitation and irrigation are presented in Table 7.1.2.2.1-10 and Table 7.1.2.2.1-11.

Table 7.1.2.2.1-10: Summary of climatic conditions at field trial sites used to investigate the dissipation of dimoxystrobin (Spain, Italy, UK)

Trial	12/02244925-01			12/02244925-02			12/02244925-03		
Location	Zafarraya, Spain			Dugliolo di Budrio, Italy			Ardleigh, Colchester, UK		
Climatic conditions	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]
Month		∑	∑		∑	∑		∑	∑
May 12	18.9	0.0	-	21.0	0.0	-	-	-	-
Jun 12	21.1	0.0	43.6	23.6	6.2	-	-	-	-
Jul 12	21.6	0.0	73.9	25.9	0.2	28	16.7	13.8	-
Aug 12	22.8	0.0	59.6	26.3	1.0	43.6	17.7	41.4	23.8
Sep 12	17.1	77.4	22.4	19.9	134.8	33	14.0	38.0	12.8
Oct 12	13.3	141.0	-	14.7	79.8	3	10.4	77.4	10.0
Nov 12	9.4	256.8	-	10.0	80.6	33	7.1	70.2	-
Dec 12	6.6	25.2	-	1.5	24.2	-	5.0	93.2	-
Jan 13	6.2	112.2	-	2.7	110.0	-	3.6	32.2	-
Feb 13	5.4	75.2	-	2.5	93.4	-	2.8	32.8	-
Mar 13	8.5	244.6	-	7.2	110.0	-	2.8	39.4	-
Apr 13	11.1	50.0	-	13.4	58.4	26	7.7	21.2	-
May 13	13.4	32.2	-	16.6	68.2	-	10.7	34.2	18.0
Jun 13	17.4	0.0	49.3	21.4	45.4	21	14.0	18.4	33.0
Jul 13	20.6	0.0	44.2	24.9	8.0	28	18.1	24.6	44.6
Aug 13	21.2	176.6	-	24.0	45.0	9	18.1	48.2	13.3
Sept 13	17.4	47.8	-	20.2	31.0	145	14.0	35.6	10.0
Oct 13	14.8	13.2	-	15.2	109.6	29	12.6	80.2	-
Nov 13	7.6	4.6	-	9.5	64.6	39	6.5	27.8	22.5
Dec 13	5.2	96.0	-	3.5	10.8	-	6.3	7.4	-
Jan 14	6.8	108.2	-	5.6	94.6	-	6.2	25.8	-
Feb 14	7.1	95.2	-	7.7	84.2	-	6.8	5.0	-
Mar 14	8.1	18.4	-	10.3	54.6	-	8.1	3.2	46.9
Apr 14	13.3	7.0	-	14.4	52.2	-	10.8	0.4	47.2
May 14	15.1	0.0	15.3	16.3	16.6	-	12.5	0.2	56.6
Jun 14	18.1	0.0	-	-	-	-	15.2	11.8	49.6
Jul 14	-	-	-	-	-	-	14.6	0.0	-
Total	**Mean: 13.4**	**Sum: 1581.6**	**Sum: 308.3**	**Mean: 14.3**	**Sum: 1383.4**	**Sum: 437.6**	**Mean: 10.5**	**Sum: 782.4**	**Sum: 388.3**

Table 7.1.2.2.1-11: Summary of climatic conditions at field trial sites used to investigate the dissipation of dimoxystrobin (Germany, France)

Trial	12/02244925-04			12/02244925-05		
Location	Bakum, Germany			Heining-les-Bouzonville, France		
Climatic conditions	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]	T_{mean} Air [°C]	Prec. [mm]	Irrigation [mm]
Month		∑	∑		∑	∑
May 12	17.7	19.2	-	22.8	0.0	
Jun 12	14.7	87.2	-	15.7	104.4	
Jul 12	17.1	90.8	-	17.1	60.2	
Aug 12	18.5	51.4	20	19.3	34.4	15.2
Sep 12	13.4	40.2	10	14.2	48.6	13.0
Oct 12	9.4	76.6	-	10.1	49.0	10.1
Nov 12	6.0	37.0	-	5.9	53.8	-
Dec 12	3.4	86.6	-	3.4	93.0	-
Jan 13	1.1	56.0	-	0.9	43.6	-
Feb 13	0.8	42.0	-	-0.3	36.8	-
Mar 13	0.4	21.0	-	2.3	32.4	-
Apr 13	8.1	28.8	21	8.9	70.6	-
May 13	12.3	44.8	31	11.0	136.8	-
Jun 13	15.5	80.2	32	16.3	94.6	-
Jul 13	18.9	30.0	55	20.5	46.2	-
Aug 13	17.9	68.6	12	18.1	54.8	10.6
Sept 13	13.6	70.4	18	14.1	125.2	-
Oct 13	11.6	52.4	19	11.7	152.2	-
Nov 13	5.9	69.4	-	4.8	95.2	-
Dec 13	5.2	21.6	-	3.8	52.4	-
Jan 14	3.1	8.4	-	4.2	56.4	-
Feb 14	5.7	19.0	-	4.9	79.4	-
Mar 14	7.7	18.4	30	8.2	9.0	-
Apr 14	11.8	53.4	54	11.8	7.4	16.3
May 14	10.3	39.0	-	11.1	49.6	-
Total	**Mean: 10.0**	**Sum: 1212.4**	**Sum: 302**	**Mean: 10.2**	**Sum: 1586.0**	**Sum: 65.2**

Weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling)

Historical (long-term) weather data on precipitation and average air temperature from at least 9 years were taken from official weather stations located nearby (approximately 0.5-22 km distance to trials). The historical and actual data, each averaged over the complete duration of the individual trials, are presented in Table 7.1.2.2.1-12.

The actual air temperature recorded at the test fields during the study period was similar to the historic values. Whereas the test fields of the trials in Spain, UK and Germany received less rain during the study period compared to the historical values, rainfall was higher than the historic values in Italy and Northern France. Due to additional irrigation, the total water input at the test fields during the study was at least 91.6 % of the historical average rainfall.

Table 7.1.2.2.1-12: Summary of historical and actual weather data at field trial sites averaged over entire trial duration

Trial Country	T_{mean} Air [°C] (average over trial period)		Precipitation [mm] (sum over trial period)		Irrigation [mm]	Sum of actual precipitation and irrigation [mm]	% of historic precipitation
	Historic*	Actual	Historic*	Actual			
12/02244925-01 Spain	13.5	13.4	1684.5	1581.6	308.3	1889.9	112.2
12/02244925-02 Italy	13.5	14.3	1310.8	1383.4	437.6	1821.0	138.9
12/02244925-03 UK	10.2	10.5	1277.7	782.4	388.3	1170.7	91.6
12/02244925-04 Germany	8.8	10.0	1574.4	1212.4	302	1514.4	96.2
12/02244925-05 France (North)	9.8	10.4	1496.3	1586.0	65.2	1651.2	110.4

* at least over nine years

4. Sampling

Replicate soil specimens (8 per treated subplot and 10 or 15 per control plot) were taken at intervals up to 741 days and down to a maximum soil depth of 70 cm. At day 0, immediately after application and sand coverage, the treated plots were sampled down to 10 cm only. The detailed sampling intervals are presented in Table 7.1.2.2.1-13.

Table 7.1.2.2.1-13: Summary of sampling intervals at each field trial site

Trial	Country	Sampling intervals [days after application]
12/02244925-01	Spain	-4, 0, 8, 29, 34, 62, 90, 118, 261, 323, 359, 483, 652, 741
12/02244925-02	Italy	-3, 0, 7, 14, 27, 56, 85, 116, 175, 244*, 342, 382, 480, 602, 720
12/02244925-03	UK	-1, 0, 7, 14, 27, 61, 89, 122, 187, 249, 370, 490, 609, 712
12/02244925-04	Germany	-1, 0, 8, 13, 28, 64, 92, 120, 176, 266, 357, 470, 594, 715
12/02244925-05	France	-7, 0, 8, 35, 63, 89, 124, 230, 300, 378, 474, 594, 712

* First attempt of sampling was done on 244 DAA but had to be repeated on 342 DAA due to bad weather conditions.

Untreated specimens were collected from the control plot on three occasions, between one and seven days before application down to a depth of 70 cm, and after about one year and two years to a depth of 10 cm. The specimens were taken randomly from a separate sub-subplot of the untreated plot each time and pooled to one specimen. The 15 cores collected at the first sampling interval were taken using a common soil probe equipped with a plastic liner of 4.6 cm diameter. The 10 cores taken after about one and two years were collected with a metal tube of 11.0 cm diameter.

Treated soil specimens were taken randomly from eight points of each of the three treated subplots A – C and pooled according to subplot and depth. All soil specimens from 0-10 cm depth collected from the treated plots were taken separately using a metal tube of minimum 11.0 cm diameter which was pressed into the ground and collecting the soil with a spoon or similar device. Soil specimens deeper than 10 cm were collected through the center of the excavation hole contained by the metal tube, using a common soil corer fitted with a plastic liner of diameter 4.6 cm. Sampling of these cores was conducted in one run. After sampling, the remaining holes were filled with untreated soil from outside the plots or with sand.

In addition to the main sampling described above, a second complete sampling (double sampling) was carried out for back-up purposes. The retain specimens were stored in the freezers at the field Test Sites and shipped to the Test Facility at different dates than the main specimens or stored at the test site.

All soil specimens intended for residue analysis were stored at about -18°C within a maximum of 10 hours and 10 minutes after sampling and remained frozen until shipment to the Test Site of the analytical phase except for short term rises of temperature usually during loading and unloading operations, except for specimens of trial 12/02244925-05, which were stored at the Test Facility directly. Sample processing (segmentation and homogenization) was conducted in frozen state.

Shipment verification specimens were prepared to demonstrate stability of the residues in soil during storage and through any shipping processes. The samples were prepared at three occasions by fortification of soil with 0.05 mg/kg dimoxystrobin and were generally stored and shipped under the same conditions as the actual residue specimens. At the trials 12/02244924-01 (Spain), 12/02244924-02 (Italy) and 12/02244924-03 (UK), an additional shipping verification sampling was done (600 DAA) as on those trials one of the previous shipping verification specimens could not be used.

5. Analytical procedure

Field soil specimens, application verification specimens (Petri dish specimens) and shipping verification specimens were analyzed according to the validated BASF method L0189/01 [*CA 4.1.2/1, BASF DocID 2012/1287158*].

Field soil specimens were analyzed for dimoxystrobin and metabolites 505M98, 505M01, 505M08 and 505M09. The analytical method involved extraction of the soil with methanol/water (80/20, v/v) and final determination of the analytes by LC-MS/MS. Some deviations to method L0189/01 were made, yet without negative impact on the validity of the analysis as shown by the laboratory procedural recoveries. The limit of quantification (LOQ) was 0.002 mg kg^{-1} for each individual analyte. The limit of detection (LOD) was set at 0.0006 mg kg^{-1} (30% of LOQ). Analysis of field soil specimens originating from the treated plots was conducted down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ of 0.002 mg kg^{-1}). Analysis was performed up to a maximum of 741 days after application (DAA).

Petri dish specimens were analyzed for dimoxystrobin according to the following procedure: Approximately 50 g of the soil from the Petri dish were transferred into a 1000 mL glass bottle. The lid and base of the Petri dish were rinsed three times with methanol/ultra pure water (8/2, v/v) and the extracts analyzed by LC-MS/MS.

Shipping verification specimens were transferred into a graduated centrifugation tube and the weight recorded. The extraction solution methanol/ultra pure water (8/2, v/v) was added and the specimen was shaken for 30 minutes. After the mixture was centrifuged, the supernatant was decanted and the extraction repeated for two times. The extracts were unified, filled up to 200 mL and an aliquot was transferred into a new centrifugation tube. After centrifugation for 5 minutes, the extracts were diluted and subjected to LC-MS/MS.

Spray broth specimens were diluted to the appropriate concentration and analyzed for dimoxystrobin using HPLC-UV.

The validity of the analytical method was proven within the study by analysis of untreated control and at least two fortified specimens within each analytical specimen set.

6. Storage stability experiments

Storage stability of dimoxystrobin and metabolites 505M98, 505M01, 505M08 and 505M09 in frozen soil is investigated in a separate study [*CA 7.1.2.2.1/4, Meyer, M., BASF DocID 2014/1286541*] with soils originating from the individual trial sites of the present terrestrial field dissipation study.

7. Calculation of degradation times

No calculation of degradation times is provided in the study report. A detailed kinetic evaluation of the degradation behavior of dimoxystrobin and its metabolites 505M98, 505M01, 505M08 and 505M09 in the five European field soils is presented in a separate modeling report [*CA 7.1.2.2.1/3, Budde, E., BASF DocID 2015/1001161*].

II. RESULTS AND DISCUSSION

1. Spray broth concentration and application verification

Spray broth homogeneity was confirmed by visual check for all trials. In addition, spray mixtures sampled before and after application of each subplot were analyzed for dimoxystrobin. Analyzed concentrations averaged across the individual trial sites were in the range of 233 to 353 mg L^{-1} corresponding to 93-106% of the target concentration of the respective trials. The analytical results were not corrected for procedural recoveries and confirm the integrity of the test item used in the trials.

Procedural recovery experiments were conducted with untreated soil along with the analysis of the applied petri dish specimens from the field that served as application monitors. Procedural recoveries for dimoxystrobin ranged from 95.6% to 102.9% with an average across all trials of 101.0%.

Residue levels of dimoxystrobin achieved on extraction and analysis of the application monitors (Petri dishes filled with soil) were not corrected for procedural recoveries but directly converted into residue rates (in g ha^{-1}) taking into account the area of the Petri dishes (91.6 cm^2) used. As a result, the obtained rates averaged for the individual trials ranged from 85.4 to 112.2 g ha^{-1} representing 85.4–112.2% of the target application rate (see Table 7.1.2.2.1-9 for individual figures). The applied amount determined via the application monitors in these trials is in good agreement with the nominal value of 100 g ha^{-1} and the results from spray broth analysis.

2. Residues in field soil samples

Untreated soil specimens (control specimens) of the respective soil depths from each trial were analyzed for residues of dimoxystrobin and its metabolites 505M98, 505M01, 505M08 and 505M09. No residues above LOQ of any analyte were detected in any of the control specimens proving that there were no interferences of the untreated soil material with the analytical procedures used. Procedural recovery experiments performed with untreated field soil specimens spiked with a mix of the five analytes at concentration levels of 0.002, 0.01, 0.02, 0.1 and 0.2 mg kg^{-1} yielded overall mean recovery rates for the individual analytes between 91.7 and 97.6%, confirming the validity of the analytical method used in this study. Detailed results are summarized in Table 7.1.2.2.1-14.

Table 7.1.2.2.1-14: Method procedural recoveries

Analyte	Fortification level [mg/kg]	N	Mean [%]*	SD [%]	RSD[%]
BAS 505 F	0.002	62	96.8	5.4	5.6
	0.01	2	91.0	n.c.	n.c.
	0.02	52	97.6	6.3	6.5
	0.1	13	97.3	7.5	7.7
	0.2	21	98.9	3.2	3.2
	Overall	**150**	**97.3**	**5.7**	**5.9**
505M98	0.002	62	95.9	6.0	6.2
	0.01	2	97.1	n.c.	n.c.
	0.02	52	97.8	5.9	6.0
	0.1	13	96.7	7.0	7.3
	0.2	21	99.4	3.7	3.7
	Overall	**150**	**97.1**	**5.8**	**6.0**
505M01	0.002	62	98.4	7.6	7.7
	0.01	2	96.9	n.c.	n.c.
	0.02	52	97.0	8.5	8.7
	0.1	13	97.3	6.6	6.8
	0.2	21	96.7	4.5	4.7
	Overall	**150**	**97.6**	**7.9**	**8.1**
505M08	0.002	62	91.2	8.5	9.3
	0.01	2	87.7	n.c.	n.c.
	0.02	52	93.1	7.9	8.5
	0.1	13	94.8	6.1	6.5
	0.2	21	95.4	5.7	5.9
	Overall	**150**	**92.7**	**7.8**	**8.4**
505M09	0.002	62	92.3	11.2	12.2
	0.01	2	114.3	n.c.	n.c.
	0.02	52	90.1	11.0	12.2
	0.1	13	91.6	10.0	10.9
	0.2	21	92.2	9.3	10.1
	Overall	**150**	**91.7**	**11.0**	**12.0**

* mean values are across all soils and soil depths.
n.c. not calculated

These data prove that the analytical method applied was suitable to accurately determine residues of dimoxystrobin and its metabolites in soil down to a concentration of 0.002 mg kg^{-1} for each analyte.

Field soil specimens from the treated plots were analyzed down to a depth until at least two consecutive soil segments were free of quantifiable residues (< LOQ of 0.002 mg kg^{-1}, maximum depth of 40 cm). If specimens were analyzed in duplicate, the individual numbers were averaged to produce a mean for the respective soil specimen. When one of the values was below the LOQ, it was set to half of LOQ and an average was built as well. For all trials, the 0 DAA double specimens of the 0-10 cm soil layer were analyzed as well, in order to account for the importance of the day 0 value, and the final data were obtained by averaging the mean values of the respective main and retain specimens.

All residue values presented are related to the dry weight of the soil and were not corrected for procedural recoveries. Residue levels of the analytes in mg kg^{-1} dry soil (when above the LOQ) were converted to residue rates in g ha^{-1} taking into account the actual dry soil density of the field specimens, and were summed up for all depths between 0 and 40 cm analyzed. Results are presented in Table 7.1.2.2.1-15 to Table 7.1.2.2.1-18.

Table 7.1.2.2.1-15: Total residues of dimoxystrobin in soil converted to g ha^{-1} and summed up for all depths between 0 and 40 cm analyzed**

12/02244925-01 Zafarraya, Spain				12/02244925-02 Dugliolo di Budrio, Italy				12/02244925-03 Ardleigh, Colchester, UK			
	Subplot A	Subplot B	Subplot C		Subplot A	Subplot B	Subplot C		Subplot A	Subplot B	Subplot C
DAA	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	DAA	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	DAA	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]
0	60.4	92.9	82.5	0	94.2	90.8	95.5	0	109.3	86.7	102.9
8	64.5	88.8	101.9	7	81.3	84.3	81.5	7	59.4	63.7	65.1
29	7.9	26.8	21.7	14	58.2	68.0	74.8	14	60.2	110.3	76.0
34	10.6	20.7	35.2	27	51.5	58.5	40.5	27	51	70.2	62.7
62	13.9	17.4	23.6	56	44.0	44.3	47.5	61	50.1	31.9	49.0
90	15.1	20.5	32.3	85	32.4	30.7	29.6	89	31.8	37.5	32.8
118	13.5	40.9	20.2	116	32.7	36.6	24.1	122	48.2	45.1	34.1
261	15.7	28.2	23.8	175	28.8	28.9	28.8	187	50.2	26.9	46.2
323	16.0	18.1	15.0	342	24.7	23.5	24.3	249	23.2	32.3	39.0
359	10.7	20.6	21.6	382	19.5	20.6	20.9	370	14.4	19.9	14.5
483	8.3	14.2	6.9	480	15.9	16.9	19.1	490	10.8	10.9	9.3
652	6.3	5.9	8.0	602	13.9	20.6	21.3	609	8.3	9.8	9.7
741	0	0	0	720	11.1	9.9	12.3	712	5.4	5.7	5.5

12/02244925-04 Bakum, Germany				12/02244925-05 Heining-les-Bouzonville, France			
	Subplot A	Subplot B	Subplot C		Subplot A	Subplot B	Subplot C
DAA	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]	DAA	[g ha^{-1}]	[g ha^{-1}]	[g ha^{-1}]
0	98.6	89.9	111.7	0	91.7	136.7	103.8
8	97.3	99.2	83.9	8	61.2	65.7	84.2
13	80.1	91.0	74.3	35	42.6	30.1	36.4
28	68.8	83.1	87.3	63	31.8	28.4	31.2
64	71.4	65.0	73.6	89	21.8	26.4	23.8
92	61.3	58.8	51.8	124	15.0	21.9	18.6
120	43.6	54.2	57.6	230	14.2	9.7	14.6
176	53.8	58.4	44.2	300	10.5	9.7	14.6
266	46.7	43.6	39.1	378	8.4	8.4	7.5
357	40.7	39.1	42.9	474	5.9	4.7	8.2
470	22.9	27.7	34.9	594	3.0	3.2	2.8
594	25.5	26.3	27.6	712	3.0	0	2.0
715	24.4	21.9	17.8				

** calculations are based on actual dry soil density for individual soil layers; for residue values <2 µg/kg (<LOQ) no conversions to g/ha were made (reported as 0 = not calculated)

DAA days after application

Table 7.1.2.2.1-16: Total residues of 505M08 in soil specimens of trial 12/02244925-05 (France) converted to g ha^{-1} and summed up for all depths between 0 and 40 cm analyzed**

Sampling No.	**9**	**10**	**12**	**14**	**15**	**16**	**19**	**27**	**21**	**22**	**23**	**25**
DAA	**0**	**8**	**35**	**63**	**89**	**124**	**230**	**300**	**378**	**474**	**594**	**712**
	Subplot A: Residues of 505M08 [g/ha] – calculated**											
depth [cm] 0 - 10	0*	0	2.3	0	2.6	0	0	0	0	0	0	0
10 - 20	-	0	0	0	0	0	0	0	0	0	0	0
20 - 30	-	0	0	0	0	0	0	0	0	0	0	0
30 - 40	-	--	--	--	--	--	--	--	--	--	--	--
total residues in soil profile [g/ha]	**0**	**0**	**2.3**	**0**	**2.6**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Subplot B: Residues of 505M08 [g/ha] – calculated**											
depth [cm] 0 - 10	0*	0	0	0	0	0	0	0	0	0	0	0
10 - 20	-	0	0	0	0	0	0	0	0	0	0	0
20 - 30	-	0	0	0	0	0	0	0	0	0	0	0
30 - 40	-	--	--	--	--	--	--	--	--	--	--	--
total residues in soil profile [g/ha]	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Subplot C: Residues of 505M08 [g/ha] – calculated**											
depth [cm] 0 - 10	0*	0	0	0	0	0	0	0	0	0	0	0
10 - 20	-	0	0	0	0	0	0	0	0	0	0	0
20 - 30	-	0	0	0	0	0	0	0	0	0	0	0
30 - 40	-	--	--	--	--	--	--	--	--	--	--	--
total residues in soil profile [g/ha]	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

- no specimen taken
-- specimen not analyzed
* mean value of double determinations of each, mean and double specimens (in total from 4 values)
** calculations are based on actual dry soil density for individual soil layers; for residue values <2 µg/kg (<LOQ) no conversions to g/ha were made and values are reported as zero.
DAA days after application

Table 7.1.2.2.1-17: Total residues of 505M09 in soil specimens of trial 12/02244925-03 (UK) converted to g ha^{-1} and summed up for all depths between 0 and 40 cm analyzed**

Sampling No.	9	10	11	12	14	15	16	17	19	21	22	23	25
DAA	**0**	**7**	**14**	**27**	**61**	**89**	**122**	**187**	**249**	**370**	**490**	**609**	**712**
	Subplot A: Residues of 505M09 [g/ha] – calculated**												
depth [cm] 0 - 10	0	0	0	3.4	3.9	0	3.1	0	0	0	0	0	0
10 - 20	-	0	0	0	0	0	0	0	0	0	0	0	0
20 - 30	-	0	0	0	0	0	0	0	0	0	0	0	0
30 - 40	-	--	--	--	--	--	--	--	--	--	0	0	0
total residues in soil profile [g/ha]	**0**	**0**	**0**	**3.4**	**3.9**	**0**	**3.1**	**0**	**0**	**0**	**0**	**0**	**0**
	Subplot B: Residues of 505M09 [g/ha] – calculated**												
depth [cm] 0 - 10	0	0	2.9	3.6	2.3	0	0	0	0	0	0	0	0
10 - 20	-	0	0	0	0	0	0	0	0	0	0	0	0
20 - 30	-	0	0	0	0	0	0	0	0	0	0	0	0
30 - 40	-	--	--	--	--	--	--	--	--	--	0	0	0
total residues in soil profile [g/ha]	**0**	**0**	**2.9**	**3.6**	**2.3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Subplot C: Residues of 505M09 [g/ha] – calculated**												
depth [cm] 0 - 10	0	0	2.9	3.5	4.0	0	0	0	0	0	0	0	0
10 - 20	-	0	0	0	0	0	0	0	0	0	0	0	0
20 - 30	-	0	0	0	0	0	0	0	0	0	0	0	0
30 - 40	-	--	--	--	--	--	--	--	--	--	0	0	0
total residues in soil profile [g/ha]	**0**	**0**	**2.9**	**3.5**	**4.0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

- no specimen taken
-- specimen not analyzed
* mean value of double determinations of each, mean and double specimens (in total from 4 values)
** calculations are based on actual dry soil density for individual soil layers; for residue values <2 µg/kg (<LOQ) no conversions to g/ha were made and values are reported as zero.
DAA days after application

Table 7.1.2.2.1-18: Total residues of 505M09 in soil specimens of trial 12/02244925-05 (France) converted to g ha^{-1} and summed up for all depths between 0 and 40 cm analyzed**

Sampling No.	9	10	12	14	15	16	19	27	21	22	23	25
DAA	**0**	**8**	**35**	**63**	**89**	**124**	**230**	**300**	**378**	**474**	**594**	**712**
	Subplot A: Residues of 505M09 [g/ha] – calculated**											
depth [cm] 0 - 10	0*	0	3.8	3.5	3.4	2.5	2.7	0	0	0	0	0
10 - 20	-	0	0	0	0	0	0	0	0	0	0	0
20 - 30	-	0	0	0	0	0	0	0	0	0	0	0
30 - 40	-	--	--	--	--	--	--	--	--	--	--	--
total residues in soil profile [g/ha]	**0**	**0**	**3.8**	**3.5**	**3.4**	**2.5**	**2.7**	**0**	**0**	**0**	**0**	**0**
	Subplot B: Residues of 505M09 [g/ha] – calculated**											
depth [cm] 0 - 10	0*	0	0	4.3	3.3	2.8	0	0	0	0	0	0
10 - 20	-	0	0	0	0	0	0	0	0	0	0	0
20 - 30	-	0	0	0	0	0	0	0	0	0	0	0
30 - 40	-	--	--	--	--	--	--	--	--	--	--	--
total residues in soil profile [g/ha]	**0**	**0**	**0**	**4.3**	**3.3**	**2.8**	**0**	**0**	**0**	**0**	**0**	**0**
	Subplot C: Residues of 505M09 [g/ha] – calculated**											
depth [cm] 0 - 10	0*	0	3.1	0	3.5	0	0	0	0	0	0	0
10 - 20	-	0	0	0	0	0	0	0	0	0	0	0
20 - 30	-	0	0	0	0	0	0	0	0	0	0	0
30 - 40	-	--	--	--	--	--	--	--	--	--	--	--
total residues in soil profile [g/ha]	**0**	**0**	**3.1**	**0**	**3.5**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

- no specimen taken
-- specimen not analyzed
* mean value of double determinations of each, mean and double specimens (in total from 4 values)
** calculations are based on actual dry soil density for individual soil layers; for residue values <2 µg/kg (<LOQ) no conversions to g/ha were made and values are reported as zero.
DAA days after application

Dimoxystrobin degraded at all five European field sites. The total amount of dimoxystrobin residues detected in the soil profiles decreased from an average of 96.5 g ha^{-1} at day 0 to an average of 7.9 g ha^{-1} after 24 months. In one of the five field trials, trial 12/02244925-01 (Spain), no residues above the LOQ (0.002 mg kg^{-1}) were detected after 24 months.

Residues of dimoxystrobin were only measured in the top 0-10 cm soil layer, except for trial 12/02244925-01 (Spain) where residues could be detected also in the 10-20 cm layer. No residues above the LOQ were detected below 20 cm in any specimen. Altogether, it can be concluded that dimoxystrobin does not show any significant tendency to move into deeper soil layers indicating low potential for dimoxystrobin residues to leach to groundwater.

Metabolites 505M98 and 505M01 were not detected at levels above the LOQ in any specimen.

Metabolite 505M08 was sporadically detected in trace amounts close to the LOQ at one trial site (France), where it was detected in a total of two samples. In all further trials, no residues at levels above the LOQ were detected in any specimen. Metabolite 505M08 was exclusively found in the top 0-10 cm soil layer. No residues above the LOQ were observed in deeper soil layers in any specimen at any site.

Metabolite 505M09 was detected in significant amounts just at the trial sites in UK and France. It was detected from 14 DAA to 61 DAA, reaching maximum values of 4.0 g ha^{-1} at 61 DAA in trial 12/02244925-03 (UK), and from 35 DAA to 230 DAA, reaching maximum concentrations of 4.3 g ha^{-1} at 63 DAA in trial 12/02244925-05 (France). Thereafter, residues of 505M09 declined again and were no longer detected after 300 DAA at the latest. The metabolite was exclusively found in the top 0-10 cm soil layer. No residues above the LOQ were observed in deeper soil layers in any specimen at any site.

3. Shipment verification specimens

Shipping verification specimens spiked with dimoxystrobin were analyzed to check stability of the residues in soil during storage at the test site and through any shipping processes. Concentrations of dimoxystrobin were not corrected for procedural recoveries.

The analytical results demonstrated no significant losses from the shipping verification samples. The average amount of dimoxystrobin from the spiked field specimens was 97.6% across all trials. It was concluded that dimoxystrobin was stable in all soils under the storage and shipping conditions used.

4. Time of storage

The predominant part of the specimens was analyzed within 1 year. Very few individual specimens typically foreseen for re-analysis or originating from deeper soil layers were stored for a longer time period prior to analysis. The maximum period any soil specimen from the present field soil dissipation study was stored from the time of sampling to extraction was 614 days. Petri dish specimens, spray broth specimens as well as Shipment verification specimens were stored for up to six months after sampling before analysis.

III. CONCLUSION

Dimoxystrobin degraded well under field conditions in soil at all five European field sites. The total amount of dimoxystrobin residues detected in the soil profiles decreased from an average of 96.5 g ha^{-1} at day 0 to an average of 7.9 g ha^{-1} after 24 months. In one of the five field trials (trial 12/02244925-01 in Spain), no residues above the LOQ (0.002 mg kg^{-1}) were detected after 24 months. DT_{50} values will be calculated in a separate modeling report.

Dimoxystrobin residues were exclusively detected in the upper 20 cm of the soils. No residues above the LOQ were detected below 20 cm in any specimen. Altogether, it can be concluded that dimoxystrobin does not show any significant tendency to move into deeper soil layers indicating low potential for dimoxystrobin residues to leach to groundwater.

Metabolites 505M98, 505M01, 505M08 and 505M09 were also monitored during the study: No residues of 505M98 and 505M01 above the LOQ were detected in any specimen of any trial.

Residues of 505M08 were exclusively detected in trial 12/02244925-05 (France) in small amounts (max. 2.6 g ha^{-1}). Detects of 505M08 occurred only sporadically and no residues above LOQ were observed later than 89 days.

Metabolite 505M09 was detected in significant amounts only in trial sites UK and France. Residues of 505M09 were detected between 14 and 230 DAA reaching a maximum of 4.3 g ha^{-1}. Residues declined again and were no longer detected after 300 DAA at the latest.

Metabolites 505M08 and 505M09 were exclusively found in the top 0-10 cm soil layer. No residues of 505M08 and 505M09 above the LOQ were observed in deeper soil layers in any specimen at any site.

Report: CA 7.1.2.2.1/3
Studenroth S.,Budde E., 2015a
Kinetic evaluation of a field dissipation study with BAS 505 F - Dimoxystrobin conducted between 2012 and 2014 in Europe: Determination of trigger and modeling endpoints according to FOCUS
2015/1001161

Guidelines: FOCUS Degradation Kinetics (2006) SANCO/10058/2005 version 1.1 of December 2014, EFSA Guidance to obtain DegT50 values in soil (2014)

GLP: no

Executive Summary

The dissipation behavior of BAS 505 F – dimoxystrobin in soil has been investigated in a field dissipation study [CA 7.1.2.2.1/2, *BASF DocID 2014/1289336*] including five field trials. The purpose of this evaluation was to analyze the degradation kinetics of dimoxystrobin observed in the five soils according to the current guidance of the FOCUS workgroup on degradation kinetics, under consideration of the recommendations provided in the EFSA guidance to obtain $DegT_{50}$ values in soil for modeling purposes.

The field trials were situated in different regions of Europe (Spain, Italy, United Kingdom, Germany, and France), considering a range of different soils and climatic conditions. Surface processes were excluded by covering the plots with a thin layer of sand (3 to 7 mm) immediately after application of the test item.

The appropriate kinetic models to derive degradation endpoints were identified considering the procedures and kinetic models proposed by the FOCUS kinetics guidance. The best-fit kinetic model to derive non-normalized field dissipation endpoints as well as degradation endpoints valid for modeling was selected based on a visual and statistical assessment. Modeling endpoints were normalized to reference conditions.

The kinetic evaluation showed that the dissipation behavior of dimoxystrobin in all field trials was best described using the bi-phasic DFOP (double first order in parallel) model. The non-normalized field half-lives for dimoxystrobin ranged from 14.0 to 184.7 days, with corresponding DT_{90} values between 283.2 and >1000 days.

Normalized (pF2, 20°C) field half-lives ($DegT_{50}$, modeling endpoints) for dimoxystrobin ranged from 42.3 to 279.7 days.

I. MATERIAL AND METHODS

The dissipation of dimoxystrobin was investigated in five European soils [CA 7.1.2.2.1/2, *BASF DocID 2014/1289336*]. The test substance was applied as formulation (BAS 540 01 F) to bare soil using a calibrated boom sprayer at an intended application rate of 100 g a.s. ha^{-1}. Immediately after application, the control plot and treated replicates were covered with a thin layer of sand (3 to 7 mm) to protect the applied product from surface processes like photolysis or volatilization. Replicate soil samples were taken at 12-14 sampling times up to 741 days after application and down to a maximum soil depth of 70 cm.

Kinetic modeling strategy

Kinetic evaluation was performed in order to derive best-fit field dissipation half-lives as well as modeling endpoints. The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 (November 2011), 436 pp*].
The best-fit kinetic model for the non-normalized data was selected based on visual and statistical assessment under consideration of the FOCUS decision scheme for deriving trigger endpoints.
The degradation endpoints suitable for modeling were derived by a time-step normalization procedure, and appropriate $DegT_{50}$ values for use in environmental fate models were selected depending on the kinetic model.

Kinetic models included in the evaluations

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the best-fit model, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC), and the bi-exponential (DFOP) kinetics. The respective model descriptions and corresponding equations for calculating endpoints are shown in the FOCUS Kinetics guidance [*FOCUS (2006)*].

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling and software for kinetic evaluation

The experimental data were derived from the study reports and adjusted according to FOCUS [*FOCUS (2006)*].

As surface processes had been excluded by covering the soil with sand in the field study, all data points were considered in this evaluation regardless of the 10 mm rain criterion described by EFSA [*EFSA (2014): EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT$_{50}$ values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662*].

The measured data as well as resulting datasets (converted to g ha^{-1}) submitted to kinetic analysis are provided in the original evaluation reports.

The software package KinGUI version 2.2014.224.1704 was used for parameter fitting. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to 1x10^{-6} and 100, respectively.

Normalization to reference conditions

Evaluation of the suitability of field dissipation data for normalization was performed according to the evaluation criteria for normalization compiled by the Dutch regulatory authority (CTB criteria).

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily soil temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20°C using the Q_{10} approach as described in the report of the FOCUS soil modeling working group [*FOCUS (1997): Soil persistence models and EU registration. The final report of the work of the Soil Modeling Work group of FOCUS*]. The Q_{10} response function was applied for temperatures above 0°C and below field temperatures of 0°C it was assumed that no degradation occurs (Equation 7.1.2.2.1-2c). A Q_{10} value of 2.58 was included in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture at field capacity (pF 2) (Equation 7.1.2.2.1-2d).

Equation 7.1.2.2.1-2:Calculation of normalized day length based on combination of soil moisture and soil temperature correction factors

a) $$D_{norm} = D * f_{temp} * f_{moisture}$$

b) $$t_i = \sum_{t=1}^{i-1} D_{norm}$$

with:	t_i =	Time from application till sampling at day i [d]
	D_{norm} =	Normalized day length (20°C, pF2) [d]
	i =	Time span between application and sampling [d]

c) $$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact-Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

d) $$f_{moist} = \begin{cases} (\frac{\theta_{act}}{\theta_{ref}})^B & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:	D_{norm} =	normalized day length (temperature and moisture)
	f_{temp} =	temperature correction factor (-)
	f_{moist} =	moisture correction factor (-)
	D =	1 d (days)
	T_{act} =	actual soil temperature (°C)
	T_{ref} =	reference temperature (20 °C)
	Q_{10} =	factor of increase of degradation rate with an increase in temperature of 10°C (Q_{10} = 2.58) (-)
	θ_{act} =	actual soil moisture (vol. water content) (m^3 m^{-3})
	θ_{ref} =	reference soil moisture at pF2 (m^3 m^{-3})
	B =	exponent of the moisture response function, B = 0.7 (-)

The trial data were normalized by time-step correction (varying the ‘day length’ virtually by applying the correction factors to the time given in days). The corrected day lengths for the time-step normalization are shown in Table 7.1.2.2.1-19.

Table 7.1.2.2.1-19 Time-step normalized sampling days

12/02244925-01 – Spain		12/02244925-02 – Italy		12/02244925-03 – UK	
DAT	D_{norm}	DAT	D_{norm}	DAT	D_{norm}
0	0	0	0	0	0
8	6.7	7	7.8	7	6.5
29	26.3	14	15.5	14	12.8
34	32.1	27	34.4	27	23.5
62	60.1	56	78.6	61	48.7
90	93.5	85	129.4	89	60.8
121	116.7	116	165.3	122	72.5
261	168.9	175	202.1	187	87.5
323	190.5	244	216.7	249	100.0
359	208.1	342	254.8	370	160.4
483	311.7	382	288.8	490	234.2
652	375.1	480	419.6	609	267.9
741	419.5	602	473.7	712	318.4
		720	529.0		
12/02244925-04 – Germany		**12/02244925-05 – France**			
DAT	**D_{norm}**	**DAT**	**D_{norm}**		
0	0	0	0		
8	5.8	8	5.0		
13	7.9	35	24.2		
30	17.9	63	45.8		
64	42.9	89	69.6		
92	68.2	124	89.9		
120	86.5	230	118.8		
176	107.8	300	127.1		
266	123.1	378	159.7		
357	143.9	474	240.4		
470	226.3	594	281.3		
594	272.4	712	311.0		
715	309.3				

II. RESULTS AND DISCUSSION

The derived non-normalized best-fit field half-lives for dimoxystrobin are summarized in Table 7.1.2.2.1-20. The kinetic evaluation showed that the DFOP model is appropriate to derive trigger endpoints for additional work in all five soils.

Table 7.1.2.2.1-20 Best-fit field dissipation endpoints for dimoxystrobin

Field trial	Soil type (USDA)	Best-fit kinetic model	χ^2 error	DT_{50} [d]	DT_{90} [d]
12/02244925-01 (ES)	Loam	DFOP	29.7	17.9	650.6
12/02244925-02 (IT)	Sandy loam	DFOP	6.0	36.7	946.6
12/02244925-03 (UK)	Silt loam	DFOP	15.2	62.7	625.1
12/02244925-04 (DE)	Sandy loam	DFOP	4.5	184.7	> 1000
12/02244925-05 (FR)	Silt loam	DFOP	4.5	14.0	283.2

The degradation behavior of dimoxystrobin in the field trials was analyzed in a stepwise approach to derive modeling endpoints.
The field half-lives ($DegT_{50}$) adequate to be used in environmental fate modeling are summarized in Table 7.1.2.2.1-21. The visual fits are good for all trials; the χ^2 error is > 15% for two trials resulting from scattering of the measured values at early sampling times.

Table 7.1.2.2.1-21 Modeling endpoints for dimoxystrobin

Field trial	Soil type (USDA)	Kinetic model	χ^2 error	$DegT_{50}$ [d]
12/02244925-01 (ES)	Loam	FOMC	33.1	101.9 [a]
12/02244925-02 (IT)	Sandy loam	DFOP	6.3	279.7 [b]
12/02244925-03 (UK)	Silt loam	SFO	15.5	65.5
12/02244925-04 (DE)	Sandy loam	SFO	6.5	126.6
12/02244925-05 (FR)	Silt loam	DFOP	3.2	42.3 [a]

[a] $DegT_{50} = DegT_{90}/3.32$, as 10% of initial concentration were reached
[b] $DegT_{50} = \ln 2/k_2$

III. CONCLUSION

Field dissipation endpoints were derived for dimoxystrobin from one field dissipation study including five field trials.
The non-normalized best-fit field half-lives for dimoxystrobin ranged from 14.0 to 184.7 days, with corresponding DT_{90} values between 283.2 and >1000 days.
Normalized (pF2, 20°C) field half-lives ($DegT_{50}$, modeling endpoints) for dimoxystrobin ranged from 42.3 to 279.7 days.

Summary table field diss. kinetic data - trigger + modelling endpoints

Table 7.1.2.2.1-22: Summary of best-fit field half-lives of dimoxystrobin

Study	Trial	Location	pH ($CaCl_2$)	Org. C [%]	Best-fit DT_{50} / DT_{90} [d]	Kinetic model	χ^2 error level
2000/1000122 2013/1335916	ALO/03/98	Spain	7.5	0.7	1.7 / 526.7 [a]	DFOP	5.2
	ALO/04/98	Spain	7.6	0.9	2.7 / 794.1 [a]	DFOP	9.2
	HUS/09/98	Sweden	5.8	1.3	104.4 / >1000 [a]	DFOP	11.5
1999/11287 2013/1335916	D05/03/97	Germany	6.3	1.08	29.3 / >1000 [a]	DFOP	1.9
	DU3/04/97	Germany	5.3	0.63	63.0 / >1000 [a]	DFOP	4.8
	DU2/03/97	Germany	6.4	1.29	25.1 / >1000 [a]	DFOP	6.7
2014/1289336 2015/1001161	12/02244925-01	Spain	7.33	0.93	17.9 / 650.6 [b]	DFOP	29.7
	12/02244925-02	Italy	7.59	0.45	36.7 / 946.6 [b]	DFOP	6.0
	12/02244925-03	UK	6.58	1.64	62.7 / 625.1 [b]	DFOP	15.2
	12/02244925-04	Germany	5.89	2.07	184.7 / >1000 [b]	DFOP	4.5
	12/02244925-05	France	4.85	0.81	14.0 / 283.2 [b]	DFOP	4.5

[a] field soil study including surface loss processes; best-fit endpoints serve as trigger endpoints for additional work
[b] best-fit endpoints should not be used as triggers for additional work due to exclusion of surface loss processes [*EFSA, 2010*]

Table 7.1.2.2.1-23: Summary of normalized field half-lives of dimoxystrobin suitable for modeling

Study	Trial	Location	pH ($CaCl_2$)	Org. C [%]	$DegT_{50}$ (20°C, pF2) [d]	Kinetic model	χ^2 error level
2000/1000122 2013/1335916	ALO/03/98	Spain	7.5	0.7	- [a]	-	-
	ALO/04/98	Spain	7.6	0.9	- [a]	-	-
	HUS/09/98	Sweden	5.8	1.3	79.8	SFO	11.5
1999/11287 2013/1335916	D05/03/97	Germany	6.3	1.08	346.6 [b]	HS	1.9
	DU3/04/97	Germany	5.3	0.63	62.0	SFO	4.8
	DU2/03/97	Germany	6.4	1.29	158.9 [b]	DFOP	6.7
2014/1289336 2015/1001161	12/02244925-01	Spain	7.33	0.93	101.9 [c]	FOMC	33.1
	12/02244925-02	Italy	7.59	0.45	279.7 [b]	DFOP	6.3
	12/02244925-03	UK	6.58	1.64	65.5	SFO	15.5
	12/02244925-04	Germany	5.89	2.07	126.6	SFO	6.5
	12/02244925-05	France	4.85	0.81	42.3 [c]	DFOP	3.2
Geometric mean					**111.8**		

[a] not suitable for normalization
[b] calculated from slow rate as $DegT_{50} = \ln2/k_2$
c calculated as $DegT_{50} = DegT_{90}/3.32$, as 10% of initial concentration were reached

Report:	CA 7.1.2.2.1/4 Meyer M., 2015a Determination of the storage stability of Dimoxystrobin (BAS 505 F) and its four metabolites, namely 505M01, 505M08, 505M09 and 505M98 in soil 2014/1286541
Guidelines:	OECD-DOC ENV/MC/CHEM(98)17 Paris 1998, EEC 7032/VI/95 rev. 5, SANCO/3029/99 rev. 4 (11 July 2000), OECD 506 (Oct. 2007)
GLP:	yes (certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The storage stability of dimoxystrobin (BAS 505 F) and its four metabolites 505M01, 505M08, 505M09 and 505M98 in five soils was investigated under frozen conditions (approx. -18°C) over a period of 18 months. The soils were derived from five field dissipation trials of a parallel field study [*BASF DocID 2014/1289336*]. Trials were conducted in Spain, Italy, UK, Germany and France.

The soil samples were fortified at a concentration level of 0.1 mg kg^{-1} (10fold LOQ) dry soil, calculated as the single compound. Each soil aliquot was only spiked with a single test item (two replicates per soil type and analyzed time point). Soil samples without test item served as control and fortification samples and were stored as treated samples (4 samples per soil type and time point; 2 were used for the preparation of the freshly prepared fortified specimens). At different intervals (0, 1, 2, 3, 4, 8, 12 and 18 months) soil samples were analyzed using BASF method L0189/01. The limit of quantitation of the method is 0.01 mg kg^{-1} for each analyte. Procedural recoveries analyzed within each analytical series proved the validity of the analytical method and were used for recovery correction of stored samples.

The storage stability of dimoxystrobin and its metabolites 505M08, 505M09 and 505M98 was proved to be stable in all five tested soils over a period of at least 557 days at –18 °C. The metabolite 505M01 was only stable in the soils from trials in Spain and Italy for at least 557 days at –18 °C. In the other three soils, 505M01 was found to be not stable.

I. MATERIAL AND METHODS

A. MATERIALS

Test materials:	BAS 505 F	505M01	505M08	505M09	505M98
Batch No.:	01171-55	01893-8	01196-241	01196-245/ L80-82	1520-181
Purity:	99.7%	99.6%	97.8%	99.6%/ 96.3%	99.5%
CAS #:	149961-52-4	133409-73-1	-	-	149923-06-8

B. STUDY DESIGN

1. Experimental Conditions

Five soils from a related field soil dissipation study [CA 7.1.2.2.1/2, Gut T. – BASF DocID 2014/1289336] were used in the storage stability study for dimoxystrobin and its metabolites. The analytes were dosed at 0.1 mg kg-1 to soil samples (approximately 5 g) and then stored frozen for 0, 1, 2, 4, 8, 12 and 18 months. All samples were stored at ≤ -18 °C protected from light. Plastic bottles were used as storage containers. Additionally, soil samples without treatment were stored deep-frozen to serve as control and fortification samples at sampling.

2. Description of analytical procedures

For determination of dimoxystrobin and metabolites 505M01, 505M08, 505M09 and 505M98, BASF Method No. L0189/01 was used. Therefore, 5 g of soil is extracted with 50 mL of methanol/water (80/20, v/v) by mechanical shaking for 30 min at 225 rpm. An aliquot of 10 mL of the extract is centrifuged for 5 min at 4000 rpm (20 °C). The extract is taken directly or diluted with methanol/water (80/20, v/v) to the appropriate final volume and measured by HPLC-MS/MS. The limit of quantitation (LOQ) of the method was 0.01 mg kg-1 for each analyte.

The accuracy of the analytical method was proven by simultaneous analysis of two freshly prepared fortified specimens from each trial on each date of analysis. The fortification level was the same as for the storage stability specimens. The mean values of these recovery rates (Rmean) for dimoxystrobin and its metabolites were in the range of 81.6 to 100.5 % with relative standard deviations (RSD) ranging from 2.8 to 9.2 %.

II. RESULTS AND DISCUSSION

The storage stability of dimoxystrobin and its metabolites 505M01, 505M08, 505M09 and 505M98 in soil at ≤ -18 °C was determined by means of spiked specimens which were stored for and analysed at certain time periods. From the results, recovery rates were calculated which were corrected for the mean recovery rates obtained from the respective freshly fortified specimens (same sampling date and soil).

The recovery values in percent of dimoxystrobin and its metabolites 505M01, 505M08, 505M09 and 505M98 at the various dates of analysis (i.e. over the whole storage time period) normalised by the uncorrected mean recovery values of the freshly fortified specimens are shown in Table 7.1.2.2.1-24 to Table 7.1.2.2.1-28.

Table 7.1.2.2.1-24: Recovery values of dimoxystrobin and its metabolites 505M01, 505M08, 505M09 and 505M98 in soil samples of Trial 12/02244925-01 (Spain) normalised by the mean recovery values of the freshly fortified specimens

	Time [months]						
	0	1	2	4	8	12	18
BAS 505 F [%]	94 / 101	90 / 89	98 / 96	92 / 84	96 / 94	106 / 97	95 / 93
mean value [%]	97	90	97	88	95	101	94
505M01 [%]	93 / 98	85 / 90	90 / 91	91 / 85	83 / 91	100 / 96	92 / 89
mean value [%]	96	88	90	88	87	98	91
505M08 [%]	110 / 104	90 / 95	93 / 88	80 / 82	88 / 90	102 / 93	99 / 93
mean value [%]	107	92	91	81	89	98	96
505M09 [%]	101 / 103	101 / 91	98 / 103	96 / 86	94 / 104	86 / 93	99 / 97
mean value [%]	102	96	101	91	99	90	98
505M98 [%]	95 / 98	89 / 90	91 / 97	88 / 88	92 / 90	99 / 99	96 / 92
mean value [%]	96	90	94	88	91	99	94

Table 7.1.2.2.1-25: Recovery values of dimoxystrobin and its metabolites 505M01, 505M08, 505M09 and 505M98 in soil samples of Trial 12/02244925-02 (Italy) normalised by the mean recovery values of the freshly fortified specimens

	Time [months]						
	0	1	2	4	8	12	18
BAS 505 F [%]	98 / 97	89 / 93	97 / 95	94 / 95	97 / 96	94 / 98	97 / 103
mean value [%]	98	91	96	94	96	96	100
505M01 [%]	91 / 89	87 / 86	78 / 75	76 / 84	83 / 83	98 / 79	79 / 81
mean value [%]	90	86	77	80	83	88	80
505M08 [%]	103 / 102	93 / 96	92 / 88	98 / 96	82 / 84	87 / 93	91 / 84
mean value [%]	102	94	90	97	83	90	88
505M09 [%]	89 / 94	84 / 87	98 / 108	106 / 105	85 / 80	85 / 93	94 / 89
mean value [%]	92	85	103	106	83	89	92
505M98 [%]	93 / 93	95 / 94	92 / 94	91 / 99	84 / 89	94 / 86	88 / 93
mean value [%]	93	94	93	95	87	90	90

Table 7.1.2.2.1-26: Recovery values of dimoxystrobin and its metabolites 505M01, 505M08, 505M09 and 505M98 in soil samples of Trial 12/02244925-03 (UK) normalised by the mean recovery values of the freshly fortified specimens

	Time [months]								
	0	1	2	2 spare	3	4	8	12	18
BAS 505 F [%]	89 / 94	90 / 91	100 / 94	97 / 98	97 / 10	99 / 95	95 / 94	100 / 103	94 / 95
mean value [%]	92	90	97	98	100	97	95	102	94
505M01 [%]	96 / 92	86 / 81	59 / 53	61 / 71	73 / 68	64 / 68	63 / 72	76 / 78	68 / 64
mean value [%]	94	84	56	66	71	66	67	77	66
505M08 [%]	95 / 91	90 / 96	91 / 89	110 / 108	97 / 89	89 / 95	90 / 100	93 / 86	78 / 87
mean value [%]	93	93	90	109	93	92	95	89	82
505M09 [%]	86 / 86	97 / 83	77 / 91	91 / 86	93 / 87	103 / 103	89 / 78	88 / 83	91 / 84
mean value [%]	86	90	84	88	90	103	84	85	87
505M98 [%]	93 / 97	83 / 87	98 / 101	120 / 115	94 / 99	99 / 98	97 / 94	90 / 98	93 / 94
mean value [%]	95	85	99	118	97	99	95	94	94

Table 7.1.2.2.1-27: Recovery values of dimoxystrobin and its metabolites 505M01, 505M08, 505M09 and 505M98 in soil samples of Trial 12/02244925-04 (Germany) normalised by the mean recovery values of the freshly fortified specimens

	Time [months]								
	0	**1**	**2**	**2 spare**	**3**	**4**	**8**	**12**	**18**
BAS 505 F [%]	88 / 91	97 / 92	96 / 103	88 / 88	104 / 97	99 / 100	100 / 97	93 / 94	94 / 93
mean value [%]	90	95	99	88	100	100	99	94	93
505M01 [%]	86 / 86	55 / 49	29 / 28	47 / 47	43 / 40	59 / 55	40 / 51	38 / 40	28 / 37
mean value [%]	86	52	29	47	41	57	46	39	33
505M08 [%]	103 / 95	85 / 83	94 / 90	98 / 95	90 / 96	88 / 94	93 / 91	86 / 84	96 / 90
mean value [%]	99	84	92	97	93	91	92	85	93
505M09 [%]	93 / 91	88 / 86	99 / 99	97 / 90	98 / 98	107 / 100	83 / 81	98 / 86	90 / 89
mean value [%]	92	87	99	94	98	104	82	92	90
505M98 [%]	92 / 91	84 / 93	101 / 100	93 / 92	93 / 92	93 / 95	95 / 91	88 / 96	89 / 90
mean value [%]	91	88	101	93	93	94	93	92	89

Table 7.1.2.2.1-28: Recovery values of dimoxystrobin and its metabolites 505M01, 505M08, 505M09 and 505M98 in soil samples of Trial 12/02244925-05 (France) normalised by the mean recovery values of the freshly fortified specimens

	Time [months]								
	0	**1**	**2**	**2 spare**	**3**	**4**	**8**	**12**	**18**
BAS 505 F [%]	93 / 95	90 / 93	98 / 102	91 / 96	93 / 98	94 / 97	96 / 95	99 / 95	93 / 95
mean value [%]	94	92	100	93	96	95	95	97	94
505M01 [%]	101 / 95	49 / 46	27 / 27	27 / 30	36 / 45	32 / 41	37 / 50	33 / 31	31 / 37
mean value [%]	98	48	27	28	41	36	43	32	34
505M08 [%]	97 / 96	99 / 96	92 / 93	84 / 89	80 / 78	88 / 90	84 / 76	81 / 83	94 / 88
mean value [%]	97	97	92	87	79	89	80	82	91
505M09 [%]	92 / 91	93 / 98	89 / 95	78 / 74	84 / 77	84 / 86	83 / 80	76 / 78	89 / 87
mean value [%]	92	96	92	76	81	90	81	77	88
505M98 [%]	90 / 97	94 / 96	96 / 99	90 / 88	89 / 88	100 / 91	82 / 84	96 / 98	94 / 92
mean value [%]	93	95	98	89	89	95	83	97	93

The results of the storage stability study showed that the concentrations of dimoxystrobin (BAS 505 F) and its metabolites 505M08, 505M09 and 505M98 remained stable in the treated soil samples from the trials 12/02244925-01 (Spain), 12/02244925-02 (Italy), 12/02244925-03 (UK), 12/02244925-04 (Germany) and 12/02244925-05 (France) when stored at –18 °C over a period of at least 557 days. The corrected mean recovery values ranged from 76% to 118%.

The metabolite 505M01 was only stable in the soils from trial 12/02244925-01 (Spain) and 12/02244925-02 (Italy) for at least 557 days at –18 °C. The corrected mean recovery values ranged from 77% to 98%. In the soils from 12/02244925-03 (UK), 12/02244925-04 (Germany) and 12/02244925-05 (France), 505M01 was found to be not stable with corrected mean recovery values between 27% and 98%.

III. CONCLUSION

The results obtained from the storage stability study showed that dimoxystrobin and its metabolites 505M08, 505M09 and 505M98 were stable in the five tested soils over a period of at least 557 days at –18 °C. The metabolite 505M01 was only stable in the soils from trials in Spain and Italy for at least 557 days at –18 °C. In the other three soils, 505M01 was found to be not stable.

CA 7.1.2.2.2 Soil accumulation studies

Report: CA 7.1.2.2.2/1
Kellner O. et al., 2004a
Accumulation behaviour of BAS 505 F under field conditions
2004/1009165

Guidelines: BBA IV 4-1, SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995), IVA Leitlinie fuer Rueckstands-versuche Teil V (1993)

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

Executive Summary

The accumulation behavior of dimoxystrobin under field conditions was investigated over a four-year-period from 1998 to 2002. Two trials were conducted in Germany, one site in Nordrhein-Westfalen and the other in Rheinland-Pfalz.

The product BAS 505 01 F, formulated as SC, was broadcast applied on cereals at growth stages between 31 and 33 once a year, each on three identically treated plots with a plot size of 132 m² in the site Nordrhein-Westfalen and of 60 m² in the site in Rheinland-Pfalz. The nominal rate for each application was 200 g a.s. ha^{-1}. The actual application rates, determined by quantifying the amount of spray discharged, ranged from 192 to 211 g a.s. ha^{-1}.

Cultivation of the soils was not deeper than 30 cm. After harvest, the straw of the cereals was left on the fields and incorporated into the soils. The additionally applied pesticides did not belong to the chemical class of the strobilurines, in order to avoid microbial adaption and interferences with the analysis of dimoxystrobin and its metabolites. Climatic conditions were recorded by means of weather stations.

Soil cores were taken to a depth of 50 cm twice a year, once before application and once after harvest. The storage temperature of the samples was always less than -18°C. The soil cores were sectioned into segments of 0-10 cm, 10-25 cm and 25-50 cm either in frozen or unfrozen state and segments were pooled according to depth for the control plot and each treated replicate subplot separately.

Soil samples were analyzed for parent dimoxystrobin and metabolites 505M01 (BF 505-4), 505M08 (BF 505-7) and 505M09 (BF 505-8). No correction, neither for recoveries nor blanks, has been made, but all results were corrected for moisture content of soil. The validity of the analytical method was proven within the present study by analysis of fortified samples.

Metabolites of dimoxystrobin were generally (1 exception at the LOQ) not detected in any of the analyzed soil layers.

The highest dimoxystrobin concentrations (cumulated over all layers) in soil were found in trial D08/05/98 (Nordrhein-Westfalen) in the year 2000 with 0.036 mg kg^{-1} soil and in trial DU4/06/98 (Rheinland-Pfalz) in the year 2001 with 0.034 mg kg^{-1}. Nearly all residues could be detected in the 0-25 cm soil layers.

When considering that at growth stages 31 to 33 cereals are supposed to have an interception of 50% to the applied spray broth (FOCUS 2002), only about 100 g dimoxystrobin (active substance) will reach the soil per application. The measured soil concentrations of in spring and autumn were generally below that value. This means that after 5 years of annual application, the measured soil concentrations of dimoxystrobin are not higher than the residues immediately after application.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

Test item (formulation): BAS 505 01 F
Active substance : BAS 505 F
Type of formulation: SC (suspension concentrate)
Batch no.: 01171-55 (PCP04273), reanalysis: PCP05108, PCP06318
Content of active substance: 170 g L^{-1} (nominal)

2. Test sites

The accumulation behavior of BAS 505 F under field conditions was investigated at two field sites in Germany, one in Nordrhein-Westfalen and the other in Rheinland-Pfalz. The site characteristics including the basic soil parameters are presented in Table 7.1.2.2.2-1.

Table 7.1.2.2.2-1: Characteristics of the trial sites used to investigate the accumulation behavior of BAS 505 F

Trial	**D08/05/98**	**DU4/06/98**
Region	Nordrhein-Westfalen	Rheinland-Pfalz
Location	59510 Lippetal-Brockhausen	67227 Studernheim
Soil properties	unknown	0 - 25 cm
Soil texture (DIN 4220) sand [%] silt [%] clay [%]	Sandy loam / loam[A] 43 45 12	sandy loam[B] 56 26 18
Organic C [%] Organic matter [%]	1.6 2.8	1.0 1.7
pH [$CaCl_2$]	7.4	7.8
CEC [meq/100g dry weight]	15	13
MWHC [g/100g dry weight]	52	38

[A] according to USDA; silty loamy sand according to DIN 4220
[B] according to USDA; sandy loam according to DIN 4220
* organic matter = organic carbon x 1.724
CEC = cation exchange capacity; MWHC = maximum water holding capacity

On each field, a sequence of cereals was grown and application of BAS 505 F was performed on cereals in 1998, 1999, 2000, 2001 and 2002. The cereals grown as well as previous crops, fertilization and additional crop protection measures are described in Table 7.1.2.2.2-2. No product containing the active substance dimoxystrobin had been used on the test plots in the year prior to the start of the trial.

Table 7.1.2.2.2-2: Fertilizer, crops and pesticide use at trial sites in previous cropping seasons prior to start of trials

Trial/ Location	Year	Fertilizer	Crops grown	Pesticides applied
D08/05/98 Nordrhein-Westfalen	1995	not known	maize	
	1996	not known	barley	
	1997	not known	maize	Artett, Motivell
DU4/06/98 Rheinland-Pfalz	1995	not known	onions	Decis, Ridomil
	1996	not known	savoy cabbage	Decis, Metasystox, Euparen
	1997	not known	parsley	Afalon, Butisan, Stomp

B. STUDY DESIGN

Experimental setup and treatments

Each trial area comprised 4 plots, one untreated control plot (treatment 1) and three identically treated plots (treatments 2, 3 and 4). The whole area of plots 2, 3 and 4 were treated, but samples were only taken from core areas within the plots. Each plot was subdivided into 20 subplots. The subplot numbers corresponded to a sampling date, e.g. in all subplots with the number one, soil samples were taken before the first application. In each subplot, samples were taken only once. During the complete test period from 1998 to 2002 cereals were grown on the fields each year (see Table 7.1.2.2.2-4). Only soil samples were taken in the fields. No samples of cereals were collected.

From 1998 to 2002, dimoxystrobin was applied to the treated plots in the formulation BAS 505 01 F. This formulation is an SC formulation with a nominal content of dimoxystrobin of 170 g L^{-1}. The formulation batches were certified and the storage stabilities verified. The product was broadcast applied onto cereals once a year using a knapsack sprayer, operating with compressed air and an attached spray boom. The nominal rate for each application was 200 g dimoxystrobin ha^{-1}.

Applications were always made on cereals between growth stages 31 to 33. The actual application rates, determined by quantifying the amount of spray discharged, ranged from 192 to 211 g a.s./ha^{-1} (Table 7.1.2.2.2-3).

Table 7.1.2.2.2-3: Application parameters of the field trial sites in Germany, treated with BAS 505 F

Application number	Test item/ Nominal content/ Formulation type	Application date	DAFT**	No. of treated plots	Application method	Application rate per treatment	
						nominal [g a.s. ha^{-1}]	actual* [g a.s. ha^{-1}]
Trial D08/05/98, Nordrhein-Westfalen							
1	BAS 505 01 F 170 g a.s. L^{-1} /SC	20-May-98	0	3 (132 m² each)	broadcast spray onto cereals	200	202
2	BAS 505 01 F 170 g a.s. L^{-1} /SC	07-May-99	352				196
3	BAS 505 01 F 170 g a.s. L^{-1} /SC	08-May-00	719				205
4	BAS 505 01 F 170 g a.s. L^{-1} /SC	27-Apr-01	1073				198
5	BAS 505 01 F 170 g a.s. L^{-1} /SC	07-May-02	1448				201
Trial DU4/06/98, Rheinland-Pfalz							
1	BAS 505 01 F 170 g a.s. L^{-1}/SC	11-May-98	0	3 (60 m² each)	broadcast spray onto cereals	200	192
2	BAS 505 01 F 170 g a.s. L^{-1}/SC	07-Jun-99	392				211
3	BAS 505 01 F 170 g a.s. L^{-1}/SC	18-May-00	738				211
4	BAS 505 01 F 170 g a.s. L^{-1}/SC	12-Jun-01	1128				211
5	BAS 505 01 F 170 g a.s. L^{-1}/SC	17-May-02	1467				192

* determined by calculation of spray liquid applied
** DAFT = days after first treatment

The cultivation of the soils was not deeper than 30 cm. The straw of the cereals was left on the fields and incorporated into the soils. The additionally applied pesticides did not belong to the chemical class of the strobilurines, in order to avoid microbial adaption and interferences with the analysis of BAS 505 F and its metabolites. Information on additional pesticides and fertilizers applied during the study is presented in Table 7.1.2.2.2-4.

Table 7.1.2.2.2-4: Fertilizer, crops and pesticide use at trial sites during the dimoxystrobin soil accumulation study

Trial/ Location	Year	Fertilizer	Crops grown	Pesticides applied
D08/05/98 Nordrhein-Westfalen	1998	Phosphatkali, KAS	oat	Basagran DP,Cycocel, IPU, Stomp
	1999	AHL	winter wheat	Cycocel, Starane, IPU, Stomp
	2000	AHL	winter wheat	Cycocel, Stomp, IPU, Opus Top
	2001	AHL	winter barley	Cycocel,Primus
	2002	AHL, 40 er Kali	triticale	Cycocel,Primus, Moddus
DU4/06/98 Rheinland-Pfalz	1998	Nitro rot	winter wheat	Basaaran, Cvcocel, Gladio
	1999	Nitrophoska	spring wheat	Basagran, Starane, Gladio
	2000	Nitro rot	spring wheat	Basaaran Plus, CCC, Gladio
	2001	Nitro blau	spring wheat	Basagran, CCC, Gladio
	2002	Nitro blau	spring wheat	Basagran, CCC

Climatic conditions were recorded for trial D08/05/98 with a private weather station and for trial DU4/06/98 from the weather station Frankenthal Moersch. Monthly summary results on temperature and precipitation are presented in Table 7.1.2.2.2-5.

Table 7.1.2.2.2-5: Summary of climatic conditions at trial sites during the dimoxystrobin soil accumulation study

Year	1998		1999		2000		2001		2002	
Climatic conditions / Month	T_{mean} [°C]	PP [mm]	T_{mean} [°C]	PP [mm]	T_{mean} [°C]	PP [mm]	T_{mean} [°C]	PP [mm]	T_{mean} [°C]	PP [mm]
Trial D08/05/98, Nordrhein-Westfalen										
Jan	-	-	5.2	79.2	2.5	67.1	0.6	58.1	3.4	59.7
Feb	-	-	2.4	77.6	4.9	89.4	3.0	58.9	6.8	123.2
Mar	-	-	7.6	57.2	5.6	114.7	5.1	92.8	6.6	45.9
Apr	9.5	74.2	10.4	88.3	9.9	25.5	7.5	53.0	8.3	57.1
May	14.5	67.4	13.9	58.8	14.0	63.6	14.3	70.7	13.8	3.3
Jun	16.7	100.8	15.1	47.7	15.8	65.8	15.1	97.7	17.2	5.7
Jul	16.4	61.2	19.7	104.3	15.0	96.0	18.8	60.6	17.5	8.5
Aug	17.5	74.6	18.4	99.9	16.8	83.2	19.4	57.9	19.0	8.6
Sep	15.0	136.2	18.5	58.9	14.8	81.0	13.1	118.7	-	-
Oct	9.8	190.6	9.6	45.3	10.6	49.5	14.3	35.8	-	-
Nov	4.0	62.8	4.7	58.2	7.2	25.9	5.4	96.8	-	-
Dec	3.5	46.6	3.4	110.2	3.3	28.5	1.4	83.2	-	-
Trial DU4/06/98, Rheinland-Pfalz										
Jan	-	-	4.4	30.8	3.2	21.0	2.6	40.4	1.1	11.4
Feb	-	-	2.7	35.2	6.2	37.6	5.3	45.8	7.6	66.0
Mar	-	-	7.6	58.4	8.2	51.8	7.9	110.0	8.0	29.2
Apr	10.7	82.6	11.4	35.8	12.2	37.2	9.7	44.8	11.3	29.6
May	16.1	47.4	16.0	61.0	16.8	203.0	17.2	40.4	16.2	74.0
Jun	19.0	113.6	17.5	124.8	19.3	152.6	17.0	69.0	20.3	18.2
Jul	19.1	57.8	21.7	68.8	17.4	160.7	21.3	28.0	20.0	110.0
Aug	20.2	14.2	20.0	31.0	20.6	55.2	21.0	40.8	20.9	121.2
Sep	15.5	77.0	19.3	41.6	16.3	41.8	13.5	56.0	18.6	32.4
Oct	11.2	87.0	10.7	36.6	12.2	54.6	14.1	37.2	-	-
Nov	3.5	53.4	5.1	27.6	8.3	62.0	4.8	75.6	-	-
Dec	3.1	16.6	3.8	55.0	5.1	29.8	2.0	15.2	-	-

Tmean = Mean air temperature; PP = precipitation

4. Sampling, storage and processing

Soil samples were taken twice a year, once before application and once after harvest. At all samplings the sampling depth was 50 cm, in trial DU4/06/98 at sampling 9, treatment no. 4, only a depth of 25 cm was technically possible. Details are summarized in Table 7.1.2.2.2-6.

Soil specimens were taken with a Humax auger from 10 points (within a defined subplot) of each of the three treated plots as well as from the untreated plot. Soil samples were either directly shipped to the sample receipt laboratory at BASF Limburgerhof at the dates of sampling or deep frozen immediately before final transport to the sample receipt laboratory. The storage temperature was always less than -18°C. The soil cores were sectioned into segments of 0-10 cm, 10-25 cm and 25-50 cm either in frozen or unfrozen state and segments were pooled according to depth for the control plot and each treated plot separately.

The frozen soil segments were ground up together with dry ice and representative aliquots of the homogenized soil specimens were taken for residue analysis. All soil specimens remained frozen at temperatures around or below -18°C until final analysis.

Table 7.1.2.2.2-6: Sampling details for trial sites during the dimoxystrobin soil accumulation study

Sampling Number	DAFT	Sampling date	Sampled plots
Trial D08/05/98, Nordrhein-Westfalen			
1	-2	18-May-98	1, 2, 3, 4
2	89	17-Aug-98	1, 2, 3, 4
3	348	3-May-99	1, 2, 3, 4
4	455	18-Aug-99	1, 2, 3, 4
5	700	19-Apr-00	1, 2, 3, 4
6	810	7-Aug-00	1, 2, 3, 4
7	1072	26-Apr-01	1, 2, 3, 4
8	1156	19-Jul-01	1, 2, 3, 4
9	1448	7-May-02	1, 2, 3, 4
10	1562	29-Aug-02	1, 2, 3, 4
Trial DU4/06/98, Rheinland-Pfalz			
1	-4	7-May-98	1, 2, 3, 4
2	86	5-Aug-98	1, 2, 3, 4
3	385	31-May-99	1, 2, 3, 4
4	457	11-Aug-99	1, 2, 3, 4
5	710	20-Apr-00	1, 2, 3, 4
6	833	21-Aug-00	1, 2, 3, 4
7	1106	21-May-01	1, 2, 3, 4
8	1275	6-Nov-01	1, 2, 3, 4
9	1466	16-May-02	1, 2, 3, 4
10	1577	4-Sep-02	1, 2, 3, 4

DAFT = Days after first treatment

5. Description of analytical procedure

Field soil specimens were analyzed for parent dimoxystrobin and metabolites 505M01 (BF 505-4), 505M08 (BF 505-7) and 505M09 (BF 505-8) using BASF analytical method No. 427. The analytical method involved extraction with 80% aqueous methanol, concentration of the extract to the aqueous phase, pH adjustment to pH 1-2 with HCI and partition of the residues into ethyl acetate, followed by evaporation of the ethyl acetate extract to dryness and methylation with diazomethane. An additional silica gel column clean up step was necessary prior to final GC/MS analysis.

The limit of quantification (LOQ) was 0.01 mg kg^{-1} for each of the four analytes. No correction (neither for recoveries nor blanks) has been made, but all results were corrected for moisture content of soil. The validity of the analytical method was proven within the present study by analysis of fortified samples.

As the residue results had to be calculated for dry soil and moist soil samples were analyzed, the water content of the soil samples was calculated in %.

II. RESULTS AND DISCUSSION

A. PROCEDURAL RECOVERIES

Untreated field soil specimens of the respective soil depths from the control plots were analyzed for residues of dimoxystrobin and its metabolites 505M01, 505M08 and 505M09. No residues above the LOQ of any analyte were detected in any of the control samples proving that there were no interferences of the untreated soil material with the analytical procedures used. Procedural recovery experiments performed with untreated field soil specimens spiked with a mix of the four analytes at concentration levels of 0.01 and 1.0 mg kg^{-1} yielded overall mean recovery rates for the individual analytes between 95.1 and 101.6%, confirming the validity of the analytical method used in this study. The results are summarized in Table 7.1.2.2.2-7.

Table 7.1.2.2.2-7: Method procedural recoveries

Analyte	Fortification levels [mg kg^{-1}]	Average recovery [%] ± Standard deviation*	n
dimoxystrobin	--, 0.01,1.0	95.5 ± 8.3	52
505M01 (BF 505-4)		95.8 ± 24.7	
505M08 (BF 505-7)		101.6 ± 13. 7	
505M09 (BF 505-8)		95.1 ± 13.4	

* mean values are across all fortification levels and samplings

B. FINDINGS

Control samples from untreated plots were analyzed from sampling before application. They were free of residues. Additionally the samples of treated plots 2, 3 and 4 were analyzed before the first application. The data demonstrated that no interferences of the sample material with the analytical procedure occurred and that the control plots were free of residues of dimoxystrobin and its metabolites (see results of first sampling in Table 7.1.2.2.2-8 and Table 7.1.2.2.2-9).

Samples of the treated plots were analyzed for dimoxystrobin and its metabolites 505M01, 505M08 and 505M09. No corrections were made for recoveries or blanks, but all results were corrected for moisture content of soil. Samples from sampling 10 were not analyzed because the study was terminated due to the low residue concentrations of dimoxystrobin.

The analytical average results of dimoxystrobin for the treated plots 2, 3 and 4 are summarized in Table 7.1.2.2.2-8 and Table 7.1.2.2.2-9. Residue levels of dimoxystrobin in mg kg^{-1} dry soil were converted to residue rates in g ha^{-1} taking into account the standard soil density of 1.5 g cm^{-3}, and were summed up for all depths between 0 and 50 cm analyzed.

Table 7.1.2.2.2-8: Dimoxystrobin concentration (mg kg^{-1}) in soil of treated plots 2, 3 and 4 and cumulated values over total soil profile (g ha^{-1}), trial D08/05/98, Nordrhein-Westfalen

	Residues of dimoxystrobin [mg kg^{-1}]		
	Treated plot 2	**Treated plot 3**	**Treated plot 4**
Depth [cm]	**Sampling no. 1 (before treatment, 18.05.1998)**		
0 – 10	< 0.01	< 0.01	< 0.01
10 – 25	< 0.01	< 0.01	< 0.01
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**0.0**		
Depth [cm]	**Sampling no. 2 (17.08.1998)**		
0 – 10	0.0433	0.0179	0.0311
10 – 25	< 0.01	< 0.01	< 0.01
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**46.5**		
Depth [cm]	**Sampling no. 3 (03.05.1999)**		
0 – 10	< 0.01	< 0.01	< 0.01
10 – 25	< 0.01	< 0.01	< 0.01
25 – 50	0.0108	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**13.5**		
Depth [cm]	**Sampling no. 4 (18.08.1999)**		
0 – 10	0.0343	0.0377	0.0172
10 – 25	< 0.01	< 0.01	< 0.01
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**45.0**		
Depth [cm]	**Sampling no. 5 (19.04.2000)**		
0 – 10	< 0.01	< 0.01	< 0.01
10 – 25	0.012	< 0.01	< 0.01
25 – 50	< 0.01	0.0359	< 0.01
Mean cumulated [g ha^{-1}]**	**54.0**		

Table 7.1.2.2.2-8: Dimoxystrobin concentration (mg kg^{-1}) in soil of treated plots 2, 3 and 4 and cumulated values over total soil profile (g ha^{-1}), trial D08/05/98, Nordrhein-Westfalen

	Residues of dimoxystrobin [mg kg^{-1}]		
	Treated plot 2	**Treated plot 3**	**Treated plot 4**
Depth [cm]	**Sampling no. 6 (07.08.2000)**		
0 – 10	0.0350	0.0456	0.0432
10 – 25	< 0.01	< 0.01	< 0.01
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**61.5**		
Depth [cm]	**Sampling no. 7 (26.04.2001)**		
0 – 10	< 0.01	< 0.01	< 0.01
10 – 25	< 0.01	0.012	0.011
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**18.0**		
Depth [cm]	**Sampling no. 8 (19.07.2001)**		
0 – 10	0.026	0.025	0.029
10 – 25	0.012	0.010	0.011
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**64.5**		
Depth [cm]	**Sampling no. 9 (07.05.2002)**		
0 – 10	0.015	0.014	0.013
10 – 25	0.012	0.011	0.011
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**46.5**		

**calculated assuming standard soil density of 1.5 g cm^{-3} for individual soil layers

Table 7.1.2.2.2-9: Dimoxystrobin concentration (mg kg^{-1}) in soil of treated plots 2, 3 and 4 and cumulated values over total soil profile (g ha^{-1}), trial DU4/06/98, Rheinland-Pfalz

	Residues of dimoxystrobin [mg kg^{-1}]		
	Treated plot 2	**Treated plot 3**	**Treated plot 4**
Depth [cm]	**Sampling no. 1 (before treatment, 07.05.1998)**		
0 – 10	< 0.01	< 0.01	< 0.01
10 – 25	< 0.01	< 0.01	< 0.01
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**0.0**		
Depth [cm]	**Sampling no. 2 (05.08.1998)**		
0 – 10	0.0299	0.0538	0.0389
10 – 25	< 0.01	< 0.01	< 0.01
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**61.5**		

Table 7.1.2.2.2-9: Dimoxystrobin concentration (mg kg^{-1}) in soil of treated plots 2, 3 and 4 and cumulated values over total soil profile (g ha^{-1}), trial DU4/06/98, Rheinland-Pfalz

	Residues of dimoxystrobin [mg kg^{-1}]		
	Treated plot 2	**Treated plot 3**	**Treated plot 4**
Depth [cm]	**Sampling no. 3 (31.05.1999)**		
0 – 10	< 0.01	< 0.01	< 0.01
10 – 25	< 0.01	< 0.01	0.0149
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**10.5**		
Depth [cm]	**Sampling no. 4 (11.08.1999)**		
0 – 10	0.0875	0.101	0.104
10 – 25	0.0106	0.0113	0.0134
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**172.5**		
Depth [cm]	**Sampling no. 5 (20.04.2000)**		
0 – 10	< 0.01	< 0.01	< 0.01
10 – 25	0.0148	0.0126	0.0164
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**33.0**		
Depth [cm]	**Sampling no. 6 (21.08.2000)**		
0 – 10	0.0344	0.0473	0.0507
10 – 25	< 0.01	0.0245	< 0.01
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**84.0**		
Depth [cm]	**Sampling no. 7 (21.05.2001)**		
0 – 10	0.012	0.012	0.016
10 – 25	0.013	0.016	0.012
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**51.0**		
Depth [cm]	**Sampling no. 8 (06.11.2001)**		
0 – 10	0.013	0.016	0.012
10 – 25	0.015	0.015	0.015
25 – 50	< 0.01	< 0.01	< 0.01
Mean cumulated [g ha^{-1}]**	**54.0**		
Depth [cm]	**Sampling no. 9 (16.05.2002)**		
0 – 10	0.013	< 0.01	0.015
10 – 25	< 0.01	< 0.01	0.010
25 – 50	< 0.01	< 0.01	n.c.
Mean cumulated [g ha^{-1}]**	**21.0**		

**calculated assuming standard soil density of 1.5 g cm^{-3} for individual soil layers

Metabolites of dimoxystrobin were generally not detected in any of the analyzed soil layers. All analytical results were <0.01 mg kg^{-1} (below LOQ), with one exception of 0.0103 mg kg^{-1} of 505M09 (BF 505-8) at 0-10 cm (in one of the three replicates).

The highest concentrations at a spring sampling in soil cumulated over all layers were found in trial D08/05/98 with 0.036 mg kg^{-1} soil and in trial DU4/06/98 with 0.034 mg kg^{-1}. Nearly all residues could be detected in the 0-25 cm soil layers. Neither in the spring samples nor in the autumn samples was a significant increase of the dimoxystrobin soil concentration observed.

III. CONCLUSION

In two trials, dimoxystrobin was applied from 1998 to 2002 on cereals with an annual nominal application rate of 200 g active substance ha^{-1}. The growth stages according to BBCH code were 31 to 33. At these growth stages an interception (according to FOCUS) of 50% can be anticipated, i.e. only ca. 100 g dimoxystrobin reach the soil per application. The measured soil concentrations of dimoxystrobin were generally below that value of a single application. No accumulation in soil occurred over time.

CA 7.1.3 Adsorption and desorption in soil

CA 7.1.3.1 Adsorption and desorption

CA 7.1.3.1.1 Adsorption and desorption of the active substance

The adsorption values as listed in the dimoxystrobin EU dossier (Oct. 2001), the dimoxystrobin DAR (July 2003) and the EFSA Scientific Report (Aug. 2005) are shown in Table 7.1.3.1.1-1.

Table 7.1.3.1.1-1: Adsorption of dimoxystrobin on different soils

Soil	Soil type	OC [%]	pH ($CaCl_2$) [-]	K_f [mL g^{-1}]	K_{foc} [mL g^{-1}]	1/n [-]
Minto	loam	2.26	8.1	18.62	831.6	0.894
Fuquai Varina	sand	0.29	6.9	0.58	200.0	0.903
Red River	clay	1.86	6.8	17.38	935.3	0.942
Lufa 2.2.	loamy sand	2.3	5.8	7.11	309.2	0.923
Bruch West	sandy loam	1.8	7.5	3.52	195.8	0.902
Li 35b	loamy sand	0.9	6.5	3.31	368.2	0.925
Borstel	loamy sand	1.2	4.6	6.76	563	0.940

Although the already peer-reviewed adsorption/desorption study with dimoxystrobin is considered still valid, a new study with additional soils was performed in order to extend and broaden the data set for leaching assessment. The new study is described below. A summary table combining the old and new data set can be found at the end of chapter CA 7.1.3.1.1.

Report: CA 7.1.3.1.1/1
Sacchi R.R., 2014a
Adsorption/desorption behavior of BAS 505 F on European soils
2014/3017801
Guidelines: OECD 106 (2000), SOP-PA.1005, EPA 835.1230
GLP: yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

In laboratory experiments the adsorption/desorption behavior of dimoxystrobin was investigated on five European soils. The five tested soils covered a range of pH ($CaCl_2$) from 5.6 to 7.4, a range of organic carbon content from 0.60 to 1.85%, and five different USDA textural classes: silt loam, loam, loamy sand, sand and sandy loam.

For the determination of the adsorption isotherms, five different concentrations of test item (nominal 1.0, 0.5, 0.1, 0.05 and 0.01 µg mL^{-1}) in 0.01 M $CaCl_2$ solutions were used. The ratio of soil versus test solution was 1/5 and the adsorption equilibrium time was 48 hours on five soils. Desorption tests were performed in two steps. The concentrations of the test item in the adsorption solution and desorption solutions 1 and 2 were determined by liquid scintillation counting (LSC). The stability of ^{14}C-dimoxystrobin in the test systems was checked by radio-HPLC analysis of the sample solutions at different phases.

No relevant degradation was observed during 48 h of shaking. Mass balance of ^{14}C-dimoxystrobin for the test soils ranged from 93.3 to 95.9% of the total applied radioactivity (TAR).

The indirect method was applied to determine the adsorption coefficients. The Freundlich adsorption coefficients (K_F) ranged from 1.82 to 9.14 mL g^{-1}. The K_{FOC} values ranged from 303.6 mL g^{-1} to 593.9 mL g^{-1}, and the Freundlich exponent (1/n) ranged from 0.93 to 0.99.

I. MATERIAL AND METHODS

B. STUDY DESIGN

1. Test Material

BAS code: BAS 505 F (Dimoxystrobin)
Reg. No.: 285028
Chemical name (IUPAC): (E)-o-(2,5-dimethylphenoxymethyl)-2-methoxyimino-N-methylphenylacetamide
Molar mass: 326.39 g mol^{-1}(unlabelled)
Position of radiolabel: Benzyl-U-^{14}C
Specific radioactivity of a.i.: 7.41 MBq mg^{-1}
Batch No.: 596-3101
Radiochemical purity: 99.0%

2. Soils

The study was conducted with five different European soils. The properties of the soils are provided in Table 7.1.3.1.1-2.

Table 7.1.3.1.1-2: Characterization of soils used to determine the adsorption/desorption behavior of dimoxystrobin

Soil designation Origin	Nierswalder Wildacker (Germany)	Fiorentino Poggio Renatico 1 (Italy)	Li10 (Germany)	Lufa 2.1 (Germany)	Lufa 2.3 (Germany)
Textural class (USDA scheme)	Silt Loam	Loam	Loamy Sandy	Sand	Sandy Loam
Soil texture [%], (USDA) Clay Silt Sand	 8.8 73.5 17.7	 16.7 33.9 49.4	 4.3 12.2 83.5	 2.3 6.9 90.8	 8.3 23.1 68.6
Organic carbon [%] (ISO 10694)	1.85	1.00	0.95	0.60	0.99
Cation exchange capacity [$cmol^+$ kg^{-1}]	3.1	11.8	5.5	-0.7	7.5
pH ($CaCl_2$) pH (water)	5.7 6.5	7.4 8.2	6.2 6.9	5.6 6.5	6.7 7.4
Max. water holding capacity [g/100g dry soil]	36.1	29.7	23.2	23.1	28.2
Bulk density [g L^{-1}]	1236	1403	1384	1381	1226

B. STUDY DESIGN

1. Test conditions

Preliminary tests: adsorption kinetics and soil/solution ratio

The adsorption of the test item to Teflon, glass and polypropylene centrifuge tubes was investigated in tubes with no soil present after an agitation period of 24 hours. Measurements of total radioactivity remaining in the aqueous solution were determined and revealed a significant adsorption of the test item to both glass and polypropylene centrifuge tubes (mean recoveries of 88% and 80%, respectively), but no adsorption to Teflon tubes (mean recovery of 100%). Therefore, Teflon tubes were used throughout further experiments.

To find the optimal soil/solution ratio for the main test, a preliminary experiment was run for 24 h with two soils (Nierswalder Wildacker and Lufa 2.1) showing the highest and lowest organic carbon value of the chosen test soils (0.60% to 1.85%). Three different soil/solution ratios were tested: 1/1, 1/5 and 1/10 using a concentration of 1.0 µg mL^{-1} dimoxystrobin in 0.01 M $CaCl_2$. The ratio 1/5 was chosen, because it provided adsorption between 30 and 60%, enabling enough radioactivity in both phases (soil and solution) for best accurate measures.

A preliminary experiment was run with all five soils to determine the time needed to establish equilibrium conditions. The equilibrium time experiments were performed at a soil/solution ratio of 1/5 and at a test substance concentration of 1.0 µg mL^{-1} in 0.01 M $CaCl_2$. The test was performed in centrifuge tubes containing 1 g of soil and 5 mL application solution. The tubes were protected from light and shaken at 250 rpm at 20 ± 2°C for 4, 8, 24, 32, and 48 hours. The test showed an increase of adsorption until 32 hours for all five soils. After 32 hours, no significant further increase occurred. Nevertheless, the longest shaking time of 48 hours was chosen for conducting the isotherm test.

For testing the stability of the test item in the five soils, the soil residue of the highest test concentration after the adsorption and two desorption steps was further extracted with methanol and methanol/water. The $CaCl_2$ solutions as well as soil extracts were analyzed by LSC and radio-HPLC. The results proved that the radioactivity could be completely recovered (material balance 93 - 96% TAR) and that the test item was stable in $CaCl_2$ as well as in soil (extracts).

Determination of Freundlich adsorption and desorption isotherms

The adsorption isotherm determination was performed with five concentration levels (1.0, 0.5, 0.1, 0.05 and 0.01 µg mL^{-1}) in five soils. The soil/solution ratio was 1/5. For all samples 1 g of soil and 5 mL solution of each concentration level were added to the test centrifuge tubes. Each experiment (one soil and one solution) was done in duplicate. All samples were shaken at 250 rpm on a horizontal shaker at 20 ± 2°C for 48 hours. The soil/solution suspension was then centrifuged and aliquots of the supernatants were assayed by LSC to determine the % TAR. Aliquots from the highest dose sample supernatants were analyzed directly by radio-HPLC to determine the nature of the radioactivity.

The desorption step were performed by replacing the $CaCl_2$ supernatant from the adsorption test with an equal volume of $CaCl_2$ solution without test item. The new mixture was shaken again 48 hours at 250 rpm. After centrifugation, aliquots of the supernatants were assayed by LSC to estimate the radioactivity present in the desorption solutions. Aliquots of the highest dose samples were furthermore analyzed by radio-HPLC to determine the nature of the radioactivity. The second desorption step was performed in analogous manner.

2. Analytical procedure

Soil samples of the 1.0 µg mL^{-1} concentration level (preliminary test and isotherm test) were extracted by shaking the soils with 15 mL of methanol on a mechanical shaker for 30 minutes. After shaking, the samples were centrifuged. This procedure was repeated twice with 15 mL methanol / water (50/50; v/v) solution. The extracts were combined in a 50 mL volumetric flask and the volume was completed. Aliquots of the extracts were assayed by LSC and radio-HPLC. The mass balance was calculated as sum of radioactivity in adsorption and desorption solutions and soil extracts.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

A mass balance determination was carried out for samples of the highest concentration level (nominal 1.0 µg mL^{-1}). Good mass balances were obtained. Mean recovery values (2 replicates) ranged from 93.3 to 95.9% of the total applied radioactivity (TAR).

B. FINDINGS

Adsorption Equilibrium Test

The adsorption equilibrium test carried out with ^{14}C-dimoxystrobin at a soil/solution ratio 1:5 showed an increase of adsorption over 32 hours for all five soils tested. No large difference was observed between 32 and 48 hours. Nevertheless, 48 hours was chosen for conduction of the main isotherm test. Adsorption values for the five soils were in a range of 35.2% for Lufa 2.1 soil to 67.0% for Nierswalder Wildacker soil after 48 h shaking. No degradation was observed on samples after 48 h of shaking.

Freundlich Adsorption Isotherm Determination

The indirect method was applied for determination of the adsorption coefficients.

The Freundlich adsorption coefficients K_F ranged from 1.82 mL g^{-1} (Lufa 2.1 soil) to 9.14 mL g^{-1} (Nierswalder Wildacker soil). The K_{FOC} values ranged from 303.6 mL g^{-1} (Lufa 2.1 soil) to 593.9 mL g^{-1} (Fiorentino Poggio Renatico 1 soil) and 1/n values ranged from 0.93 (Lufa 2.1 soil) to 0.99 (Nierswalder Wildacker soil). The values obtained are summarized in Table 7.1.3.1.1-3.

Table 7.1.3.1.1-3 Adsorption of dimoxystrobin based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	Org. C [%]	pH $CaCl_2$ (pH water)	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Nierswalder Wildacker	Silt Loam	1.85	5.7 (6.5)	9.14	0.99	493.8
Fiorentino Poggio Renatico 1	Loam	1.00	7.4 (8.2)	5.94	0.95	593.9
Li10	Loamy Sandy	0.95	6.2 (6.9)	3.33	0.94	350.6
LUFA 2.1	Sand	0.60	5.6 (6.5)	1.82	0.93	303.6
LUFA 2.3	Sandy Loam	0.99	6.7 (7.4)	3.45	0.94	348.1

Freundlich Desorption Isotherm Determination

The desorption values are summarized in Table 7.1.3.1.1-4.

Table 7.1.3.1.1-4: Desorption of dimoxystrobin based on Freundlich isotherms in five soils

Soil	Desorption 1			Desorption 2		
	K_F [mL/g]	1/n	K_{FOC} [mL/g]	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Nierswalder Wildacker	13.21	0.99	713.8	15.99	0.96	864.4
Fiorentino Poggio Renatico 1	8.38	0.95	837.7	12.97	0.94	1297.3
Li10	4.72	0.95	497.0	7.51	0.96	790.4
LUFA 2.1	3.28	1.00	546.3	6.78	1.06	1129.9
LUFA 2.3	5.47	0.94	552.7	9.36	0.95	945.0

III. CONCLUSION

The adsorption and desorption behavior of ^{14}C-dimoxystrobin was determined on five different European soils, which covered a range of pH from 5.6 to 7.4, a range of organic carbon content from 0.6% to 1.85% and five different USDA textural classes. The Freundlich adsorption coefficients K_F covered a range from 1.82 to 9.14 mL g^{-1} for the five soils. The K_{FOC} values ranged from 303.6 to 593.9 mL g^{-1}.

Adsorption summary for active substance dimoxystrobin

The combined adsorption data set (old and new data) for leaching assessment of dimoxystrobin is summarized in Table 7.1.3.1.1-5.

Table 7.1.3.1.1-5: Summary of adsorption values of dimoxystrobin on different soils

Soil	Soil type (USDA)	OC [%]	pH $CaCl_2$ (pH water) [-]	K_f [mL g^{-1}]	K_{foc} [mL g^{-1}]	1/n [-]
Minto	loam	2.26	8.1 [b]	18.62	831.6	0.894
Fuquai Varina	sand	0.29	6.9 [b]	0.58	200.0 [a]	0.903 [a]
Red River	clay	1.86	6.8 [b]	17.38	935.3	0.942
Lufa 2.2.	loamy sand	2.3	5.8 (6.4 [c])	7.11	309.2	0.923
Bruch West	sandy loam	1.8	7.5 (8.0 [c])	3.52	195.8	0.902
Li 35b	loamy sand	0.9	6.5 (7.0 [c])	3.31	368.2	0.925
Borstel	loamy sand	1.2	4.6 (5.6)	6.76	563	0.940
Nierswalde	Silt Loam	1.85	5.7 (6.5)	9.14	493.8	0.99
Fiorentino	loam	1.00	7.4 (8.2)	5.94	593.9	0.95
Li 10	loamy sand	0.95	6.2 (6.9)	3.33	350.6	0.94
Lufa 2.1	sand	0.60	5.6 (6.5)	1.82	303.6	0.93
Lufa 2.3	sandy loam	0.99	6.7 (7.4)	3.45	348.1	0.94
				Geometric mean	**435.2**	
				Arithmetic mean		**0.93**

[a] Not considered for mean, as organic carbon content does not fulfil guideline requirements.
[b] if determined in H_2O or in $CaCl_2$ not stated in report
[c] estimated as proposed by *FOCUS (2000, 2014)*: pH-water=0.953*pH-CaCl2+0.85

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

The adsorption/desorption data of the dimoxystrobin metabolites 505M08 (BF 505-7), 505M09 (BF 505-8) and 505M01 (BF 505-4) were already evaluated during the previous Annex I listing process.

After the previous dossier submission, a report amendment was written for the adsorption/desorption study of metabolite 505M08 (BF 505-7). This report amendment was not submitted or peer-reviewed yet. It does not contain any changes in results and is listed here only for sake of completeness.

Report:	CA 7.1.3.1.2/1 Seher A., 1999b Soil adsorption/desorption study of 354562 (BF 505-7) on soils 1999/11089
Guidelines:	OECD 106, EPA 163-1
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	CA 7.1.3.1.2/2 Zirnstein M., 2002b Report amendment No. 1 to final report: Soil adsorption / desorption study of 354 562 (BF 505-7) on soils 2002/1016728
Guidelines:	OECD 106, EPA 163-1
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

The report amendment of this study only contains a correction of the chemical name of the test substance 505M08 (BF 505-7), since in the original report it was erroneously stated the wrong position of ring constituents.

Original version:	(E)-o-[(5-Hydroxycarbonyl-2-methyl)phenoxymethyl]-2-methoxyimino-N-methylphenylacetamide-[phenyl-U-14C]
Corrected version:	(E)-o-[(**2**-Hydroxycarbonyl-**5**-methyl)phenoxymethyl]-2-methoxyimino-N-methylphenylacetamide-[phenyl-U-14C]

Overall, the adsorption/desorption studies performed with dimoxystrobin metabolites 505M08, 505M09 and 505M01 are considered still valid. The adsorption values as given in the Draft Assessment Report (2003) are presented in Table 7.1.3.1.2-1 - Table 7.1.3.1.2-3.

Table 7.1.3.1.2-1: Adsorption of 505M08 (BF 505-7) on different soils as listed in the DAR (2003)

Soil	Soil type	OC [%]	pH ($CaCl_2$) [-]	K_f [-]	1/n	K_{foc} [mL g^{-1}]
Lufa 2.2	sand /loamy sand	2.5	5.8	0.4992	0.9458	20.0
Bruch West	loamy sand	1.5	7.5	0.0570	1.2249	3.8
Li 35 b	loamy sand	1.1	6.5	0.0860	0.9857	7.8
Borstel	loamy sand	1.4	5.8	0.3503	0.9305	25.0
USA 538-30-5	loamy sand	0.4	5.8	0.1075	0.9463	26.9
USA 538-31-2	silty loamy sand	0.5	5.2	0.6652	0.9509	133.0
Canada	sandy loam	3.4	7.5	0.6888	0.9130	20.3

Table 7.1.3.1.2-2: Adsorption of 505M09 (BF 505-8) on different soils as listed in the DAR (2003)

Soil	Soil type	OC [%]	pH [-]	K_f [-]	1/n	K_{foc} [mL g^{-1}]
Lufa 2.2	sand /loamy sand	2.5	5.8	1.111	0.915	44.4
Bruch West	loamy sand	1.5	7.5	0.135	0.808	9.0
Li 35 b	loamy sand	1.1	6.5	0.142	0.812	12.9
Borstel	loamy sand	1.4	5.8	0.647	0.880	46.2
USA 538-30-5	loamy sand	0.4	5.8	0.154	0.824	38.6
USA 538-31-2	silty loamy sand	0.5	5.2	0.595	0.892	119.0
Canada	sandy loam	3.4	7.5	1.771	0.873	52.1

Table 7.1.3.1.2-3: Adsorption of 505M01 (BF 505-4) on different soils as listed in the DAR (2003)

Soil	Soil type	OC [%]	pH [-]	K_f [-]	1/n	K_{foc} [mL g^{-1}]
Lufa 2.2	sand /loamy sand	2.5	5.8	0.097	0.921	3.9
Bruch West	loamy sand	1.5	7.5	0.031	0.911	2.0
Li 35 b	loamy sand	1.1	6.5	0.034	0.933	3.1
Borstel	loamy sand	1.4	5.8	0.157	0.814	11.2
USA 538-30-5	loamy sand	0.4	5.8	0.023	0.878	5.8
USA 538-31-2	silty loamy sand	0.5	5.2	0.151	0.928	30.2
Canada	sandy loam	3.4	7.5	1.208	0.736	35.5

Since the data indicate some mobility in soil, additional new soil adsorption data were produced for all three soil metabolites in order to produce a broader data basis for groundwater leaching assessment. Tables showing the combined data sets of adsorption values for each metabolite (old and new) are shown at the end of this chapter.

Report: CA 7.1.3.1.2/3
Sacchi R.R., 2014c
Amended final report - Adsorption/desorption behavior of 505M08 (Reg.No. 354562) on European soils
2014/3017802
Guidelines: OECD 106 (2000), SOP-PA.1005, EPA 835.1230, POP-PA.1005
GLP: yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

In laboratory experiments the adsorption/desorption behavior of the dimoxystrobin metabolite 505M08 (Reg.No. 354562) was investigated on five European soils. The five tested soils covered a range of pH ($CaCl_2$) from 5.6 to 7.4, a range of organic carbon content from 0.60 to 1.85%, and five different USDA textural classes: silt loam, loam, loamy sand, sand and sandy loam.

For the determination of the adsorption isotherms, five different concentrations of test item (nominal 1.0, 0.5, 0.1, 0.05 and 0.01 µg mL^{-1}) in 0.01 M $CaCl_2$ solutions were used. The soil/solution ratio was 1/1 and the tests were performed at a adsorption equilibrium time of 24 hours. For one test soil (Nierswalder Wildacker), two desorption steps were performed. The concentrations of the test item in the adsorption and desorption solutions as well as in soils extracts were determined by liquid scintillation counting. The stability of ^{14}C-505M08 in the test system was checked by radio-HPLC analysis.

Mass balance (mean recovery values) of ^{14}C-505M08 for the test soil ranged from 95.6% (Fiorentino Poggio Renatico 1 soil) to 99.2% (Lufa 2.1 soil).

The direct method was applied to determine the adsorption coefficients for Fiorentino Poggio Renatico 1, Li 10, Lufa 2.1 and Lufa 2.3 soils and the indirect method for Nierswalder Wildacker soil. The Freundlich adsorption coefficients K_F ranged from 0.06 to 0.75 mL g^{-1}. The K_{FOC} values ranged from 6.03 to 40.56 mL g^{-1} and the Freundlich exponent (1/n) values ranged from 0.96 to 1.0.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item: ^{14}C-505M08
Reg. No.: 354562
Chemical name (IUPAC): (E)-o-[(2-hydroxycarbonyl-5-methyl)phenoxymethyl]-2-methoxyimino-N-methylphenylacetamide
Molar mass: 356.38 g mol^{-1}(unlabelled)
Position of radiolabel: benzyl-U-^{14}C
Specific radioactivity of a.i.: 7.43 MBq mg^{-1}
Batch No.: 695-2038
Radiochemical purity: 99.3%

2. Soils

The study was conducted with five different soils originating from Europe. The physico-chemical properties of the soils are provided in Table 7.1.3.1.2-4.

Table 7.1.3.1.2-4: Characterization of soils used to determine the adsorption / desorption behavior of metabolite 505M08

Soil designation Origin	Nierswalder Wildacker (Germany)	Fiorentino Poggio Renatico 1 (Italy)	Li10 (Germany)	Lufa 2.1 (Germany)	Lufa 2.3 (Germany)
Textural class (USDA scheme)	Silt Loam	Loam	Loamy Sand	Sand	Sandy Loam
Soil texture [%], (USDA) Clay Silt Sand	 8.8 73.5 17.7	 16.7 33.9 49.4	 4.3 12.2 83.5	 2.3 6.9 90.8	 8.3 23.1 68.6
Organic carbon [%] (ISO 10694)	1.85	1.00	0.95	0.60	0.99
Cation exchange capacity [cmol$^+$ kg^{-1}]	3.1	11.8	5.5	-0.7	7.5
pH ($CaCl_2$) pH (water)	5.7 6.5	7.4 8.2	6.2 6.9	5.6 6.5	6.7 7.4
Max. water holding capacity [g/100g dry soil]	36.1	29.7	23.2	23.1	28.2
Bulk density [g L^{-1}]	1236	1403	1384	1381	1226

B. STUDY DESIGN

1. Experimental conditions

Preliminary tests: adsorption kinetics and soil/solution ratio

The adsorption of the test item to Teflon, glass and polypropylene centrifuge tubes was investigated (without soil) after an agitation period of 24 hours. Mean recovery rates of the test item in the solution were 106.4%, 99.9% and 102.2% for the adsorption test in Teflon, glass and polypropylene tubes, respectively. Since no relevant adsorption of the test item on the vessels occurred, all tested materials were considered suitable to conduct the study. Polypropylene tubes were chosen for the study.

To find the optimal soil/solution ratio for the adsorption/desorption tests a preliminary experiment was run for 24 h with two non-sterilized soils. Nierswalder Wildacker and Lufa 2.1 soils were selected to cover the whole organic carbon-range from 0.60% to 1.85% of the present soils. Three different soil/solution ratios were tested: 1/1, 1/5 and 1/10 using a test concentration of 1.0 µg mL^{-1} in 0.01 M $CaCl_2$. The ratio 1/1 was chosen, because it provided adsorption closer to 50%, enabling enough radioactivity in both phases (soil and solution) for best accurate measures.

The equilibrium time experiments were performed for all 5 soils at a soil/solution ratio of 1/1 and at a test concentration of 1.0 µg mL^{-1} in 0.01 M $CaCl_2$. The test was performed in centrifuge tubes containing 5 g of soil and 5 mL application solution. The tubes were protected from light and shaken at 250 rpm at a temperature of 20 ± 2°C. Sampling times were 4, 8, 24, 32, and 48 hours. The soil/solution suspensions were then centrifuged and the supernatants were isolated for analysis. Aliquots were radioassayed to determine the concentration of test item in the supernatants. The test showed no significant increase of adsorption after 24 hours for all five soils tested. Therefore, 24 hours was chosen for conducting the isotherms tests.

Stability of the test substance

The test was performed as described for the equilibrium time test, additionally analysing the soil following extraction (also see section on extraction below). For the Li 10, Lufa 2.1 and Lufa 2.3 soils, the mass balance of solution (supernatant of adsorption) plus soil (extract) was less than 90% and for the Fiorentino Poggio Renatico 1 soil, the K_d value was less than 0.3 cm^3 g^{-1}. Therefore, the direct method was used for those four soils. Only for Nierswalder Wildacker soil, the indirect method was applied for the adsorption test.

Determination of Freundlich adsorption and desorption isotherms

The adsorption isotherm determination was performed for all five soils at five concentration levels (nominal concentrations: 1.0, 0.5, 0.1, 0.05 and 0.01 µg mL^{-1}) at a soil/solution ratio of 1:1. For all samples 5 g of soil and 5 mL solution of each concentration level were added to the test centrifuge tubes. Each experiment was done in duplicate. All samples were shaken at 250 rpm on a horizontal shaker in a dark room at 20 ± 2°C for 24 hours. The soil/solution suspension was then centrifuged and aliquots of the supernatants were assayed by LSC. Aliquots from the highest dose sample supernatants were furthermore analyzed by radio-HPLC.

The desorption test could only be performed with the Nierswalder Wildacker soil. Desorption was performed by replacing the removed supernatant from the adsorption test with equal volume of fresh, untreated $CaCl_2$ solution and shaking again for 24 hours at 250 rpm. Then the suspensions were centrifuged and aliquots of the supernatants were assayed by LSC. Aliquots from the highest dose samples supernatants were further analyzed by radio-HPLC. The second desorption step was performed in analogous manner. To show the stability of the test item, the soils of the highest concentration level (nominal 1.0 µg mL^{-1}) remaining after the latter desorption step were extracted.

2. Description of analytical procedures

Extraction

For the soils Li10, Lufa 2.2, Lufa 2.3, and Fiorentino Poggio Renatico1, soil samples of all concentration levels remaining after the adsorption step were extracted. Samples of the Nierswalder Wildacker soil (highest concentration of 1.0 µg mL^{-1}) were also extracted after the last desorption step. The soils were extracted once with 15 mL of methanol and twice with 15 mL methanol / water (50/50; v/v) on a mechanical shaker for 30 minutes. After centrifugation, the extracts were combined in a 50 mL volumetric flask and the volume was completed. Aliquots of the extracts were assayed by LSC and radio-HPLC. The mass balance was calculated as sum of radioactivity in adsorption and desorption solutions and soil extracts.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

A mass balance determination was carried out for samples of the highest concentration level (1.0 µg mL^{-1}). Good mass balances were obtained. Mean recovery values (2 replicates) ranged from 95.6% to 99.2%.

B. FINDINGS

Adsorption Equilibrium Test

The adsorption equilibrium test carried out with ^{14}C-505M08 at a soil/solution ratio of 1:1 showed only a very slow increase of adsorption during the test period for all five soils tested. After 24 hours, no significant further increase was observed and therefore, 24 hours was chosen for conducting the isotherm tests. Adsorption values for the five soils were in a range of 10.1% for Li 10 soil to 44.1% for Nierswalder Wildacker soil after 24 h shaking. No relevant degradation was observed after 24 h of shaking.

Freundlich Adsorption Isotherm Determination

The results of the adsorption tests with metabolite 505M08 are summarized in Table 7.1.3.1.2-5.

Table 7.1.3.1.2-5: Adsorption of metabolite 505M08 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	Org. C [%]	pH $CaCl_2$ (pH water)	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Nierswalder Wildacker*	Silt loam	1.85	5.7 (6.5)	0.75	0.96	40.56
Fiorentino Poggio Renatico 1**	Loam	1.00	7.4 (8.2)	0.08	1.00	8.28
Li10**	Loamy sand	0.95	6.2 (6.9)	0.09	1.00	9.60
LUFA 2.1**	Sand	0.60	5.6 (6.5)	0.13	1.00	21.98
LUFA 2.3**	Sandy loam	0.99	6.7 (7.4)	0.06	0.99	6.03

* Indirect method
** Direct method

Freundlich Desorption Isotherm Determination

The desorption constants for Nierswalder Wildacker soil were:
K_{Fdes1} = 0.96 mL g^{-1}.
K_{Fdes2} = 1.22 mL g^{-1}.
$K_{FOCdes1}$ = 51.72 mL g^{-1}.
$K_{FOCdes2}$ = 66.08 mL g^{-1}.

III. CONCLUSION

The adsorption and desorption behavior of ^{14}C-505M08 was determined on five different European soils, covering a pH range ($CaCl_2$) of 5.6 to 7.4, an organic carbon range from 0.6% to 1.85% and five different USDA textural classes. The Freundlich adsorption coefficients K_F covered a range from 0.06 to 0.75 mL g^{-1} for the five soils. The K_{FOC} values ranged from 6.03 to 40.56 mL g^{-1}. Freundlich exponents (1/n) ranged from 0.96 to 1.00.

Report: CA 7.1.3.1.2/4
Sacchi R.R., 2014d
Amended final report - Adsorption/desorption behavior of 505M09 (Reg.No. 354563) on European soils
2014/3017803
Guidelines: OECD 106 (2000), POP-PA.1005, SOP-PA.1005, EPA 835.1230
GLP: yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

In laboratory experiments the adsorption behavior of the dimoxystrobin metabolite 505M09 (Reg.No. 354563) was investigated on five European soils. The five tested soils covered a pH range ($CaCl_2$) of 5.6 to 7.4, an organic carbon range from 0.6 to 1.85%, and five different USDA textural classes: silt loam, loam, loamy sand, sand and sandy loam.

For the determination of the adsorption isotherms, five different concentrations of test item (nominal 1.0, 0.50, 0.1, 0.05 and 0.01 µg mL^{-1}) in 0.01 M $CaCl_2$ were used. The soil/solution ratio was 1/1. The tests were performed at the adsorption equilibrium time of 24 hours on the five soils. The concentrations of the test item in the adsorption solutions and soils extracts were determined by liquid scintillation counting. The stability of ^{14}C-505M09 in the test system was checked by radio-HPLC analysis. Mass balance (mean recovery values) of ^{14}C-505M09 for the soil ranged from 95.7 to 97.5%.

The direct method was applied to determine the adsorption coefficients. The Freundlich adsorption coefficients K_F ranged from 0.16 to 1.17 mL g^{-1}. The K_{FOC} values ranged from 16.56 to 63.22 mL g^{-1} and the Freundlich exponent (1/n) values ranged from 0.92 to 1.0.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item: ^{14}C-505M09
Reg. No.: 354563
Chemical name (IUPAC): (E)-o-[(5-hydroxycarbonyl-2-methyl)phenoxymethyl]-2-methoxyimino-N-methylphenylacetamide
Molar mass: 356.38 g mol^{-1}(unlabelled)
Position of radiolabel: benzyl-U-^{14}C
Specific radioactivity of a.i.: 7.55 MBq mg^{-1}
Batch No.: 664-2005
Radiochemical purity: 99.2%

2. Soils

The study was conducted with five different soils originating from Europe. The physico-chemical properties of the soils are provided in Table 7.1.3.1.2-6.

Table 7.1.3.1.2-6: Characterization of soils used to determine the adsorption behavior of 505M09

Soil designation Origin	Nierswalder Wildacker (Germany)	Fiorentino Poggio Renatico 1 (Italy)	Li10 (Germany)	Lufa 2.1 (Germany)	Lufa 2.3 (Germany)
Textural class (USDA scheme)	Silt Loam	Loam	Loamy Sandy	Sand	Sandy Loam
Soil texture [%], (USDA)					
Clay	8.8	16.7	4.3	2.3	8.3
Silt	73.5	33.9	12.2	6.9	23.1
Sand	17.7	49.4	83.5	90.8	68.6
Organic carbon [%] (ISO 10694)	1.85	1.00	0.95	0.60	0.99
Cation exchange capacity [$cmol^+ kg^{-1}$]	3.1	11.8	5.5	-0.7	7.5
pH ($CaCl_2$)	5.7	7.4	6.2	5.6	6.7
pH (water)	6.5	8.2	6.9	6.5	7.4
Max. water holding capacity [g/100g dry soil]	36.1	29.7	23.2	23.1	28.2
Bulk density [g L^{-1}]	1236	1403	1384	1381	1226

B. STUDY DESIGN

1. Experimental conditions

Preliminary tests: adsorption kinetics and soil/solution ratio

The adsorption of the test item to Teflon, glass and polypropylene centrifuge tubes was investigated (without soil) after an agitation period of 24 hours. Mean recovery rates of test item in the solution were 104.2%, 101.0% and 101.9% for the adsorption test in Teflon, Glass and polypropylene tubes, respectively. Since no relevant adsorption of the test item occurred on the vessels, all were considered suitable to conduct the study. Teflon tubes were chosen for the study.

To find the optimal soil/solution ratio for the adsorption tests, a preliminary experiment was run for 24 h with two non-sterilized soils. Nierswalder Wildacker and Lufa 2.1 soils were selected to cover the whole organic carbon range from 0.60% to 1.85% of the present soils. Three different soil/solution ratios were tested: 1/1, 1/5 and 1/10 using a test concentration of 1.0 µg mL^{-1} in 0.01 M $CaCl_2$. The ratio 1/1 was chosen, because it provided adsorption closer to 50%, enabling enough radioactivity in both phases (soil and solution) for best accurate measures.

The equilibrium time experiments were performed for all 5 soils at a soil/solution ratio of 1/1 and at a test concentration of 1.0 µg mL^{-1} in 0.01 M $CaCl_2$. The test was performed in centrifuge tubes containing 5 g of soil and 5 mL of application solution. The tubes were protected from light and shaken at 250 rpm at a temperature of 20 ± 2°C. Sampling times were 4, 8, 24, 32, and 48 hours. The soil/solution suspension was then centrifuged and the supernatant isolated for analysis. Aliquots were radioassayed by LSC to determine the concentration of test item in the supernatants. The test showed no significant further increase of adsorption after 24 hours for all five soils tested. Therefore, 24 hours was chosen for conducting the isotherm tests.

Stability of the test substance

The test was performed as described for the equilibrium time test, additionally analysing the soil following extraction. For the Nierswalder Wildacker, Li 10 and Lufa 2.1 soils, the balance of solution (supernatant of adsorption) and soil (extract) was less than 90% and therefore the direct method was used for the adsorption test. For the Fiorentino Poggio Renatico 1 and Lufa 2.3 soils, the K_d value was less than 0.3 and, therefore, the direct method was also used.

Determination of Freundlich adsorption isotherms

The adsorption isotherm determination was performed for all five soils at all concentration levels (nominal concentrations: 1.0, 0.5, 0.1, 0.05 and 0.01 µg mL^{-1}) at a soil/solution ratio of 1/1. For all samples 5 g of soil and 5 mL solution of each concentration level were added to the test centrifuge tubes. Each experiment was done in duplicate. All samples were shaken at 250 rpm on a horizontal shaker at 20 ± 2°C for 24 hours. The soil/solution suspension was then centrifuged and aliquots of the supernatants were assayed by LSC. Aliquots from the highest dose sample supernatants were furthermore analyzed by radio-HPLC.

Since the direct method was used for all five soils, the soil residues after removing the $CaCl_2$ supernatants were extracted as described below.

2. Description of analytical procedures

Soil samples of all concentration levels remaining after the adsorption step were extracted.. The soils were extracted once with 15 mL of methanol and twice with 15 mL methanol / water (50/50; v/v) on a mechanical shaker for 30 minutes. After centrifugation, the extracts were combined in a 50 mL volumetric flask and the volume was completed. Aliquots of the extracts were assayed by LSC and radio-HPLC. The mass balance was calculated as sum of radioactivity in adsorption solutions and soil extracts (for soil Nierswalder Wildacker also combustion results of the extracted soil residue were included).

II. RESULTS AND DISCUSSION

A. MASS BALANCE

A mass balance determination was carried out for samples of the highest concentration level (1.0 µg mL^{-1}). Good mass balances were obtained. Mean recovery values (2 replicates) ranged from 95.7% to 97.5%.

B. FINDINGS

Adsorption Equilibrium Test

The adsorption equilibrium test carried out with ^{14}C-505M09 at a soil/solution ratio of 1.1 showed no significant further increase of adsorption after 24 hours shaking for all five soils tested. Therefore, 24 hours was the time chosen for conducting of isotherms test. Adsorption values for the five soils were in a range of 19.0% for Li 10 soil to 63.5% for Nierswalder Wildacker soil after 24 h shaking. No relevant degradation was observed on samples within 24 hours of shaking.

Freundlich Adsorption Isotherm Determination

For all soils, the direct method was applied to determine the adsorption coefficients. Therefore, no desorption steps were performed.

The results of the adsorption tests with metabolite 505M09 are summarized in Table 7.1.3.1.2-7.

Table 7.1.3.1.2-7: Adsorption of metabolite 505M09 based on Freundlich isotherms in five soils

Soil	Soil Type (USDA)	Org. C [%]	pH $CaCl_2$ (pH water)	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Nierswalder Wildacker	Silt loam	1.85	5.7 (6.5)	1.17	0.95	63.22
Fiorentino Poggio Renatico 1	Loam	1.00	7.4 (8.2)	0.20	0.94	20.37
Li10	Loamy sand	0.95	6.2 (6.9)	0.18	0.96	19.07
LUFA 2.1	Sand	0.6	5.6 (6.5)	0.28	1.00	45.85
LUFA 2.3	Sandy loam	0.99	6.7 (7.4)	0.16	0.92	16.56

III. CONCLUSION

The adsorption behavior of ^{14}C-505M09 was determined on five different European soils, which covering a pH range ($CaCl_2$) of 5.6 to 7.4, an organic carbon range from 0.6% to 1.85% and five different USDA textural classes. The Freundlich adsorption coefficients K_F covered a range from 0.16 to 1.17 mL g^{-1} for the five soils. The K_{FOC} values ranged from 16.56 to 63.22 mL g^{-1}. Freundlich exponents ranged from 0.92 to 1.00.

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Sacchi R.R., 2014e
Amended final report - Adsorption/desorption behavior of 505M01 (Reg.No. 358104) on European soils
2014/3017804

Guidelines: OECD 106 (2000), POP-PA.1005, SOP-PA.1005, EPA 835.1230

GLP: yes
(certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

In laboratory experiments the adsorption/desorption behavior of 505M01 (Reg.No. 358104) was investigated on five European soils. The five tested soils covered a range of pH ($CaCl_2$) from 5.6 to 7.4, a range of organic carbon content from 0.60 to 1.85%, and five different USDA textural classes: silt loam, loam, loamy sand, sand and sandy loam.

For the determination of the adsorption isotherms, five different concentrations of test item (nominal 1.0, 0.5, 0.1, 0.05 and 0.01 µg mL^{-1}) in 0.01 M $CaCl_2$ solutions were used. The soil/solution ratio was 1/1 and the tests were performed at an adsorption equilibrium time of 48 hours on five soils. Since adsorption of the test substance proved to be quite low in the preliminary tests, the direct method was chosen for the isotherm test and no desorption steps were performed. The concentration of the test item in the adsorption solutions and soil extracts was determined by liquid scintillation counting. The stability of ^{14}C-505M01 in the test systems was checked by radio-HPLC.

Mass balance (mean recovery values) of ^{14}C-505M01 for the test soil ranged from 95.9% (Fiorentino Poggio Renatico 1 soil) to 99.5% (Li 10 soil).

The direct method was applied to determine the adsorption coefficients. The Freundlich adsorption coefficients (K_F) ranged from 0.04 mL g^{-1} to 0.25 mL g^{-1}. The K_{FOC} values ranged from 5.38 mL g^{-1} to 24.67 mL g^{-1} and the Freundlich exponent (1/n) ranged from 0.91 to 1.0.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Test item: ^{14}C-505M01
Reg. No.: 358104
Chemical name (IUPAC): (E)-2-(2-hydroxymethylphenyl)-2-methoxyimino-N-methyl-acetamide
Molar mass: 222.24 g mol^{-1}(unlabelled)
Position of radiolabel: benzyl-U-^{14}C
Specific radioactivity of a.i.: 12.3 MBq mg^{-1}
Batch No.: 665-2003
Radiochemical purity: 99.2%

2. Soils

The study was conducted with five different soils originating from Europe. The physico-chemical properties of the soils are provided in Table 7.1.3.1.2-8.

Table 7.1.3.1.2-8: Characterization of soils used to determine the adsorption behavior of metabolite 505M01

Soil designation Origin	Nierswalder Wildacker (Germany)	Fiorentino Poggio Renatico 1 (Italy)	Li10 (Germany)	Lufa 2.1 (Germany)	Lufa 2.3 (Germany)
Textural class (USDA scheme)	Silt Loam	Loam	Loamy Sandy	Sand	Sandy Loam
Soil texture [%], (USDA)					
Clay	8.8	16.7	4.3	2.3	8.3
Silt	73.5	33.9	12.2	6.9	23.1
Sand	17.7	49.4	83.5	90.8	68.6
Organic carbon [%] (ISO 10694)	1.85	1.00	0.95	0.60	0.99
Cation exchange capacity [$cmol^+$ kg^{-1}]	3.1	11.8	5.5	-0.7	7.5
pH ($CaCl_2$)	5.7	7.4	6.2	5.6	6.7
pH (water)	6.5	8.2	6.9	6.5	7.4
Max. water holding capacity [g/100g dry soil]	36.1	29.7	23.2	23.1	28.2
Bulk density [g L^{-1}]	1236	1403	1384	1381	1226

B. STUDY DESIGN

1. Experimental conditions

Preliminary tests: adsorption kinetics and soil/solution ratio

The adsorption of the test item to Teflon, glass and polypropylene centrifuge tubes was investigated (without soil) after an agitation period of 24 hours. Mean recovery rates were 108.9%, 101.3% and 105.4% for the adsorption test in Teflon, Glass and polypropylene tubes, respectively. Since no relevant adsorption of the test item occurred on the vessels, all tested materials were considered suitable to conduct the study. Glass tubes were chosen for the study.

To find the optimal soil/solution ratio for the adsorption tests, a preliminary experiment was run for 24 h with two non-sterilized soils. Nierswalder Wildacker and Lufa 2.1 soils were selected to cover the whole organic carbon-range of the present soils (0.6% to 1.85%). Three different soil/solution ratios were tested: 1/1, 1/5 and 1/10 using a test concentration of 1.0 µg mL^{-1} in 0.01 M $CaCl_2$. The ratio 1/1 was chosen, because it provided adsorption closer to 50%, enabling enough radioactivity in both phases (soil and solution) for best accurate measures.

The equilibrium time experiments were performed for all five soils at a solution to soil ratio of 1/1 and a test concentration of 1.0 µg mL^{-1} in 0.01 M $CaCl_2$. The test was performed in centrifuge tubes containing 5 g soil and 5 mL application solution. The tubes were protected from light and shaken at 250 rpm at a temperature of 20 ± 2°C. Sampling time were 4, 8, 24, 32, 48 hours. The soil/solution suspensions were then centrifuged and the supernatants were isolated for analysis. Aliquots were radioassayed to determine the concentration of test item in the supernatants. The test showed a slight increase of adsorption still after 32 hours. Therefore, 48 hours was chosen for conduction of isotherms test.

Stability of the test substance

The test was performed as described for the equilibrium time test, additionally analysing the soil following extraction (also see section on extraction below).

For the Li 10 and Lufa 2.1 soils, the balance of solution (supernatant of adsorption) and soil (extract) was less than 90%. For the Nierswalder Wildacker, Fiorentino Poggio Renatico 1 and Lufa 2.3 soils, the K_d values were between 0.1 and 0.3. The direct method was therefore applied for all soils.

Determination of Freundlich adsorption isotherms

The adsorption isotherm determination was performed for all five soils at all five concentration levels (nominal concentrations: 1.0, 0.5, 0.1, 0.05 and 0.01 µg mL^{-1}) at a soil/solution ratio of 1/1. For all samples 5 g of soil and 5 mL solution of each concentration level were added to the test centrifuge tubes. Each experiment was done in duplicate. All samples were shaken at 250 rpm on a horizontal shaker in a dark room at 20 ± 2°C for 48 hours. The soil/solution suspension was then centrifuged and aliquots of the supernatants were assayed by LSC to determine the % TAR in the supernatants. Aliquots from the highest dose sample supernatants were analyzed directly by Radio-HPLC to determine the nature of the radioactivity.

2. Description of analytical procedures

Soil samples of all concentration levels were extracted (preliminary test and isotherm test). The soils were extracted once with 15 mL of methanol and twice with 15 mL methanol / water (50/50; v/v) on a mechanical shaker for 30 minutes. After centrifugation, the extracts were collected in a 50 mL volumetric flask and the volume was completed. Aliquots of the extracts were assayed by LSC and radio-HPLC. The mass balance was calculated as sum of radioactivity in adsorption solutions and soil extracts.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

A mass balance determination was carried out for samples of the highest concentration level(1.0 µg mL^{-1}). Good mass balances were obtained. Mean recovery values (2 replicates) ranged from 95.9% to 99.5%.

For the Li 10 and Lufa 2.1 soils, the balance of solution (supernatant of adsorption) and soil (extract) was less than 90% in the stability test. For the Nierswalder Wildacker, Fiorentino Poggio Renatico 1 and Lufa 2.3 soils, the Kd values were less than 0.3. Therefore, the direct method was used for all soils in the isotherm test.

B. FINDINGS

Adsorption Equilibrium Test

The adsorption equilibrium test carried out with ^{14}C-505M01 at a soil/solution ratio of 1:1 showed only a very slow increase of adsorption during the test period for all five soils tested. After 24 hours, still some increase was observed and therefore, 48 hours was chosen for conducting the isotherm tests. Adsorption values for the five soils were in a range of 5.1% for Lufa 2.1 soil to 20.4% for Fiorentino Poggio Renatico 1 soil after 48 hours shaking. Negligible degradation was observed in samples in presence of soil after 48 h of shaking and not further considered for calculation.

Freundlich Adsorption Isotherm Determination

The direct method was applied to determine the adsorption coefficients in all soils.

The results of the adsorption tests with metabolite 505M01 are summarized in Table 7.1.3.1.2-9.

Table 7.1.3.1.2-9: Adsorption of metabolite 505M01 based on Freundlich isotherms on five soils

Soil	Soil Type (USDA)	Org. C [%]	pH $CaCl_2$ (pH water)	K_F [mL/g]	1/n	K_{FOC} [mL/g]
Nierswalder Wildacker	Silt loam	1.85	5.7 (6.5)	0.18	0.99	9.79
Fiorentino Poggio Renatico 1	Loam	1.00	7.4 (8.2)	0.25	0.91	24.67
Li10	Loamy sand	0.95	6.2 (6.9)	0.05	0.99	5.38
LUFA 2.1	Sand	0.60	5.6 (6.5)	0.04	0.93	6.24
LUFA 2.3	Sandy loam	0.99	6.7 (7.4)	0.10	1.00	10.47

III. CONCLUSION

The adsorption behavior of ^{14}C-505M01 was determined on five different European soils, which covered a pH range ($CaCl_2$) of 5.6 to 7.4, an organic carbon range from 0.60% to 1.85% and five different USDA textural classes. The Freundlich adsorption coefficients K_F covered a range from 0.04 to 0.25 mL g^{-1} for the five soils. The K_{FOC} values ranged from 5.38 to 24.67 mL g^{-1}. The Freundlich exponent (1/n) ranged from 0.91 to 1.00.

Summary of adsorption values for dimoxystrobin metabolites

The updated (combined new and old) data sets for soil adsorption of dimoxystrobin metabolites are shown in Table 7.1.3.1.2-10 - Table 7.1.3.1.2-12.

For metabolites 505M08 and 505M09, a pH-dependent sorption was observed. The sorption behaviour of metabolite 505M01 is considered pH independent.

Table 7.1.3.1.2-10: Soil adsorption values of aerobic soil metabolite 505M08 (BF 505-7)

Soil	Soil type	Org. C [%]	pH $CaCl_2$ (pH water) [-]	K_f [-]	1/n	K_{foc} [mL g^{-1}]
USA 538-31-2	silty loamy sand	0.5	5.2 (5.8 [a])	0.665	0.95	133.0
LUFA 2.1	sand	0.6	5.6 (6.5)	0.130	1.00	22.0
Nierswalder Wildacker	silt loam	1.9	5.7 (6.5)	0.750	0.96	40.6
Lufa 2.2	sand / loamy sand	2.5	5.8 (6.4 [a])	0.499	0.95	20.0
Borstel	loamy sand	1.4	5.8 (6.5)	0.350	0.93	25.0
USA 538-30-5	loamy sand	0.4	5.8 (6.4 [a])	0.108	0.95	26.9
Li10	loamy sand	1.0	6.2 (6.9)	0.090	1.00	9.6
Li 35 b	loamy sand	1.1	6.5 (7.0 [a])	0.086	0.99	7.8
LUFA 2.3	sandy loam	1.0	6.7 (7.4)	0.060	0.99	6.0
Fiorentino Poggio Renatico 1	loam	1.0	7.4 (8.2)	0.080	1.00	8.3
Bruch West	loamy sand	1.5	7.5 (8.0 [a])	0.057	1.22 [b]	3.8
Canada	sandy loam	3.4	7.5 (8.0 [a])	0.689	0.91	20.3
Geometric mean (soils with pH (water) > 6.5)						**8.13**
Geometric mean (soils with pH (water) ≤ 6.5)						**34.18**
Arithmetic mean (soils with pH (water) > 6.5)					**0.98**	
Arithmetic mean (soils with pH (water) ≤ 6.5)					**0.96**	

[a] estimated as proposed by *FOCUS (2000, 2014)* pH-water=0.953*pH-$CaCl_2$+0.85
[b] set to 1.00 for averaging, since 1/n values above 1 are scientifically not reasonable

Table 7.1.3.1.2-11: Soil adsorption values of aerobic soil metabolite 505M09 (BF 505-8)

Soil	Soil type	OC [%]	pH $CaCl_2$ (pH water) [-]	K_f [-]	1/n	K_{foc} [mL g^{-1}]
USA 538-31-2	silty loamy sand	0.5	5.2 (5.8 [a])	0.595	0.89	119.0
LUFA 2.1	sand	0.6	5.6 (6.5)	0.280	1.00	45.9
Nierswalder Wildacker	silt loam	1.9	5.7 (6.5)	1.170	0.95	63.2
Lufa 2.2	sand / loamy sand	2.5	5.8 (6.4 [a])	1.111	0.92	44.4
Borstel	loamy sand	1.4	5.8 (6.4 [a])	0.647	0.88	46.2
USA 538-30-5	loamy sand	0.4	5.8 (6.4 [a])	0.154	0.82	38.6
Li10	loamy sand	1.0	6.2 (6.9)	0.180	0.96	19.1
Li 35 b	loamy sand	1.1	6.5 (7.0 [a])	0.142	0.81	12.9
LUFA 2.3	sandy loam	1.0	6.7 (7.4)	0.160	0.92	16.6
Fiorentino Poggio Renatico 1	loam	1.0	7.4 (8.2)	0.200	0.94	20.4
Bruch West	loamy sand	1.5	7.5 (8.0 [a])	0.135	0.81	9.0
Canada	sandy loam	3.4	7.5 (8.0 [a])	1.771	0.87	52.1
Geometric mean (soils with pH (water) > 6.5)						**18.41**
Geometric mean (soils with pH (water) ≤ 6.5)						**54.88**
Arithmetic mean (soils with pH (water) > 6.5)					**0.89**	
Arithmetic mean (soils with pH (water) ≤ 6.5)					**0.91**	

[a] estimated as proposed by *FOCUS (2000, 2014)* pH-water=0.953*pH-$CaCl_2$+0.85

Table 7.1.3.1.2-12: Soil adsorption values of soil photolysis metabolite 505M01 (BF 505-4)

Soil	Soil type	OC [%]	pH $CaCl_2$ (pH water) [-]	K_f [-]	1/n	K_{foc} [mL g^{-1}]
USA 538-31-2	silty loamy sand	0.5	5.2 (5.8 [a])	0.151	0.93	30.2
LUFA 2.1	sand	0.6	5.6 (6.5)	0.040	0.93	6.2
Nierswalder Wildacker	silt loam	1.9	5.7 (6.5)	0.180	0.99	9.8
Lufa 2.2	sand / loamy sand	2.5	5.8 (6.4 [a])	0.097	0.92	3.9
USA 538-30-5	loamy sand	0.4	5.8 (6.4 [a])	0.023	0.88	5.8
Borstel	loamy sand	1.4	5.8 (6.4 [a])	0.157	0.81	11.2
Li10	loamy sand	1.0	6.2 (6.9)	0.050	0.99	5.4
Li 35 b	loamy sand	1.1	6.5 (7.0 [a])	0.034	0.93	3.1
LUFA 2.3	sandy loam	1.0	6.7 (7.4)	0.100	1.00	10.5
Fiorentino Poggio Renatico 1	loam	1.0	7.4 (8.2)	0.250	0.91	24.7
Bruch West	loamy sand	1.5	7.5 (8.0 [a])	0.031	0.91	2.0
Canada	sandy loam	3.4	7.5 (8.0 [a])	1.208	0.74	35.5
				Geometric mean		**8.5**
				Arithmetic mean	**0.91**	

[a] estimated as proposed by *FOCUS (2000, 2014)* pH-water=0.953*pH-$CaCl_2$+0.85

CA 7.1.3.2 Aged sorption

No experimental data are available. They are not considered necessary for leaching assessment of dimoxystrobin. PECgw calculations were performed with the regular Freundlich sorption coefficients as presented in M-CA 7.1.3.1.1.

CA 7.1.4 Mobility in soil

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

No new experimental data are available. The column leaching study already peer-reviewed during the previous Annex I inclusion process is considered still valid. Dimoxystrobin showed no tendency of leaching through soil columns (4 soils tested). The total amounts of radioactivity found in leachates were always < 1% AR.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

No new experimental data are available. The aged soil column leaching study already peer-reviewed during the previous Annex I inclusion process is considered still valid. The total amount of radioactivity in the leachate was 2.5% AR. More than 90% AR was retained in the upper 10 cm soil segment.

CA 7.1.4.2 Lysimeter studies

No new experimental data are available. The soil mobility of dimoxystrobin and its metabolites was evaluated during the previous Annex I inclusion process in a lysimeter study performed in Schmallenberg, NRW, Germany with 3 replicate soil cores.

Two of the three soil cores were treated 2 x with 250 g ^{14}C-dimoxystrobin/ha in the first year. The third soil core was treated 2 x with 250 g/ha in the first year and 2 x with 200 g/ha in the second year. Compared to the current valid GAP (application of 1 x 200 g/ha/year), all three soil cores can be considered highly overdosed.

Dimoxystrobin and the soil photolysis metabolite 505M01 never exceeded 0.1 µg/L in yearly average over the three year leaching period. Metabolite 505M08 reached a yearly average max. concentration of 2.3 µg/L and 505M09 of 2.0 µg/L.

A relevance assessment was performed for both metabolites according to EU guidance document SANCO/221/2000. Both metabolites proved to be non-relevant.

CA 7.1.4.3 Field leaching studies

No field leaching study was performed. The leaching assessment in the current renewal dossier is based on PECgroundwater calculations performed with updated endpoints according to the newest guidelines and guidance documents.

CA 7.2 Fate and behaviour in water and sediment

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

Except for the new data requirement "aquatic mineralization" (accord. to OECD 309), no new experimental data were produced for the aquatic environment. The kinetic evaluation of the already peer-reviewed studies from the previous Annex I listing was however updated according to the newest guidelines and guidance documents.

An overview on the degradation rates obtained for parent, as well as on the maximum occurrences of metabolites and their degradation rates (where feasible) can be found at the end of this chapter.

CA 7.2.1.1 Hydrolytic degradation

No new experimental data on hydrolysis of Dimoxystrobin were produced. The old hydrolysis study is considered still valid. Dimoxystrobin proved to be stable at all tested pH values (pH 4 - pH 9) and temperatures (25 and 50°C).

CA 7.2.1.2 Direct photochemical degradation

No new experimental data on direct photolysis of Dimoxystrobin was produced. The already peer-reviewed study is considered still valid.

Dimoxystrobin showed a very slow degradation under photolytic conditions. After 15 days irradiation, it still amounted to 77% (phenyl-label) and 79% AR (benzyl-label). Besides slight shift from E- to Z-isomer (max. 6.8% AR), the cleavage product 505M01 (BF 505-4) was formed in amounts of 7.8% AR. Most of the other photolysis products occurred only in trace amounts and none of them exceeded 4% AR at any sampling time.

Although not used in risk assessment, new kinetic evaluations of the two direct aqueous photolysis experiments with the two radiolabels were performed.

Figure 7.2.1.2-1: Proposed route of degradation of dimoxystrobin during aqueous photolysis (from dimoxystrobin dossier 2001)

BAS 505 F

BAS 505 F
(Z)-isomer

BF 505-13
(505M03)

BF 505-4
(505M01)

BF 505-12
(505M04)

CO_2

Report: CA 7.2.1.2/1
Budde E., 2014b
Kinetic evaluation of two studies on aqueous photolysis of BAS 505 F - Dimoxystrobin according to FOCUS Degradation Kinetics
2014/1263218
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0
GLP: no (not applicable)

Executive Summary

The aqueous photolysis of BAS 505 F - dimoxystrobin has been investigated in two studies, one of them performed in sterile aqueous buffer solution, the other one performed with natural water. The purpose of this evaluation was to analyze the degradation kinetics of dimoxystrobin observed in the studies, taking into account the current guidance of the FOCUS workgroup on degradation kinetics.

The best-fit $DegT_{50}$ were 61.5 and 63.8 days in the sterile buffer solutions, and 14.0 days in the natural water system. In all test systems, the SFO kinetic model provided an appropriate fit to the measured data.

I. MATERIAL AND METHODS

The aqueous photolysis of dimoxystrobin was investigated in one study with a sterile buffer solution [*Singh, M. (1998): Photolysis of 14C-BAS 505 F in aqueous media. BASF DocID 1997/5286*] and in one study with natural water [*Goetz, A., Moss, I. (1998): Natural water photolysis of BAS 505 F. BASF DocID 1998/5428*].

In the study by Singh, the photolytic degradation of ^{14}C-labeled dimoxystrobin was investigated at pH 7 in sterile aqueous buffer solution, using benzyl- or phenyl-labeled test substance. The concentration of dimoxystrobin was 2.03 mg L^{-1} for the benzyl-label and 1.87 mg L^{-1} for the phenyl-label. The treated buffer solutions were continuously exposed to artificial sunlight for about 355 hours, while being maintained at 22±1°C. Dimoxystrobin degraded slowly, with 78.8%TAR and 76.6%TAR remaining after 355 hours (=15 days) in the benzyl- and phenyl-labeled systems, respectively. The experimental data of the irradiated systems used for kinetic analysis are given in the table below. The data of the dark control system were not evaluated, as dimoxystrobin proved to be stable.

Table 7.2.1.2-1 Data for kinetic evaluation of the aqueous photolysis study (irradiated systems)

Day [a]	Experimental data [%TAR]		Input data according to FOCUS [%TAR]	
	Benzyl label	Phenyl label	Benzyl label	Phenyl label
0	99.1	99.4	100.0 [b]	100.0 [b]
3	93.5	99.4	93.5	99.4
7	90.3	94.3	90.3	94.3
11	85.5	93.9	85.5	93.9
15	84.6	83.3	84.6	83.3

TAR = Total applied radioactivity
[a] reported sampling times in hours converted to days
[b] set to material balance

In the study by Goetz and Moss, the photolytic degradation of dimoxystrobin was investigated in natural water. The water had a pH of 8.6, organic matter content of 6 mg L^{-1}, and a nitrate content of 4 mg L^{-1}. The study was performed with non-labeled dimoxystrobin at a concentration of 2 mg L^{-1}. Samples were irradiated for 357 hours and maintained at 22°C during irradiation. Dimoxystrobin degraded moderately, with 46.9% TAR remaining at the end of the study. The experimental data of the irradiated system used for kinetic analysis are given in the following table. The data of the dark control system were not evaluated, as dimoxystrobin proved to be stable.

Table 7.2.1.2-2 Data for kinetic evaluation of the natural water photolysis study (irradiated system)

Day [a]	Irradiated system [%AD]
0	96.4
1	90.5
2	90.3
3	83.7
6	71.2
8	64.9
10	55.1
13	53.0
15	46.9

AD = Applied dose
[a] reported sampling times in hours converted to days

Kinetic modelling

Kinetic evaluation was performed in order to derive degradation parameters as triggers for additional work (trigger endpoints). The appropriate kinetic model for deriving trigger endpoints was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 1.0 (November 2011), 436pp*]. The best-fit model was selected based on visual and statistical assessment and the corresponding $DegT_{50}$ and $DegT_{90}$ values are reported as trigger endpoints.

Kinetic models included in the assessment

The kinetic models employed for this evaluation were described by the FOCUS workgroup on degradation kinetics:

- Single-First-Order (SFO); Box 5-1, in *FOCUS (2006)*,
- Gustafson and Holden (FOMC): Box 5-2, in *FOCUS (2006)*,
- Double first-order in parallel (DFOP); Box 5-4, in *FOCUS (2006)*.

The appropriateness of a distinct kinetic model to describe degradation can be tested with the following checks recommended by FOCUS [*FOCUS (2006)*, chapter 6.3.1]:

- Visual assessment of goodness-of-fit
- Estimation of the error percentage at which the χ^2 test is passed (Equation 6-2 in *FOCUS (2006)*)
- t-test to evaluate whether estimated degradation parameters differ from zero

A kinetic model is considered appropriate if the residuals are randomly distributed around zero, the χ^2 - error value is < 15 % and the estimated degradation parameters differ from zero as outlined by FOCUS [*FOCUS (2006)*, chapter 6.3.1].

Data handling and software for kinetic evaluation

The experimental data were derived from the study reports and adjusted according to FOCUS [*FOCUS (2006)*]. The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting [*SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. BASF DocID 2007/1062781; Schmitt, W., Gao, Z., Meyer, H. (2011) KinGUII, Version 2.2012.320.1629 Bayer CropScience AG*]. The error tolerance and the number of iterations of the optimization tool were set to 1×10^{-6} and 100, respectively.

II. RESULTS AND DISCUSSION

The datasets for each system were analyzed considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. Graphical presentations of the tested kinetic models and the results of the χ^2 - test and all other statistical endpoints used in the decision-making process are given in the original study report.

An overview of the estimated trigger endpoints for dimoxystrobin from the two studies is given in Table 7.2.1.2-3.

Table 7.2.1.2-3 Kinetic endpoints for the degradation of dimoxystrobin in the aqueous photolysis studies

Study matrix	Test system	Kinetic model	χ^2 error	k [d^{-1}]	Kinetic endpoints	
					$DegT_{50}$ [d]	$DegT_{90}$ [d]
Sterile buffer solution	Benzyl label	SFO	1.2	0.0113	61.5	204.3
	Phenyl label	SFO	2.0	0.0109	63.8	212.0
Natural water	Irradiated	SFO	2.0	0.0495	14.0	46.5

III. CONCLUSION

The degradation of dimoxystrobin in two aqueous photolysis studies was investigated and evaluated according to the recommendations of the FOCUS workgroup on degradation kinetics. The SFO model proved to be appropriate to derive kinetic endpoints for dimoxystrobin from the aqueous photolysis studies, with $DegT_{50}$ values ranging from 14.0 to 63.8 days and $DegT_{90}$ values ranging from 46.5 to 212.0 days. For the evaluated systems, visual assessment and goodness-of-fit statistics of the SFO model indicate plausible fit and therefore, the resulting values can be considered reliable.

CA 7.2.1.3 Indirect photochemical degradation

No new experiments on indirect photochemical degradation of dimoxystrobin were performed. Since the existing and already peer-reviewed study with dimoxystrobin was done with non-labelled test item, no further information on the route of degradation in the aquatic environment can be given. The half-life was re-evaluated according to FOCUS (2006) and is reported in Table 7.2.1.2-3.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

CA 7.2.2.1 “Ready biodegradability”

The already peer-reviewed study is considered still valid. Dimoxystrobin was found to be not readily degradable according to OECD guideline 301 F.

CA 7.2.2.2 Aerobic mineralisation in surface water

Report: CA 7.2.2.2/1
Yeomans P., 2014a
^{14}C-Dimoxystrobin (BAS 505 F): Aerobic mineralisation in surface water
2014/1031018

Guidelines: OECD 309 (April 2004)

GLP: yes
(certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

The purpose of this study was to determine the mineralisation and degradation rates of the fungicidal active substance dimoxystrobin (BAS 505 F) in an aquatic system under dark conditions. The study was performed according to the OECD guideline 309 (Aerobic mineralization in surface water – Simulation biodegradation test). The pelagic test system was chosen for this study.

The test was performed at two different dimoxystrobin concentrations (10 µg L^{-1} and 90 µg L^{-1}), using two differently ^{14}C-labelled test items (phenyl and benzyl label). Sterile samples were tested for each label of the higher concentration. The test vessels were attached to a flow-through system for continuous aeration and incubated at a temperature of 20 ± 2°C in the dark. Samples were taken at 0, 3, 7, 14, 21, 35 and 59 days after treatment.

The amount and nature of radioactivity in the water samples was determined by liquid scintillation counting (LSC) and chromatographic methods (radio-HPLC). Volatiles were trapped in 2M sodium hydroxide and were also analyzed by LSC. Parent substance and metabolite identification was done by co-chromatography with the corresponding reference items on HPLC.

From the obtained results it could be concluded that dimoxystrobin was not significantly degraded in the natural water environment provided in the test. After 59 days, 89 to 97% AR (applied radioactivity) was recovered as unchanged active substance.

Some trace amounts (<1%) of the known metabolites 505M08 and 505M09 were detected and assigned by retention time comparison on HPLC analysis. Other degradation products were detected only in minor amounts (≤1.5% AR, highest single value).

Radioactivity in the volatile traps did not exceed 1% AR indicating a low rate of mineralization. No differences in the test item behaviour were observed regarding radiolabel or concentration level.

Overall, the compound was stable in the test system. Degradation kinetics were not calculated as no significant degradation was observed.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS-Code: BAS 505 F (Dimoxystrobin)
Chemical name: (E)-o-(2,5-dimethylphenoxymethyl)-2-methoxyimino-N-methylphenylacetamide
Molecular formula: $C_{19}H_{22}N_2O_3$
Molar mass: 326.4 g mol^{-1} (unlabeled)

Label 1 (phenyl label)
Label: phenyl-U-^{14}C
Batch No.: 597-1501
Specific radioactivity: 4.67 MBq mg^{-1}
Radiochemical purity: 99.9%
Chemical purity: 98.7%

Label 2 (benzyl label)
Label: benzyl-U-^{14}C
Batch No.: 596-3101
Specific radioactivity: 7.41 MBq mg^{-1}
Radiochemical purity: 99.0%
Chemical purity: 96.4%

2. Test system

Water and sediment were collected from The Lake at Studley Royal (Ripon, United Kingdom).

Prior to use the sediment and water were stored together in the dark at 4 ± 2°C with free access to air. Water was filtered through a 0.1 mm sieve and sediment was passed through a 2 mm sieve prior to use and characterisation. The physico-chemical properties of the system are summarized in Table 7.2.2.2-1.

Table 7.2.2.2-1: Characterization of the water/sediment system

Designation Origin		Fountains Abbey The Lake, Studley Royal, Ripon, UK	
Water			
Temperature	[°C]	4.8*	
pH water	-	8.99*	
Oxygen content	[%]	12.46*	
Redox potential (Eh)	[mV]	134.1*	
Hardness	[mg $CaCO_3$ L^{-1}]	90	
Total organic carbon	[ppm]	8.15	
Total N	[%]	0.00375	
Total P	[mg L^{-1} PO_4^{3-}]	0.27	
Sediment			
Textural class		UK & BBA Particle Size Distribution	USDA
Sand	[%]	76	78
Silt	[%]	19	17
Clay	[%]	5	5
Soil type	-	Loamy sand	Loamy sand
pH	-	8.35*	
pH (H_2O)	-	8.1	
pH ($CaCl_2$)	-	7.5	
Redox potential (Eh)	[mV]	-182*	
Organic carbon	[%]	1.3	

* measured at sampling

B. STUDY DESIGN

1. Experimental conditions

A total of 94 test vessels were prepared for incubation: 18 test vessels for each radiolabel (phenyl and benzyl) and each nominal concentration (10 and 90 µg L^{-1}), 9 vessels for the sterile incubation (for both labels; 90 µg L^{-1}), 2 vessels as system control with sodium [^{14}C]-benzoate and 2 vessels with sodium [^{14}C]-benzoate plus treatment solvent.

The vessels were filled with about 100 mL test water, using sterile techniques where necessary. Appropriate amounts of the respective application solutions were pipetted to the water surface to achieve nominal application rates of 10 µg L^{-1} or 90 µg L^{-1}.

The systems were incubated at 20 ± 2°C in a metabolism apparatus (incubator) with a gas flow system. Each test vessel was connected to a volatile trapping system of two gas washing bottles containing trapping solutions (2x NaOH) for the ^{14}C-volatiles to be expected. Test vessels containing sterile water were also aerated, however, the air stream was led through sterile filters to avoid contamination of the test system by airborne germs. Vessels were kept in the dark and were agitated by continuous stirring on magnetic stirrers throughout the incubation period.

2. Sampling

Test vessels, including the sterile groups, were sampled at 0, 3, 7, 14, 21, 35 and 59 days after treatment (DAT). For sampling, the flasks were removed from the rigs and the conductivity, O_2 content, pH and redox potential of the water were measured.

3. Description of analytical procedures

Water
The water in the test vessels was transferred into glass jars, the test vessels were rinsed with Milli-Q water which was added to the water from the test vessel and weighed prior to LSC. The test vessels were then washed (with sonication) with acetonitrile (25 mL).

Weighed aliquots of the water for LSC were mixed with fresh acetonitrile prior to addition of scintillant.

For higher concentration samples (90 µg L^{-1}), HPLC analysis was carried out without further work-up. For lower concentration samples (10 µg L^{-1}), sub-samples of the water were partitioned with dichloromethane. The dichloromethane was concentrated to dryness and the samples reconstituted in acetonitrile or acetonitrile : water (1:1, v/v) prior to chromatography. Procedural recoveries were checked by LSC and were found to be 90% or greater.

Volatiles
Throughout the test, traps were collected for sampled vessels. Remaining test samples had their traps collected and replenished with fresh solutions at 3, 7, 14, 21, 29, 35, 43 and 51 DAT. Reference vessels had traps collected at the same intervals. Volatiles trapped in sodium hydroxide were analyzed by LSC.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The material balance and distribution of radioactivity are shown in Table 7.2.2.2-2 and Table 7.2.2.2-3.

The applied mass of test item per test vessel containing 100 mL of water was 9.2 µg (high concentration) and 1.0 µg (low concentration).

The material balance for the pelagic test ranged from 91.8 to 105.3% of the total applied radioactivity (TAR) in Lake water treated with the phenyl-^{14}C-labeled test item and from 93.1 to 102.3% TAR in Lake water treated with the benzyl-^{14}C-labeled test item. In the sterile vessels, the material balance ranged from 90.7 to 93.7 TAR (phenyl label) and from 91.2 to 97.5% TAR (benzyl label).

The radioactivity recovered from test vessels was found predominantly in the water. Adsorption to the inner test vessel surface was negligible, only up to 2 % AR was found in the acetonitrile rinsing solution. At the end of the study (59 DAT) the water accounted for 92.2 to 98.6% TAR for the viable test vessels and 91.4 to 93.9% TAR for the sterilized vessels. For all test samples and sampling time points, the radioactivity in the volatile traps remained below 1.0% TAR indicating a low rate of mineralization.

Table 7.2.2.2-2: Material balance and distribution of radioactivity after application of [phenyl-U-^{14}C] dimoxystrobin to lake water

Days after treatment	Percent of total applied radioactivity [% TAR]			
	Water	Vessel wash**	NaOH Trap (CO_2)	Material balance
Low concentration (10 µg L^{-1})*				
0	105.3	-	NA	105.3
3	99.1	-	-	99.1
7	95.2	0.9	0.1	96.2
14	99.5	1.4	0.3	101.1
21	97.0	0.8	0.2	97.9
35	96.0	-	0.5	96.4
59	98.1	0.7	0.1	98.8
High concentration (90 µg L^{-1})*				
0	92.0	0.3	NA	92.3
3	92.9	0.3	-	93.1
7	93.2	0.6	0.1	93.9
14	92.9	0.7	0.1	93.6
21	91.3	0.4	0.2	91.8
35	91.8	0.3	0.3	92.4
59	92.2	0.4	0.7	93.3
Sterilized (90 µg L^{-1})				
0	91.6	0.2	NA	91.8
3	93.7	-	-	93.7
7	91.9	0.3	-	92.2
14	90.2	0.5	-	90.7
21	91.5	0.2	-	91.7
35	91.3	0.2	0.7	92.2
59	91.4	0.3	-	91.7

NA not applicable
- not detected (or < 0.1%)
* mean of two replicates
** acetonitrile wash of the incubation vessel

Table 7.2.2.2-3: Material balance and distribution of radioactivity after application of [benzyl-U-^{14}C] dimoxystrobin to lake water

Days after treatment	Percent of total applied radioactivity [% TAR]			
	Water	Vessel wash**	NaOH Trap (CO_2)	Material balance
Low concentration (10 µg L^{-1})*				
0	102.3	-	NA	102.3
3	99.0	-	-	99.0
7	99.8	1.2	0.1	101.0
14	91.8	1.5	0.2	93.4
21	95.8	0.4	0.1	96.2
35	97.8	-	0.1	97.9
59	98.6	0.5	0.2	99.2
High concentration (90 µg L^{-1})*				
0	94.9	0.2	NA	95.1
3	92.8	0.2	0.1	93.1
7	92.8	1.1	0.1	93.9
14	92.8	0.7	0.1	93.6
21	92.8	0.5	0.1	93.4
35	93.4	0.6	0.1	94.1
59	93.7	0.5	0.2	94.4
Sterilized (90 µg L^{-1})				
0	95.5	0.3	NA	95.8
3	94.7	0.1	-	94.8
7	92.0	0.5	-	92.5
14	96.9	0.6	-	97.5
21	91.0	0.2	-	91.2
35	92.9	0.4	-	93.3
59	93.9	0.2	-	94.1

NA not applicable
- not detected (or < 0.1%)
* mean of two replicates
** acetonitrile wash of the incubation vessel

B. TRANSFORMATION OF PARENT COMPOUND

Water

The results of radio-HPLC analysis are summarized in Table 7.2.2.2-4 and
Table 7.2.2.2-5.

No significant degradation of dimoxystrobin was observed during the test. After 59 days, between 89 and 97% TAR could still be recovered as unchanged parent for the different concentrations and radiolabels. Additional peaks only appeared in trace amounts. Two of them could be assigned by retention time comparison to metabolites 505M08 and 505M09. However, none of the peaks exceeded 1.5% TAR at any sampling time.

The low amount of volatiles, metabolites and other degradation products detected indicate that only negligible microbial degradation took place.

Control samples with benzoic acid

The control vessels treated with [^{14}C]-sodium benzoate showed that the test system was microbially active both without and with the addition of acetonitrile. The total recoveries of trapped volatile radioactivity after 59 days were 96.3 and 97.8% TAR and the material balances were 100.5 and 102.2% TAR for the samples without and with acetonitrile, respectively.

A material balance was also established for intermediate sampling times, where water samples were radioassayed (after 7, 14 and 29 days). The material balance ranged from 96.3 to 100.3% TAR.

Sterilized samples

The very limited degradation observed in the viable test vessels resulted in no significant difference in test item concentration between the sterilized incubations and the viable vessels.

Table 7.2.2.2-4: Metabolite overview for the water phase after application of [phenyl-U-^{14}C] dimoxystrobin to lake water

Days After Treatment	Percent of total applied radioactivity [% TAR]					
	BAS 505 F	505M08	505M09	Unknown	Unresolved Background	Total
Low concentration (10 µg L^{-1})*						
0	104.0	-	-	-	1.2	105.3
3	98.6	-	-	-	0.4	99.1
7	94.6	-	-	-	0.6	95.2
14	98.8	-	-	-	0.6	99.5
21	95.8	-	-	-	1.1	97.0
35	95.3	-	-	-	0.6	96.0
59	96.7	-	0.7	-	0.6	98.0
High concentration (90 µg L^{-1})*						
0	91.2	-	-	-	0.8	92.0
3	91.9	-	-	-	1.0	92.9
7	91.2	-	-	0.4	1.5	93.2
14	91.7	-	-	-	1.1	92.9
21	89.4	-	0.5	-	1.4	91.3
35	89.4	0.9	0.6	-	1.0	91.8
59	90.0	-	0.4	0.7	1.1	92.2
Sterilized lake water (90 µg L^{-1})						
0	90.4	-	-	-	1.3	91.6
3	92.3	-	-	-	1.4	93.7
7	90.1	-	-	-	1.8	91.9
14	88.7	-	-	-	1.5	90.2
21	90.2	-	0.5	-	0.8	91.5
35	90.2	-	-	-	1.1	91.3
59	89.2	-	-	0.8	1.5	91.4

- not detected (or < 0.1%)
* mean of two replicates

Table 7.2.2.2-5: Metabolite overview for the water phase after application of [benzyl-U-^{14}C] dimoxystrobin to lake water

Days After Treatment	Percent of total applied radioactivity [% TAR]					
	BAS 505 F	505M08	505M09	Unknown	Unresolved Background	Total
Low concentration (10 µg L^{-1})*						
0	101.3	-	-	-	1.0	102.3
3	98.0	-	-	-	1.0	99.0
7	99.0	-	-	-	0.8	99.8
14	90.4	-	-	0.6	0.7	91.8
21	94.8	-	-	-	1.0	95.8
35	96.5	-	-	-	1.3	97.8
59	95.6	-	0.7	1.1	1.1	98.6
High concentration (90 µg L^{-1})*						
0	93.6	-	-	-	1.3	94.9
3	91.6	-	-	-	1.2	92.8
7	91.8	-	-	-	0.9	92.8
14	91.9	-	-	-	0.8	92.8
21	91.6	-	-	-	1.2	92.8
35	91.4	-	-	0.5	1.5	93.4
59	91.0	0.7	0.7	-	1.3	93.7
Sterilized lake water (90 µg L^{-1})						
0	95.2	-	-	-	0.3	95.5
3	93.9	-	-	-	0.8	94.7
7	91.7	-	-	-	0.3	92.0
14	96.5	-	-	-	0.4	96.9
21	90.5	-	0.4	-	0.1	91.0
35	90.6	-	-	0.6	1.7	92.9
59	92.0	-	-	0.9	1.0	93.9

- not detected (or < 0.1%)
* mean of two replicates

Degradation rates

Overall, the compound was stable in the test system. Degradation kinetics were not calculated as no significant degradation was observed.

III. CONCLUSION

From the obtained results it can be concluded that dimoxystrobin was not significantly degraded in the natural water environment provided in the test. After 59 days, 89 to 97% AR was recovered as the unchanged active substance. Some trace amounts (<1%) of the metabolites 505M08 and 505M09 were detected and identified by retention time comparison on HPLC analysis. Other degradation products were also detected only in minor amounts (always < 1.5% AR). Radioactivity in the volatile traps did not exceed 1.0% AR indicating a low rate of mineralization. No differences in the test item behavior were observed regarding radiolabel or concentration level. Overall, the compound was stable in the test system. Degradation kinetics were not calculated since no significant degradation was observed.

CA 7.2.2.3 Water/sediment studies

No new water/sediment study was performed. The already peer-reviewed study is considered still valid.

The behaviour in water/sediment was characterized by a rather fast movement from the water to the sediment. The radioactivity in the water decreased within 100 days to 21.9 - 31.1% AR in the two tested systems, respectively. In the sediment, the radioactivity correspondingly increased and accounted to 64.4 - 72.2% AR at the end of the incubation period.

Mineralization was low in both systems (<2.5% AR) and no other volatile degradates were detected. The bound residues in the sediment were formed only to a low extent, reaching 6.3 - 10.7% AR. The bound residues in system B were fractionated into humins, humic acids and fulvic acids. Most of the radioactivity was located in the humic acids and humins. In the fulvic acid fraction, less than 4% AR could be detected in the 100 day sample.

Dimoxystrobin was the only major compound in both water/sediment systems. The metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) could be detected only in the water phase and only in very low amounts (≤ 5% AR). In the sediment, no metabolites could be detected at any sampling time.

The half-lives of dimoxystrobin were re-calculated according to the newest guidelines and guidance documents and listed in Table 7.2.2.4-4.

CA 7.2.2.4 Irradiated water/sediment study

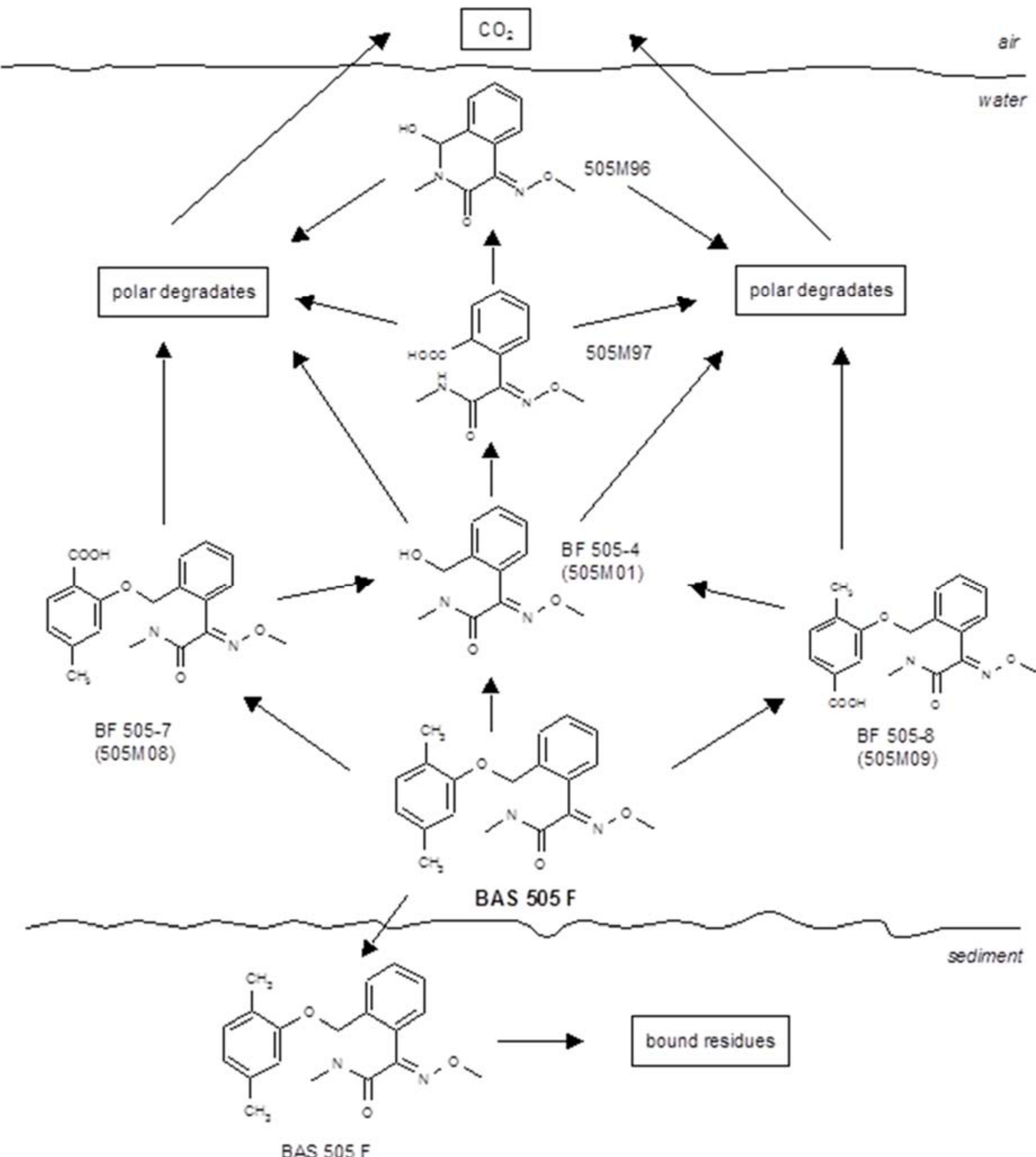
An irradiated water/sediment study was submitted and evaluated in the previous Annex I listing process. This study was initiated after it became obvious that the degradation of dimoxystrobin in water is considerably dependent on light conditions. In natural surface waters the two factors photolysis and adsorption to sediment do occur simultaneously, this additional study was designed where both factors were combined.

One water/sediment system was used for this study and the test vessels were treated with benzyl-labelled test item on July 5th, 2000. Then the test vessels were placed outdoor and exposed to natural daily sunlight and temperature variations for 120 days.

The results showed that when reaching water, dimoxystrobin undergoes a photolytical transformation forming many breakdown products and polar degradates. At the same time, adsorption to sediment takes place where dimoxystrobin is finally bound to the sediment matrix.

In the water phase, one metabolite (505M96) reached 9.6% TAR after 30 days and degraded again to 1.9% at the end of incubation. The known photolysis product 505M01 (BF 505-4) was detected in max. amount of 3.2% TAR, and the carboxylic acid metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) reached max. amounts of 3.6 and 5.3% TAR, respectively. One further metabolite 505M97 was detected with max. 3.4% TAR in the water phase. In the sediment only traces of metabolites could be detected.

Figure 7.2.2.4-1: Proposed route of degradation of dimoxystrobin in water and sediment under outdoor conditions (from dimoxystrobin dossier 2001)



Overall, the understanding of the route of degradation of dimoxystrobin in surface waters (as shown in Figure 7.2.2.4-1) did not change.

The half-lives of dimoxystrobin and metabolites (where feasible) in water/sediment under outdoor conditions were re-calculated according to the newest guidelines and guidance documents and listed in Table 7.2.2.4-8.

Report: CA 7.2.2.4/1
Budde E., 2015a
Kinetic evaluation of two water-sediment studies with BAS 505 F - Dimoxystrobin according to FOCUS Degradation Kinetics
2014/1133879
Guidelines: FOCUS Kinetics (2006) SANCO/10058/2005 version 1.0 of Nov. 2011
GLP: no

Executive Summary

The aim of the study was to evaluate the kinetics of BAS 505 F - dimoxystrobin under aerobic aquatic conditions in three water/sediment systems in two studies [*BASF DocID 2000/1000121; BASF DocID 2000/1014987*]. In the first study, two different natural systems of water and sediment were treated with phenyl-[^{14}C] and benzyl-[^{14}C] labeled dimoxystrobin and incubated in the dark under aerobic conditions for 100 days. In a higher tier approach, a natural system of water and sediment was treated with [benzyl-U-^{14}C] labeled dimoxystrobin and incubated outdoor for 120 days under aerobic conditions and exposed to natural sunlight.

The kinetic analysis was carried out following the recommendations of the FOCUS workgroup on degradation kinetics to derive trigger endpoints and modeling endpoints.
In the outdoor test system, variations in temperature were observed over the study duration. To obtain endpoints suitable for use in environmental fate models, the data were time-step normalized to a reference temperature of 20°C in order to derive normalized modeling endpoints (DT_{50}).

Kinetic evaluation for the parent substance was performed at P-I level (one-compartment approach) as well as at the P-II level (two-compartment approach: water and sediment).

Kinetic evaluation of metabolite dissipation/degradation was attempted for metabolites 505M96, 505M01, 505M08 and 505M09.

For the two laboratory systems, trigger $DegT_{50}$ values of 297.6 and 834.5 days in the total system and 13.6 and 25.3 days in the water compartment were calculated. For the outdoor system, trigger $DegT_{50}$ values of 26.7, 18.9 and 101.3 days were determined for the total system, the water compartment and the sediment phase, respectively.

Reliable modeling-$DegT_{50}$ values for the whole system were derived for system A (Kellmetschweiher) with 525.6 days, system B (Berghäuser Altrhein) with 297.6 days and outdoor system (Kellmetschweiher) with 35.2 days. For the water compartment, $DegT_{50}$ values of 81.3, 52.5 and 21.4 days were determined for System A, System B and the outdoor system, respectively. For P-II level no trigger or modeling endpoints could be calculated.

For metabolite 505M01, a trigger-$DisT_{50}$ of 31.2 days and a modeling-$DegT_{50}$ of 1.7 days was calculated, while for metabolite 505M96, a trigger-$DisT_{50}$ of 43.4 days and a modeling-$DegT_{50}$ of 16.9 days was calculated. No reliable endpoints could be derived for metabolites 505M08 and 505M09.

I. MATERIAL AND METHODS

Test systems

Laboratory study (dark study) [*BASF DocID 2000/1000121*]

The distribution and degradation of dimoxystrobin was studied in two natural systems of water and sediment. The water/sediment systems were taken from Kellmetschweiher (System A), and Berghäuser Altrhein, a pond-like side arm of a river (System B), both in Rhineland-Palatinate, Germany. The characteristics of the aquatic test systems Kellmetschweiher and Berghäuser Altrhein are described in Table 7.2.2.4-1.

Table 7.2.2.4-1: Characteristics of the test systems incubated in the dark

Designation origin	System A Kellmetschweiher Rhineland-Palatinate, Germany	System B Berghäuser Altrhein Rhineland-Palatinate, Germany
Sediment		
Sampling depth [cm]	~ 20	~ 20
Textural class (USDA)	loamy sand	sandy loam
Particle size distribution sand [%] silt [%] clay [%]	 83 8 9	 55 33 12
pH ($CaCl_2$)	7.5	7.6
Organic C [%]	1.2	1.0
Water		
pH	8.5	8.2
Hardness [mmol L^{-1}]	0.93	1.03
TOC [mg L^{-1}]	12.7	4.6
Total N [mg L^{-1}]	1	1
Total P [mg L^{-1}]	<3	<3

Two radiolabeled forms of dimoxystrobin, phenyl-[^{14}C] and benzyl-[^{14}C], were applied separately to the test systems. Dimoxystrobin was applied to the water at a rate of 30 µg a.s. per test vessel which corresponds to an application rate of 300 g a.s. ha^{-1}, when assuming direct overspray of a 30 cm deep water body. The systems were incubated in the dark at a temperature of 20 ± 2°C for 100 days. Aeration was achieved by a stream of air over the water surface.

Sampling intervals were at 0, 1, 2, 7, 14, 30, 62, and 100 days after treatment (DAT). The samples were worked up and measured by HPLC.

Dimoxystrobin was the only major compound in both water/sediment systems. The metabolites 505M08 (BF 505-7) and 505M09 (BF 505-8) could be detected only in the water phase and only in low amounts (≤ 5% TAR). In the sediment, no metabolites could be detected at any sampling time.

Higher-tier outdoor study [*BASF DocID 2000/1014987]*

The degradation of dimoxystrobin under aerobic conditions and exposed to natural sunlight was investigated in one natural water/sediment system. The water/sediment system was taken from a pond named Kellmetschweiher located in the south-western part of Germany. The characteristics of the Kellmetschweiher test system are described in Table 7.2.2.4-2.

Table 7.2.2.4-2: Characteristics of the test system incubated under outdoor conditions

Designation origin	**Kellmetschweiher Schifferstadt, Rhineland Palatinate, Germany**
Sediment	
Sampling depth [cm]	~ 20
Textural class (USDA)	loamy sand
Particle size distribution sand [%] silt [%] clay [%]	 84 4 12
Organic C [%]	1.6
Water	
pH	8.8
Total hardness [mmol L^{-1}]	1.04
TOC [mg L^{-1}]	16.8

The test systems were treated with [benzyl-U-^{14}C]-labeled dimoxystrobin at an application rate of 140 µg per test vessel, corresponding to a nominal application rate of 200 g a.s. ha^{-1}, when assuming direct overspray of a 30 cm deep water body.

The test systems were placed in big isolated plastic boxes filled with water to a distinct level to simulate a bigger water body with respective temperature compensation. Equilibration and subsequent incubation was carried out under outdoor conditions. Water temperature in one control vessel and air temperature above the test vessels were recorded with data loggers.

The vessels were exposed to natural light. In order to protect the vessels from rainfall, the vessels were placed under a plastic glass cover which was optimized concerning UV and visible light transmission. If no rainfall was forecasted the cover was removed.

Water temperatures during the test period were in the range from 9.9 to 26.5°C, with a mean temperature of 18.2°C. A decline towards the end of the study was observed, with temperature dropping to below 15 °C after day 90.

Samples were taken at 0, 1, 2, 7, 14, 30, 58, 103, and 120 DAT. One test vessel was worked up per sampling date. The water was analyzed by HPLC without further treatment. The sediment was extracted, and the extracts were analyzed by HPLC. Volatiles could not be trapped.

Numerous metabolites were formed in the water phase, but most of them never reached more than 3.5% TAR. The metabolite 505M96 was observed with 9.6% TAR after 30 days in the water phase, while metabolites 505M01 (BF 505-4), 505M08 (BF 505-7) and 505M97 were detected with less than 5% TAR. Metabolite 505M09 (BF 505-8) reached 6.4 %TAR in the total system at DAT 58, and did not decline significantly until the end of the study. In sediment, only traces of metabolites could be detected.

Kinetic modeling

The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]. The best-fit model was selected based on visual and statistical assessment, and corresponding DT_{50} and DT_{90} values are reported as *trigger endpoints*. Appropriate DT_{50} values for use in environmental fate models were derived depending on the kinetic model and are reported as *modeling endpoints*.

Experimental data of dimoxystrobin were analyzed at the P-I level (one-compartment approach) for degradation in the whole system as well as dissipation from the water phase and from the sediment phase of the test systems. At the P-II level (two-compartment approach: water and sediment), the kinetic analysis considered the degradation in water and sediment and the partitioning between both phases.

Kinetic evaluation at level M-I (dissipation) was performed for dimoxystrobin metabolites 505M96 and 505M01 observed in the outdoor test system Kellmetschweiher, and based on metabolite decline from the maximum occurrence in the total system to derive trigger and modeling endpoints. Only these two metabolites had a suitable data set, i.e., more than 3 data points when starting from the observed maximum.

In addition, kinetic evaluation at level M-I (degradation) to derive modeling endpoints was performed for metabolites 505M96, 505M01, 505M08 and 505M09. Estimation of metabolite degradation at level M-I requires fitting the data of parent and metabolites in a combined fit. This was attempted for the metabolites with maximum occurrences >5% (505M09, 505M96) and metabolites which showed a pattern of formation, plateau and decline phase (505M01, 505M08). The metabolite 505M97 did not fulfill these criteria and was therefore not included in the pathway fit. Consequently, the degradation pathway was implemented in KinGUI according to Figure 7.2.2.4-1, leaving out 505M97 (i.e., formation of 505M01, 505M08 and 505M09 from dimoxystrobin, and formation of 505M96 from 505M01).

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation reports.

Kinetic models included in the assessment

For each data set, the kinetic models proposed by FOCUS Kinetics [*FOCUS (2006)*] were tested in order to identify the best-fit model, i.e. single first order (SFO) kinetics, the Gustafson-Holden model (FOMC), and the bi-exponential (DFOP) kinetics. The respective model descriptions and corresponding equations for calculating endpoints are shown in the FOCUS Kinetics guidance [*FOCUS (2006)*].

The goodness-of-fit of the kinetic models was assessed by visual inspection and statistical measures, as recommended by the FOCUS Kinetics guidance [*FOCUS (2006)*].

Data handling

Degradation in the whole system and dissipation from the water phase of the parent compound (level P-I) were evaluated starting on the day of treatment (i.e. 0 days after treatment, DAT 0).

At level P-I of the analysis, the measured initial concentration of the parent substance in the total system or in water was set to the material balance recovered at DAT 0 as recommended by the FOCUS kinetics guidance [*FOCUS (2006)*]. At the P-II level, the total recovered amount at DAT 0 was considered as the measured initial concentration in water, while the initial concentration of the sediment phase was assumed to be zero.

At Level M-I, estimation of metabolite dissipation requires kinetics to be fitted to the corresponding decline data for each compartment, starting from the maximum observed level of the metabolite in the compartment. The dissipation of the metabolite was thus evaluated starting at the day of maximum occurrence that was defined as 0 days after maximum concentration (0 DAMC). All later time points were adjusted accordingly as days after maximum concentrations (DAMC).

Estimation of metabolite degradation at level M-I requires fitting the data of parent and metabolites in a combined fit. This was performed for metabolites 505M96, 505M01, 505M08 and 505M09. The measured initial concentration of all metabolites was set to zero at DAT 0 as recommended by the FOCUS kinetics guidance.

Values below the quantification or detection limit for parent compound and metabolites were treated as recommended by the FOCUS workgroup [*FOCUS (2006), chapter 6.1.4 and chapter 8.3.1*].

Residue data obtained from using separate labeling forms of dimoxystrobin in the dark laboratory study [*BASF DocID 2000/1000121*] were treated as replicates, as the test substance showed similar behavior with both labels.

Time-step normalization of outdoor study

To obtain endpoints suitable for use in environmental fate models, the data derived from the outdoor study were time-step normalized to derive normalized modeling endpoints. The normalization procedure was carried out by reducing or increasing day lengths depending on measured water temperature by means of a correction factor (f_{temp}). Daily water temperature values were available from the original raw data. The temperature normalization to a reference temperature of 20°C was performed using the temperature correction factor in Equation 7.2.2.4-1, and a default Q_{10} value of 2.58 was considered [FOCUS (2006)]. Daily correction factors for each day were calculated, and the cumulative time between sampling points was determined and used as input for kinetic evaluation.

Equation 7.2.2.4-1: Temperature correction factor calculation

$$f_{temp} = Q_{10}^{\frac{Tact - Tref}{10}}$$

with:			
	f_{temp}	temperature correction factor	[-]
	T_{act}	incubation temperature	[°C]
	T_{ref}	reference temperature (20°C)	[°C]
	Q_{10}	factor of increase of degradation rate with an increase in temperature of 10°C (Q_{10} = 2.58)	[-]

The normalized day lengths were derived according to Equation 7.2.2.4-2a. Normalized sampling days (DATnorm) after application were calculated by cumulatively summing up normalized day lengths according to Equation 7.2.2.4-2b.

Equation 7.2.2.4-2: Calculation of normalized day length based on temperature correction factors

a) $$D_{norm} = D * f_{temp}$$

b) $$t_i = \sum_{t=1}^{i-1} D_{norm}$$

with:		
	t_i =	Time from application till sampling at day i [d]
	D_{norm} =	Normalized day length (20°C) [d]
	i =	Time span between application and sampling [d]

c) $$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact - Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \le 0°C \end{cases}$$

with:		
	D_{norm} =	normalized day length (temperature and moisture) [d]
	f_{temp} =	temperature correction factor [-]
	D =	1 day
	T_{act} =	actual water temperature [°C]
	T_{ref} =	reference temperature, 20 °C
	Q_{10} =	factor of increase of degradation rate with an increase in temperature of 10°C, Q_{10} = 2.58 [-]

The sampling days and the corresponding normalized (20°C) day lengths based on the measured data of daily water temperature are presented in Table 7.2.2.4-3.

Table 7.2.2.4-3: Time-step normalized sampling days of the outdoor study

DAT	D_{norm}
0	0
1	1.4
2	2.8
7	7.8
14	13.1
30	31.8
58	70.0
103	102.2
120	110.4

Software for kinetic evaluation

The software package KinGUI (version 2.2012.320.1629) was used for parameter fitting [*SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. BASF DocID 2007/1062781*]. The error tolerance and the number of iterations of the optimization tool (IRLS) were set to the default values of 1×10^{-6} and 100, respectively.

II. RESULTS AND DISCUSSION

For modeling endpoints, the initial fit was performed using SFO kinetics. If the fit was not satisfactory, FOMC, DFOP and HS kinetics were tested. For trigger endpoints, SFO and FOMC kinetics were tested in a first step; if SFO was not acceptable or worse than FOMC, DFOP and HS kinetics were tried in addition. Graphical presentations of the tested kinetic models and the results of the χ^2 - test and all other statistical endpoints used in the decision-making process are given in the original study reports.

Level P-I

An overview of the estimated trigger and modeling endpoints for dimoxystrobin from three water/sediment systems is given in Table 7.2.2.4-4.

Table 7.2.2.4-4: Summary of trigger and modeling endpoints for dimoxystrobin (Level P-I)

Test system, BASF DocID	Trigger endpoints				Modeling endpoints		
Total system	**Kinetic model**	**χ^2 error**	**$DegT_{50}$ [d]**	**$DegT_{90}$ [d]**	**Kinetic model**	**χ^2 error**	**$DegT_{50}$ [d]**
Kellmetschweiher (system A), 2000/1000121	HS	0.5	834.5	>1000	SFO	1.0	525.6[a]
Berghäuser Altrhein (system B), 2000/1000121	SFO	1.5	297.6	988.7	SFO	1.5	297.6[a]
Kellmetschweiher (outdoor), 2000/1014987	HS	4.4	26.7	>1000	SFO	7.4	35.2[c]
Water compartment	**Kinetic model**	**χ^2 error**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Kinetic model**	**χ^2 error**	**$DisT_{50}$ [d]**
Kellmetschweiher (system A), 2000/1000121	DFOP	2.4	25.3	213.9	DFOP	2.4	81.3[b]
Berghäuser Altrhein (system B), 2000/1000121	DFOP	3.0	13.6	126.9	DFOP	3.0	52.5[b]
Kellmetschweiher (outdoor), 2000/1014987	HS	4.5	18.9	140.4	SFO	6.6	21.4[d]
Sediment compartment	**Kinetic model**	**χ^2 error**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Kinetic model**	**χ^2 error**	**$DisT_{50}$ [d]**
Kellmetschweiher (outdoor), 2000/1014987	FOMC[f]	3.5	101.3	>1000	SFO[f]	9.7	87.9[e]

a much longer than the study period of 100 d; interpret with care
b $DisT_{50}$ calculated from DFOP slow phase ($DisT_{50} = \ln2/k2$)
c temperature-normalized value at reference temperature of 20°C (non-normalized value: 32.5 d)
d temperature-normalized value at reference temperature of 20°C (non-normalized value: 20.2 d)
e temperature-normalized value at reference temperature of 20°C (non-normalized value: 197.1 d)
f outlier removed

Level P-II

The kinetic evaluation resulted in acceptable SFO fits for the evaluated water/sediment systems, with back-transfer rates greater than zero. However, for Kellmetschweiher (system A of dark study) and the outdoor system, the F_{sed} test was passed, but at least one degradation rate in each system failed the t-test. For Berghäuser Altrhein (system B of dark study) and the time-step normalized outdoor system, the F_{sed} test failed. Consequently, no trigger or modeling endpoints were calculated. For modeling, endpoints should be set using a default approach according to FOCUS kinetics.

Level M-I

A summary of the trigger and modeling endpoints (dissipation) (Table 7.2.2.4-5) and modeling endpoints (degradation) (Table 7.2.2.4-6) for dimoxystrobin metabolites is given below.

Table 7.2.2.4-5: Summary of estimated trigger and modeling endpoints for the metabolites of dimoxystrobin (Level M-I dissipation*, total system)

Test system	Substance	Trigger endpoints				Modeling endpoints		
		Kinetic model	χ^2 error	$DisT_{50}$ [d]	$DisT_{90}$ [d]	Kinetic model	χ^2 error	$DisT_{50}$ [d]
Kellmetschweiher (outdoor), total system	505M01	SFO	4.4	31.2	103.6	SFO	3.5	34.5[a]
	505M96	SFO	4.2	43.4	144.2	SFO	11.4	43.8[a]

* dissipation from maximum occurrence
[a] temperature-normalized value

Table 7.2.2.4-6: Summary of estimated modeling endpoints for the metabolites of dimoxystrobin (Level M-I degradation*, total system)

Test system	Substance	Kinetic model	χ^2 error	Formation fraction [-]	Std. error of FF [-]	$DegT_{50}$ [a] [d]
Kellmetschweiher (outdoor), total system	505M01	SFO	18.8	0.8809[b]	0.0844	1.7
	505M08	SFO	21.0	no reliable endpoints derived		
	505M09	SFO	24.2	no reliable endpoints derived		
	505M96	SFO	21.4	0.3770[c]	0.0873	16.9

* considering formation and degradation in a linked model
[a] temperature-normalized value at reference temperature of 20°C
[b] from parent
[c] from 505M01

III. CONCLUSION

The dissipation and degradation of dimoxystrobin in three water/sediment systems was evaluated according to the recommendations of the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*] to determine trigger and modeling endpoints. In the first study, two different natural systems of water and sediment were incubated in the dark under aerobic conditions and in the second study, a natural system of water and sediment was incubated outdoor under aerobic conditions and exposed to natural sunlight.

For the two laboratory systems, trigger $DegT_{50}$ values of 297.6 and 834.5 days in the total system and 13.6 and 25.3 days in the water compartment were calculated. For the outdoor system, trigger $DegT_{50}$ values of 26.7, 18.9 and 101.3 days were determined for the total system, the water compartment and the sediment phase, respectively.

Reliable modeling-$DegT_{50}$ values for the whole system were derived for system A (Kellmetschweiher) with 525.6 days, system B (Berghäuser Altrhein) with 297.6 days and outdoor system (Kellmetschweiher) with 35.2 days. For the water compartment, $DegT_{50}$ values of 81.3, 52.5 and 21.4 days were determined for System A, System B and the outdoor system, respectively. For P-II level no trigger or modeling endpoints could be calculated.

For metabolite 505M01, a trigger-$DisT_{50}$ of 31.2 days and a modeling-$DegT_{50}$ of 1.7 days was calculated, while for metabolite 505M96, a trigger-$DisT_{50}$ of 43.4 days and a modeling-$DegT_{50}$ of 16.9 days was calculated. No reliable endpoints could be derived for metabolites 505M08 and 505M09.

Summary of occurrences and degradation rates of dimoxystrobin and its metabolites

Table 7.2.2.4-7: Maximum occurrence of dimoxystrobin metabolites in water phase of water/sediment studies

Metabolite	Matrix	BASF DocID	System	Incubations	Parent label	Maximum % AR
505M08 (BF 505-7)	water	2000/1000121 [1]	Kellmetschweiher	dark	phenyl benzyl	0.8 [2] 0.7 [2]
			Bergh. Altrhein	dark	phenyl benzyl	2.1 [2] 2.9 [2]
		2000/1014987 [1]	Kellmetschweiher	irradiated (outdoor)	benzyl	3.6
505M09 (BF 505-8)	water	2000/1000121 [1]	Kellmetschweiher	dark	phenyl benzyl	3.4 [2] 3.7 [2]
			Bergh. Altrhein	dark	phenyl benzyl	4.7 [2] 5.2 [2]
		2000/1014987 [1]	Kellmetschweiher	irradiated (outdoor)	benzyl	5.3
505M01 (BF 505-4)	water	2000/1014987 [1]	Kellmetschweiher	irradiated (outdoor)	benzyl	3.2
505M96	water	2000/1014987 [1]	Kellmetschweiher	irradiated (outdoor)	benzyl	9.6
505M97	water	2000/1014987 [1]	Kellmetschweiher	irradiated (outdoor)	benzyl	3.4

[1] already peer-reviewed during previous EU evaluation
[2] Values taken from appendix of original report; for modeling, mean values as listed in the table section of the original report and former dossier tables are used.

Table 7.2.2.4-8: Summary table on kinetic endpoints of dimoxystrobin and metabolites obtained in water/sediment studies

Study BASF DocID	Water/ sediment system	pH water	pH sed ($CaCl_2$)	T [°C]	DT_{50} whole system [d]	Kinetic model	DT_{50} water [d]	Kinetic model	DT_{50} sediment [d]	Kinetic model
dimoxystrobin										
2000/1000121 dark	Kellmetschw.	8.5	7.5	20	834.5	HS	25.3	DFOP	-	-
2000/1000121 dark	Berghäuser Altrhein	8.2	7.6	20	297.6	SFO	13.6	DFOP	-	-
2000/1014987 irradiated	Kellmetschw.	8.8	n.r.	outdoor[a]	26.7	HS	18.9	HS	101.3	FOMC
505M01										
2000/1014987 irradiated	Kellmetschw.	8.8	n.r.	outdoor[a]	31.2 [b] 1.7 [c]	SFO SFO	-	-	-	-
505M96										
2000/1014987 irradiated	Kellmetschw.	8.8	n.r.	outdoor[a]	43.4 [b] 16.9 [c]	SFO SFO	-	-	-	-

n.r. not reported in study
[a] mean water temperature over incubation period 18.1°C (min. 8.6°C, max. 28.5°C)
[b] Level M-I dissipation, total system ($DisT_{50}$ considering decline from time of maximum occurrence)
[c] Level M-I degradation, total system ($DegT_{50}$ considering simultaneous formation and degradation of metabolite)

CA 7.2.3 Degradation in the saturated zone

Adsorption/desorption studies with dimoxystrobin showed adsorption coefficients of 0.6 - 18.6 ($K_{f,oc}$ 196 - 935 mL/g). $PEC_{groundwater}$ calculations from previous and also current dossier as well as the old lysimeter study clearly showed no risk of displacement of active substance into deeper soil layers or even groundwater. Investigations on the degradation in the saturated zone are therefore considered not necessary.

CA 7.3 Fate and behaviour in air

Dimoxystrobin is characterized by a low vapor pressure (6.0 x 10^{-7} Pa at 20°C) and a low volatilization from soil and plant surfaces (≤ 3% in 24h). Furthermore, in the air it is rapidly degraded by photochemical processes.

CA 7.3.1 Route and rate of degradation in air

No new experimental data are available, but a new calculation of the photochemical oxidative degradation in air (Atkinson) according to the newest model is provided below.

Report: CA 7.3.1/1
Hassink J., 2015a
Photochemical oxidative degradation of BAS 505 F (QSAR estimates)
2015/1001041
Guidelines: EC 1107/2009 of the European Parliament
GLP: no, not applicable

Executive Summary

The degradation rates for reactions of BAS 505 F with OH radicals and ozone in the atmosphere were calculated using the AOPWIN program based on ATKINSON's increment method. Based on the resulting degradation rate of k_{OH} = 86.6816 10^{-12} cm^3 $molecule^{-1}s^{-1}$, the atmospheric degradation half-life of the substance via this reaction route is $t_{1/2}$ = 0.123 d (12 h day). Although O_3 is likely to react with BAS 505 F, the degradation rate resulting from ozone attack could not be estimated by the OECD method due to lack of increments.

I. MATERIAL AND METHODS

Internal Code: BAS 505 F
Common Name : Dimoxystrobin
Reg. No.: 285028
CAS No. 149961-52-4
Chemical Name: (E)-o-(2,5-dimethylphenoxymethyl)-2-methoxyimino-N-methylphenyl-acetamide
Molar Mass: 326.4 g mol 1
Empirical Formula: $C_{19}H_{22}N_2O_3$

OH-radical attack

Using the computer program AOPWIN (AOPWIN Program (Atmospheric Oxidation Program for Microsoft Windows 3.1) Version 1.88, Syracuse Research Corp.) which is based on the increment system published by Atkinson, the degradation rate for reactions of dimoxystrobin with hydroxyl radicals is calculated based on the structural formula. Assuming a pseudo-first order reaction, the degradation half-life via this reaction route is calculated by taking into account the diurnally and seasonally averaged concentration of hydroxyl radicals in the troposphere.

The degradation of a compound A by OH-radicals can then be calculated by

$$-d[A]/dt = k' \cdot [A] \quad (1)$$
with $k' = k \cdot [\text{OH-radicals}]$

The half-life of this process can be calculated by equation (2):

$$t_{1/2} = \ln 2/k' = \ln 2 / (k \cdot [\text{OH-radicals}]) \quad (2)$$

Ozone attack

The degradation rate resulting from ozone attack can be determined with an increment method. The half-life for this process can then be derived as described in equation 3 by taking into account the concentration of ozone molecules in the air:

$$t_{1/2} = \ln 2/k' = \ln 2/k \cdot [\text{ozone molecules}] \quad (3)$$

II. RESULTS AND DISCUSSION

The AOPWIN program uses the SMILES notation as basis for the calculation. The SMILES code for dimoxystrobin is:

Cc1cc(c(cc1)C)OCc2ccccc2C(=NOC)C(=O)NC

For some chemical groups increment data for the Atkinson method are missing. The missing data were estimated using "assumed values" by AOPWIN. An "assumed value" is a value of a structure fragment that has not been assigned a numeric value by Atkinson or derived explicitly from experimental values.

The total rate constant is $k = 86.6816\ 10^{-12}\ cm^3\ molecule^{-1}s^{-1}$.

The weighted global average tropospheric hydroxyl radical concentration is $1.5\ 10^6\ mol\ cm^{-3}$. Conclusively, the half-life for the degradation of dimoxystrobin by OH-radicals as calculated with equation (2) is:

$t_{1/2}$ = ln2 / ($86.6816\ 10^{-12}\ 1.5\ 10^6$) s
= 1.481 h
= 0.123 d (12 h day)

Although dimoxystrobin contains reactive sites for an ozone attack no increments are available and a reasonable approximation by AOPWIN is not possible. Therefore, although O_3 is likely to react with dimoxystrobin no degradation estimation can be given.

CA 7.3.2 Transport via air

Dimoxystrobin has a very low volatilization potential and is degraded very fast by photochemical processes. Consequently, there is no risk of long-range transport of dimoxystrobin.

CA 7.3.3 Local and global effects

No effects are expected since transport via air is very unlikely (for details see above).

CA 7.4 Definition of the residue

CA 7.4.1 Definition of the residue for risk assessment

According to the results presented in M-CA 7.1 – 7.3 the following compounds have to be considered for the environmental risk assessment:

Soil:
Dimoxystrobin and its aerobic soil metabolites 505M01 (BF 505-4), 505M08 (BF 505-7), 505M09 (BF 505-8)

All three metabolites were tested in terrestrial ecotox studies. Based on the results it can be concluded that the risk of metabolites for soil organisms is negligible.

Groundwater:
Dimoxystrobin and its soil metabolites 505M01 (BF 505-4), 505M08 (BF 505-7), 505M09 (BF 505-8).

Based on the fast degradation rate in soil, metabolite 505M01 does not pose any risk of leaching to groundwater. Due the rather low sorption of the carboxylic acid metabolites 505M08 and 505M09, exceedance of the drinking water limit of 0.1 µg/L could not be excluded according to current $PEC_{groundwater}$ calculations (see chapter MCP 9.2). Therefore, a relevance assessment according to SANCO 221/2000 rev.10 was performed (see Doc N4). Both metabolites proved to be not relevant.

Surface Water:
Dimoxystrobin and its metabolites 505M08, 505M09, 505M01, 505M96

The aquatic toxicity of all metabolites was tested and was found to be three orders of magnitude lower for the most sensitive species than parent. Sufficient margins of safety were reached after FOCUS surface water step 1-2 calculations and it is concluded that the risk of metabolites for aquatic organisms is negligible.

Sediment:
Dimoxystrobin (all metabolites in sediment < 1.5% TAR)

Air:
Dimoxystrobin (no volatile metabolite detected)

CA 7.4.2 Definition of the residue for monitoring

According to the results of the risk assessment the following compounds should be considered for environmental monitoring:

Soil: Dimoxystrobin (parent only)

Ground Water: Dimoxystrobin (parent only)

Surface Water: Dimoxystrobin (parent only)

Sediment: Dimoxystrobin (parent only)

Air: Dimoxystrobin (parent only)

CA 7.5 Monitoring data

According to the knowledge of the applicant, there are currently no published environmental monitoring data available for dimoxystrobin or its metabolites, which would provide knowledge on the environmental behaviour not covered by this dossier.
– 24/Jul/2015

Dimoxystrobin

Document M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

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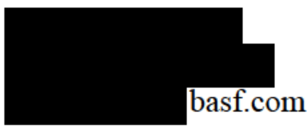
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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Dimoxystrobin (BAS 505 F), a fungicide for use in rape and sunflower, is registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included in Annex I by Commission Directive No 2006/75/EC. Inclusion entered into force on 11 September 2006.
The approval was transferred to the new Regulation (EC) No 1107/2009 in Commission Implementing Regulation (EU) No 540/2011. Approval extension was granted until 31 January 2018 by Implementing Regulation (EU) No 1136/2012.

All relevant information on the first Annex I review and the endpoints used in ecotoxicological risk assessments can be found in the monograph of dimoxystrobin, in the EFSA conclusion on dimoxystrobin (2005) and in SANCO/10531/05-Final document (EU Review Report of April 2006).

For the current registration renewal under Regulation 1107/2009, a data gap analysis according to new guidelines and new guidance documents was performed and new studies or evaluations were initiated where considered necessary. All new data are provided in this section or in the respective sections of the dossier for the new representative formulation.

Furthermore, a literature search was performed and scientific publications were evaluated for their endpoint relevance and quality. Although title and abstract of several publications indicated a potential connection to respective ecotoxicological chapters of this dossier, the detailed evaluation of these publications showed no endpoint of sufficient reliability which could be used for the required risk assessments. Consequently, for ecotoxicology, no summaries of public literature data on dimoxystrobin are provided in this section. Further information on the literature assessment and respective justifications can be found in M-CA 9.

An overview of metabolites relevant for the environment is given in M-CA 7. The table is including the different code numbers that are available for each metabolite. In the following chapters and study summaries synonym metabolite codes are given in brackets where deemed to be helpful.

CA 8.1 Effects on birds and other terrestrial vertebrates

No new studies are available on the toxicity of dimoxystrobin to birds or mammals. However, below are summaries of 3 new studies on the degradation of dimoxystrobin residues on plants. These data are used in the mammalian risk assessment in chapter M-CP 10.1.

Report: CA 8.1/1
Martin T., 2015a
Study on the residue behavior of BAS 505 F (Dimoxystrobin) on wheat (young plants) after the application of BAS 540 01 F under field conditions in Germany, Netherlands, Italy and Spain, 2013/2014
2014/1275093

Guidelines: EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, EEC 7029/VI/95 rev. 5 (July 22, 1997), EEC 7525/VI/95 rev. 9 (March 2011)

GLP: yes
(certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

Executive Summary

The objective of the study was to determine the residue behaviour of dimoxystrobin (BAS 505 F) and the metabolite 505M98 on young wheat (Martin 2015, BASF DocID 2014/1275093) plants after one application of BAS 540 01 F. Samplings were carried out directly after and at subsequent time intervals after the application. The selected application rate corresponds to the maximum single rate of the Good Agricultural Practice (critical GAP) of BAS 540 01 F.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:	BAS 540 01 F
Description:	BAS 540 01 F (dimoxystrobin)
Lot/Batch #:	(200 g/l BAS 540 01 F, SC), BAS 540 01 F: 0003354823
Purity:	BAS 505 F: 99.7%; 505M98: 99.5%
CAS#:	BAS 505 F: 149961-52-4; BAS 510 F: 188425-85-6 505M98: 149923-06-8
Crop parts(s) or Processed commodity:	wheat (young plants without roots; BBCH 11-22)
Sample size:	22.41-108.08g

B. STUDY DESIGN

Study site

During the growing seasons of 2013 and 2014, a total of eight trials were conducted in representative wheat growing areas in Germany, the Netherlands, Spain and Italy.

Test item and application

The trials consisted of a control plot (untreated, plot 1) and one treated plot (plot 2) without replication. No product containing the test item was used on the test plots during the season.

The test item BAS 540 01 F was foliar applied on plot 2 at a nominal rate of 0.1 kg dimoxystrobin/ha in a nominal spray volume of 200 L/ha at growth stage (BBCH) 11-13 according to Good Laboratory Practice.

Sampling information

For this study treated specimens were collected as wheat whole plants without roots 1 hour after the last application as well as 1, 2, 3, 4, 5, 7, 10, 12, and 14 days thereafter. Untreated specimens were collected also as whole plants without roots 1 hour after application as well as 5 and 14 days thereafter. Each specimen was collected in duplicate (“ship” and “retain”), so that reserve specimens were available in case of potential problems during sample shipment, storage or analysis.

Damaged plants were not harvested. Each specimen was collected randomly from a minimum of 12 different places within each plot.

Untreated specimens were obtained prior to treated specimens when sampling times coincided to avoid contamination. Each specimen was placed into an individual plastic bag and was subsequently double bagged with a second plastic bag. Specimen labels were fixed to the inner plastic bags detailing the specimen type, specimen number, trial and study number. The retain specimens were harvested at the same time of the original ones and were destroyed in the facilities of each test site or the Test Facility by agreement of the Study Director and Sponsor.

All specimens were stored at ≤ -18ºC and were sent to Specimen Management in BASF SE Agricultural Center Limburgerhof.

Description of analytical methods

All specimens were analyzed for BAS 505 F (Dimoxystrobin) and its Z-isomer 505M98 (M505F098) using BASF method no. L0076/08. The method has a limit of quantitation (LOQ) of 0.005 mg/kg for both analytes.

For further details on the analytical methods, please consult the consumer safety part (M-CA, section 6, chapter CA 6.3).

The results of procedural recovery experiments averaged 104% for wheat plants for BAS 505 F at fortification levels between 0.005 and 50 mg/kg and 99.2% for metabolite 505M98 at fortification levels between 0.005 and 50 mg/kg.

II RESULTS AND DISCUSSION

Dimoxystrobin

The dimoxystrobin residues in the wheat specimens taken 0 DALA (1 HALA) ranged from 3.5 - 7.0 mg/kg. They changed to 3.1-9.0 mg/kg in the specimens taken 1 DALA and further to 2.3 - 8.0 mg/kg at 2 DALA. In the specimens taken 3 DALA 1.7 - 6.2 mg/kg were determined. The residue level in the specimens taken 4 DALA was 1.9 - 5.4 mg/kg, whereas in those taken 5 DALA 1.3 - 6.1 mg/kg were found. Afterwards a steady decline was observed in the specimens taken 7 DALA (0.37 - 4.7 mg/kg), 10 DALA (0.20 - 5.5 mg/kg) and 12 DALA (0.096 – 3.6 mg/kg). At the last sampling (14 DALA they remained at this level (0.046 – 2.5 mg/kg).

No residues of dimoxystrobin above the limit of quantitation were found in any of the analysed untreated specimens.

Metabolite - 505M98

The 505M98 residues in the wheat specimens taken 0 DALA (1 HALA) ranged from < 0.005 – 0.010 mg/kg. They increased in the specimens taken at 1 DALA (< 0.005 – 0.059 mg/kg). On the second DALA the residues dropped to 0.016 – 0.12 mg/kg). At 3 DALA, specimens 0.014 – 0.12 mg/kg were found, whereas the specimens taken 4 and 5 DALA showed a slight increase to 0.018 – 0.12 mg/kg and 0.012 – 0.15 mg/kg respectively. Afterwards the residues decreased to 0.0086 – 0.15 mg/kg (7 DALA) and further to 0.0083 – 0.17 mg/kg (10 DALA). In the specimens taken 12 DALA and at the last sampling (14 DALA) they remained at this level (< 0.005 – 0.14 mg/kg and < 0.005 – 0.098 mg/kg respectively).

No residues of 505M98 above the limit of quantitation were found in any of the analysed untreated specimens.

Table 8.1-1: Summary of residues of dimoxystrobin and 505M98 in wheat (whole plant without roots) (Martin 2015, BASF DocID 2014/1275093)

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Residues (mg/kg)	
					Dimoxystrobin	505M98
Trial no. L130729 Study site: Germany	1 HALA	1	21.10.2013	11	4.0[1)]	< 0.005[1)]
	1 DALA	2	22.10.2013	11	4.4	0.038
	2 DALA	3	23.10.2013	11-12	3.7	0.040
	3 DALA	4	24.10.2013	11-12	3.2	0.037
	4 DALA	5	25.10.2013	11-12	2.5	0.038
	5 DALA	6	26.10.2013	12-13	1.3	0.032
	7 DALA	7	28.10.2013	12-13	0.43	0.0086
	10 DALA	8	31.10.2013	13	0.34	0.016
	12 DALA	9	02.11.2013	13-21	0.19	0.0055
	14 DALA	10	04.11.2013	13-21	0.088	< 0.005
Trial no. L130730 Study site: Germany	1 HALA	1	06.03.2014	13	3.9[1)]	0.007[1)]
	1 DALA	2	07.03.2014	13	4.2	0.054
	2 DALA	3	08.03.2014	13	3.6	0.057
	3 DALA	4	09.03.2014	13-14	2.8	0.051
	4 DALA	5	10.03.2014	13-21	3.2	0.062
	5 DALA	6	11.03.2014	13-22	2.7	0.061
	7 DALA	7	13.03.2014	21-22	2.2	0.060
	10 DALA	8	16.03.2014	21-22	0.59	0.015
	12 DALA	9	18.03.2014	21-22	0.47	0.018
	14 DALA	10	20.03.2014	21-22	0.19	0.0059
Trial no. L130731 Study site: Netherlands	1 HALA	1	10.03.2014	13	4.1	0.0089
	1 DALA	2	11.03.2014	13	3.3	0.020
	2 DALA	3	12.03.2014	13	3.9	0.048
	3 DALA	4	13.03.2014	14	3.9	0.069
	4 DALA	5	14.03.2014	14	1.9	0.043
	5 DALA	6	15.03.2014	14	2.4	0.046
	7 DALA	7	17.03.2014	15	1.4	0.028
	10 DALA	8	20.03.2014	18	0.59	0.017
	12 DALA	9	22.03.2014	21	0.24	0.0072
	14 DALA	10	24.03.2014	21	0.14	0.0053
Trial no. L130732 Study site: Netherlands	1 HALA	1	10.03.2014	13	4.3	0.0089
	1 DALA	2	11.03.2014	13	3.3	0.019
	2 DALA	3	12.03.2014	13	4.1	0.053
	3 DALA	4	13.03.2014	14	3.5	0.057
	4 DALA	5	14.03.2014	14	2.5	0.050
	5 DALA	6	15.03.2014	14	1.8	0.030
	7 DALA	7	17.03.2014	15	1.7	0.031
	10 DALA	8	20.03.2014	18	0.51	0.016
	12 DALA	9	22.03.2014	21	0.26	0.0059
	14 DALA	10	24.03.2014	21	0.15	0.0051
Trial no. L130733 Study site: Spain	1 HALA	1	31.10.2013	12	5.5[1)]	0.01[1)]
	1 DALA	2	01.11.2013	12	5.4	0.056
	2 DALA	3	02.11.2013	12	4.4	0.081
	3 DALA	4	03.11.2013	12-13	3.7	0.067
	4 DALA	5	04.11.2013	13	2.9	0.078
	5 DALA	6	05.11.2013	13	2.5	0.058
	7 DALA	7	07.11.2013	13	0.53	0.017
	10 DALA	8	10.11.2013	13	0.20	0.0083
	12 DALA	9	12.11.2013	14	0.096	< 0.005
	14 DALA	10	14.11.2013	14	0.046	< 0.005

Trial details	Sampling timing	Sampling no.	Date	Crop growth stage (BBCH)	Residues (mg/kg)	
					Dimoxystrobin	505M98
Trial no. L130734 Study site: Spain	1 HALA	1	18.02.2014	12	4.5[1)]	< 0.005[1)]
	1 DALA	2	19.02.2014	12	3.5	0.059
	2 DALA	3	20.02.2014	12-13	3.0	0.077
	3 DALA	4	21.02.2014	13	2.4	0.082
	4 DALA	5	22.02.2014	13	2.0	0.072
	5 DALA	6	23.02.2014	13	1.8	0.078
	7 DALA	7	25.02.2014	13	0.37	0.018
	10 DALA	8	28.02.2014	14	0.33	0.016
	12 DALA	9	02.03.2014	14	0.27	0.011
	14 DALA	10	04.03.2014	14	0.17	0.0076
Trial no. L130735 Study site: Italy	1 HALA	1	11.11.2013	12-13	3.5[1)]	< 0.005[1)]
	1 DALA	2	12.11.2013	12-13	3.1	< 0.005
	2 DALA	3	13.11.2013	13	2.3	0.016
	3 DALA	4	14.11.2013	13	1.7	0.014
	4 DALA	5	15.11.2013	13	2.0	0.018
	5 DALA	6	16.11.2013	13	1.6	0.012
	7 DALA	7	18.11.2013	13-14	1.1	0.014
	10 DALA	8	21.11.2013	13-14	1.1	0.018
	12 DALA	9	23.11.2013	14	0.24	< 0.005
	14 DALA	10	25.11.2013	14-15	0.17	< 0.005
Trial no. L130736 Study site: Italy	1 HALA	1	06.12.2013	13	7.0[1)]	< 0.005[1)]
	1 DALA	2	07.12.2013	13	9.0	0.028
	2 DALA	3	08.12.2013	13	8.0	0.12
	3 DALA	4	09.12.2013	13	6.2	0.12
	4 DALA	5	10.12.2013	13	5.4	0.12
	5 DALA	6	11.12.2013	13	6.1	0.15
	7 DALA	7	13.12.2013	13	4.7	0.15
	10 DALA	8	16.12.2013	14	5.5	0.17
	12 DALA	9	18.12.2013	14	3.6	0.14
	14 DALA	10	20.12.2013	14	2.5	0.098

HALA: hours after last application; DALA: days after last application
1 mean of two values

III. CONCLUSION

In wheat plant samples collected directly after the application (BBCH 11-13), the residues of BAS 505 F ranged between 3.5 to 7.0 mg/kg. At the last sampling at 14 DALA (BBCH 13-22) residues of BAS 505 F decreased to a range of 0.046 to 2.5 mg/kg. Residues of 505M98 were in the range of <0.005-0.010 mg/kg directly after the application (BBCH 11-13). At the last sampling at 14 DALA (BBCH 13-22) residues of 505M98 ranged between < 0.005 to 0.098 mg/kg.

Residues of BAS 505 F and 505M98 were below 0.005 mg/kg in all control specimens.

Report:	CA 8.1/2 Moreno S.,Galvez O., 2015a Study on the residue behaviour of Dimoxystrobin (BAS 505 F) on pea after treatment with BAS 540 01 F under field conditions in North and South Europe, season 2013 - 2014 2014/1001972
Guidelines:	EC 1107/2009 of the European Parliament and of the Council of 21 Oct 2009, International guidelines for distribution and pesticides application AEPLA FAO 1985, EEC 7029/VI/95 rev. 5 Appendix B (July 22, 1997), OECD 509 Crop Field Trial (2009), EEC 7525/VI/95 rev. 9 (March 2011)
GLP:	yes (certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

Executive Summary

The objective of the study was to determine the residue behaviour of dimoxystrobin (BAS 505 F) and the metabolite 505M98 on young pea plants after one application of BAS 540 01 F. Samplings were carried out directly after and at subsequent time intervals after the application. The selected application rate corresponds to the maximum single rate of the Good Agricultural Practice (critical GAP) of BAS 540 01 F.

I. MATERIAL AND METHODS

A. MATERIALS

Test Material:	BAS 540 01 F
Description:	BAS 540 01 F (dimoxystrobin)
Lot/Batch #:	(200 g/l BAS 540 01 F, SC), BAS 540 01 F: 0003354823 except in trials of pea study L130773, L130774, L130775, L130776, L130778 and L130780: FRE-000949
Purity:	BAS 505 F: 99.7%; 505M98: 99.5%
CAS#:	BAS 505 F: 149961-52-4; BAS 510 F: 188425-85-6 505M98: 149923-06-8
Crop parts(s) or Processed commodity:	peas (young plants without roots; BBCH 12-30)
Sample size:	50.3-170g

B. STUDY DESIGN

Study site

During the growing seasons of 2013 and 2014, a total of eight trials were conducted in representative pea growing areas in Germany, the Netherlands, Spain and Italy.

Test item and application

The trials consisted of a control plot (untreated) and one treated plot (plot 2) without replication. No product containing the test item was used on the test plots during the season.

The test item BAS 540 01 F was foliar applied on plot 2 at a nominal rate of 0.1 kg dimoxystrobin/ha in a nominal spray volume of 200 L/ha at a growth stage (BBCH) 12-13 in peas according to Good Laboratory Practice.

Sampling information

For this study treated specimens were collected as pea whole plants without roots 1 hour after the last application as well as 1, 2, 3, 4, 5, 7, 10, 12 and 14-15 days thereafter. Untreated specimens were collected also as whole plants without roots 1 hour after application as well as 5 and 14 days thereafter. Each specimen was collected in duplicate (“ship” and “retain”), so that reserve specimens were available in case of potential problems during sample shipment, storage or analysis.

Damaged plants were not harvested. Each specimen was collected randomly from a minimum of 12 different places within each plot.

Untreated specimens were obtained prior to treated specimens when sampling times coincided to avoid contamination. Each specimen was placed into an individual plastic bag and was subsequently double bagged with a second plastic bag. Specimen labels were fixed to the inner plastic bags detailing the specimen type, specimen number, trial and study number. The retain specimens were harvested at the same time of the original ones and were destroyed in the facilities of each test site or the Test Facility by agreement of the Study Director and Sponsor.

All specimens were stored at ≤ -18ºC and were sent to Specimen Management in BASF SE Agricultural Center Limburgerhof.

Description of analytical methods

All specimens were analyzed for BAS 505 F (Dimoxystrobin) and its Z-isomer 505M98 (M505F098) using BASF method no. L0076/08. The method has a limit of quantitation (LOQ) of 0.005 mg/kg for both analytes.

For further details on the analytical methods, please consult the consumer safety part (M-CA, section 6, chapter CA 6.3).

The results of procedural recovery experiments averaged at about 101% for pea plants for BAS 505 F at fortification levels between 0.005 and 5 mg/kg and at 98.7% for metabolite 505M98 at fortification levels between 0.005 and 5 mg/kg.

II RESULTS AND DISCUSSION

Dimoxystrobin

The dimoxystrobin residues in the pea specimens taken 0 DALA (1 HALA) ranged from 0.74 – 4.4 mg/kg. They increased to 0.99 - 3.6 mg/kg in the specimens taken 1 DALA and further to 0.28 - 3.7 mg/kg at 2 DALA. In the specimens taken 3 DALA 0.31 – 2.8 mg/kg were determined. The residue level in the specimens taken 4 DALA was 0.12 - 2.2 mg/kg, whereas in those taken 5 DALA < 0.005 - 2.1 mg/kg were found. Afterwards a steady decline was observed in the specimens taken 7 DALA (0.10 - 1.8mg/kg), 10 DALA (0.052 - 1.1mg/kg) and 12 DALA (0.011 – 0.63 mg/kg). At the last sampling (14 DALA they remained at this level (0.023 – 0.36 mg/kg).

No residues of dimoxystrobin above the limit of quantitation were found in any of the analysed untreated specimens.

Metabolite - 505M98

The 505M98 residues in the pea specimens taken 0 DALA (1 HALA) ranged from < 0.005 – 0.0095 mg/kg. They increased in the specimens taken at 1 DALA (0.0082 – 0.031 mg/kg). On the second DALA the residues increased to < 0.005 – 0.037 mg/kg). In the 3 DALA specimens < 0.005 – 0.035 mg/kg were found, whereas the specimens taken 4 and 5 DALA showed a slight increase to < 0.005 – 0.045 mg/kg and < 0.005 – 0.47 mg/kg respectively. Afterwards the residues decreased to < 0.005 – 0.035 mg/kg (7 DALA) and < 0.005 – 0.038 mg/kg (10 DALA). In the specimen taken 12 DALA and at the last sampling (14 DALA) they remained at this level (< 0.005 – 0.016 mg/kg and < 0.005 – 0.0058 mg/kg respectively).

No residues of 505M98 above the limit of quantitation were found in any of the analysed untreated specimens.

Table 8.1-2: Summary of residues of dimoxystrobin and 505M98 in pea (whole plant without roots) (Gálvez & Moreno 2015, BASF DocID 2014/1001972)

Trial details	Sampling timing	Sampling no.	Sampling date	Crop growth stage (BBCH)	Residues (mg/kg)	
					Dimoxystrobin	505M98
Trial no. L130773 Study site: Germany	1 HALA	1	25.04.2014	13	1.5	< 0.005
	1 DALA	2	26.04.2015	13	1.2	0.009
	2 DALA	3	27.04.2015	14	0.64	0.0076
	3 DALA	4	28.04.2015	14	0.60	0.012
	4 DALA	5	29.04.2015	15	0.22	< 0.005
	5 DALA	6	30.04.2015	15	0.14	< 0.005
	7 DALA	7	02.05.2014	16	0.10	< 0.005
	10 DALA	8	05.05.2014	17	0.074	< 0.005
	12 DALA	9	07.05.2014	19	0.011	< 0.005
	14 DALA	10	09.05.2014	30	0.1.1025	< 0.005
Trial no. L130774 Study site: Germany	1 HALA	1	25.04.2014	13	1.1	< 0.005
	1 DALA	2	26.04.2015	13	1.1	0.0082
	2 DALA	3	27.04.2015	14	0.51	0.0065
	3 DALA	4	28.04.2015	14	0.71	0.012
	4 DALA	5	29.04.2015	15	0.19	< 0.005
	5 DALA	6	30.04.2015	15	0.19	< 0.005
	7 DALA	7	02.05.2014	16	0.13	< 0.005
	10 DALA	8	05.05.2014	17	0.087	< 0.005
	12 DALA	9	07.05.2014	19	0.019	< 0.005
	14 DALA	10	09.05.2014	30	0.025	< 0.005
Trial no. L130775 Study site: Netherlands	1 HALA	1	05.05.2015	13	1.1	< 0.005
	1 DALA	2	06.05.2015	13	1.1	0.016
	2 DALA	3	07.05.2015	14	0.44	0.0057
	3 DALA	4	08.05.2015	14	0.31	< 0.005
	4 DALA	5	09.05.2015	15	0.16	< 0.005
	5 DALA	6	10.05.2015	15	0.10	< 0.005
	7 DALA	7	12.05.2014	16	0.12	< 0.005
	10 DALA	8	15.05.2014	17	0.094	< 0.005
	12 DALA	9	17.05.2014	18	0.062	< 0.005
	14 DALA	10	19.05.2014	19	0.041	< 0.005
Trial no. L130776 Study site: Netherlands	1 HALA	1	05.05.2015	13	0.74	< 0.005
	1 DALA	2	06.05.2015	13	0.99	0.016
	2 DALA	3	07.05.2015	14	0.28	< 0.005
	3 DALA	4	08.05.2015	14	0.32	0.0059
	4 DALA	5	09.05.2015	15	0.12	< 0.005
	5 DALA	6	10.05.2015	15	< 0.005	< 0.005
	7 DALA	7	12.05.2014	16	0.11	< 0.005
	10 DALA	8	15.05.2014	17	0.052	< 0.005
	12 DALA	9	17.05.2014	18	0.065	< 0.005
	14 DALA	10	19.05.2014	19	0.023	< 0.005
Trial no. L130777 Study site: Spain	1 HALA	1	23.01.2014	12	4.4	0.0076
	1 DALA	2	24.01.2014	12	3.6	0.018
	2 DALA	3	25.01.2014	12	3.7	0.036
	3 DALA	4	26.01.2014	12	2.8	0.035
	4 DALA	5	27.01.2014	12	2.2	0.045
	5 DALA	6	28.01.2014	13	2.1	0.047
	7 DALA	7	30.01.2014	13	1.8	0.035
	10 DALA	8	02.02.2014	14	1.1	0.038
	12 DALA	9	04.02.2014	14	0.63	0.016
	14 DALA	10	07.02.2014	14-15	0.16[1)]	< 0.005

Trial details	Sampling timing	Sampling no.	Sampling date	Crop growth stage (BBCH)	Residues (mg/kg)	
					Dimoxystrobin	505M98
Trial no. L130778 Study site: Spain	1 HALA	1	23.04.2014	12	4.1	0.0095
	1 DALA	2	24.04.2014	12	3.3	0.031
	2 DALA	3	25.04.2014	12-13	2.7	0.037
	3 DALA	4	26.04.2014	12-13	1.6	0.027
	4 DALA	5	27.04.2014	12-13	1.2	0.021
	5 DALA	6	28.04.2014	13-14	1.1	0.018
	7 DALA	7	30.04.2014	14	0.87	0.015
	10 DALA	8	02.05.2014	14-15	0.48	0.0072
	12 DALA	9	04.05.2014	15	0.40	0.0061
	14 DALA	10	07.05.2014	16	0.36	0.0058
Trial no. L130779 Study site: Italy	1 HALA	1	18.11.2013	12-13	1.1	< 0.005
	1 DALA	2	19.11.2013	12-13	1.1	0.0098
	2 DALA	3	20.11.2013	13-14	0.84	0.010
	3 DALA	4	21.11.2013	14	0.61	0.0084
	4 DALA	5	22.11.2013	14-15	0.66	0.009
	5 DALA	6	23.11.2013	14-15	0.59	0.0083
	7 DALA	7	25.11.2013	15	0.44	0.006
	10 DALA	8	28.11.2013	15-16	0.41	0.0058
	12 DALA	9	30.11.2013	15-16	0.078	< 0.005
	14 DALA	10	02.12.2012	15-16	0.039	< 0.005
Trial no. L130780 Study site: Italy	1 HALA	1	14.05.2014	12-13	1.3	< 0.005
	1 DALA	2	15.05.2014	12-13	1.038	0.012
	2 DALA	3	16.05.2014	13-14	0.77	0.010
	3 DALA	4	17.05.2014	14	0.53	< 0.005
	4 DALA	5	18.05.2014	14-15	0.73	< 0.005
	5 DALA	6	19.05.2014	14-15	0.56	< 0.005
	7 DALA	7	21.05.2014	16-17	0.45	< 0.005
	10 DALA	8	24.05.2014	17-18	0.27	< 0.005
	12 DALA	9	26.05.2014	18-19	0.077	< 0.005
	14 DALA	10	28.05.2014	19	0.040	< 0.005

HALA: hours after last application; DALA: days after last application
1 mean of two values L007: 0.163 mg/kg, L009: 0.155

III. CONCLUSION

In whole pea plant samples collected directly after the application (BBCH 12-13), the residues of BAS 505 F were in the range of 0.74-4.4 mg/kg directly and decreased to 0.023-0.36 mg/kg at 14 days after the application. Residues of 505M98 were in the range of <0.005-0.0095 mg/kg directly after the application (BBCH 12-13) and decreased to < 0.005-0.0058 mg/kg at 14 DALA (BBCH 14-30).

Residues of BAS 505 F and 505M98 were below 0.005 mg/kg in all control specimens.

Report:	CA 8.1/3 Erzgraeber B., 2015a Dissipation of BAS 505 F (Dimoxystrobin) on young plants (wheat and peas) from field trials conducted in Europe (North and South) - Calculation of DT50 / DT90 dissipation times 2015/1111337
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp., FOCUS Kinetics (2006) SANCO/10058/2005 version 2.0
GLP:	No. Study was conducted in compliance with the Codex of Good Modeling Practices.

This modelling report provides kinetic analysis and estimation of dissipation times (DT_{50} and DT_{90} values) for dimoxystrobin on young wheat and pea plants.

MATERIAL AND METHODS

Calculation of DT_{50}

The residue decline of BAS 505 F (dimoxystrobin) on young wheat and young pea plants has been studied at a range of field trials at different sites in Europe (northern and southern zone) during the growing season of the year 2013-2014. The modelling report provides kinetic analyses and estimations of the dissipation times (DT_{50}, DT_{90} values) for dimoxystrobin for each field data set. Individual dissipation parameter estimations were conducted for wheat and peas at the different field trials.

Guidance of the FOCUS workgroup on degradation kinetics was used in order to derive DT_{50} endpoints for modelling purposes. This means the selected DT_{50} values are suitable input parameters for models that require single first order (SFO) DT_{50} values or conservative substitutes. The software package KinGUII version 2.2014.224.1704 was used for parameter fitting.

The goodness-of-fit was evaluated by means of visual and statistical assessment as proposed by the FOCUS kinetics guidance. The fits are visually and statistically acceptable for all datasets.

The decline of dimoxystrobin residues on young plants was well described by the SFO kinetic model. The resulting DT_{50} values for dimoxystrobin in young wheat and young pea plants and the respective statistical indices are presented in the following table.

RESULTS

Table 8.1-3: DT_{50} values of dimoxystrobin in young wheat and pea plants

Plant	Trial	Zone	DT50 [d]	Kinetic model	χ2 error
Wheat	L130729	North	3.4	SFO	20.80
Wheat	L130730	North	5.3	SFO	14.77
Wheat	L130731	North	4.4	SFO	20.05
Wheat	L130732	North	4.3	SFO	16.67
Wheat	L130733	South	3.3	SFO	16.35
Wheat	L130734	South	3.4	SFO	10.61
Wheat	L130735	South	4.2	SFO	11.42
Wheat	L130735	South	10.9	SFO	11.68
Peas	L130773	North	1.8	SFO	14.52
Peas	L130774	North	2.3	SFO	25.15
Peas	L130775	North	1.7	SFO	26.96
Peas	L130776	North	2.1	SFO	40.23
Peas	L130777	South	4.7	SFO	7.79
Peas	L130778	South	2.7	SFO	9.72
Peas	L130779	South	4.6	SFO	12.30
Peas	L130780	South	4.0	SFO	12.81

CONCLUSION

The decline of dimoxystrobin residues on young plants was well described by single first order kinetics.

CA 8.1.1 Effect on birds

No new study available.

CA 8.1.1.1 Acute oral toxicity to birds

No new study available.

CA 8.1.1.2 Short-term dietary toxicity to birds

No new study available.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

No new study available.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

No new study available.

CA 8.1.2.1 Acute oral toxicity to mammals

No new study available.

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

No new study available.

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

No new study available.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

According to the revised data requirements under regulation 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to amphibians and reptiles shall be addressed. Nevertheless, unlike birds and mammals, toxicity tests for amphibian and reptile species are not requested. In the EU there is no guidance or validated regulatory protocols yet available neither on the type of regulatory testing necessary or on how to conduct a risk assessment for amphibian and reptiles. In the case of dimoxystrobin, there are no studies in the literature on the toxicity of this substance on amphibians and reptiles.

According to the new aquatic guidance document (EFSA, 2013) amphibian should be included in the aquatic and terrestrial risk assessment. In absence of GLP studies the assessment should be based on any existing relevant information (testing of amphibian is not recommended at first instance due to animal welfare reasons and the absence of standard guidelines for amphibian testing). With regard to the aquatic risk assessment several data analyses indicate that the risk assessment for aquatic organisms (and fish in particular) covers the risk assessment for aquatic phases of amphibians (Fryday and Thompson, 2012; Weltje et al., 2013).

Compared to aquatic studies, regulatory ecotoxicological information on amphibians based on dosing studies (LD50) is rather scarce. However, in the few cases where terrestrial stages of amphibians were tested in this kind of study as birds and mammals, the general pattern is that amphibians are less sensitive than the latter two taxa (see Table 12 and 13 in Fryday and Thompson, 2012).

In the case of reptiles there is even less information available than for amphibians (see the revision by Fryday and Thompson, 2009). The risk from dietary exposure can be assumed to be lower for reptiles than for birds and mammals (Fryday and Thompson 2009). This is because reptiles are poikilotherm (i.e., do not maintain a constant body temperature) and as a result feeding activity will peak at warm days and will be zero during hibernation or at cold days. In contrast, birds and mammals will have to maintain a constant body temperature and, hence, will need to feed every day (Fryday & Thompson 2009). Uncertainties remain on the contribution of dermal exposure to the overall exposure to reptiles. However, in contrast to amphibians the skin of reptiles is much less permeable; its functions is in general protection and barrier and not an organ used for respiration or water/mineral exchange with the environment. Accordingly, reptiles are considered less vulnerable to dermal exposure as compared to amphibians. Nevertheless, some uncertainty with respect to the risk to reptiles, i.e. whether they are sufficiently covered by other (more standard) ecotoxicological data will remain and further research is needed.

However, dimoxystrobin has been used for many years in many countries worldwide. So far, there are no publications indicating a potential risk of this compound to amphibians / reptiles and despite the long term use worldwide, we are not aware of a single findings or (incidence) reports that amphibians / reptiles were harmfully affected by applications of this substance.

References:

Commission Regulation (EU) No 283/2013 setting out data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Commission Regulation (EU) No 284/2013: setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union: 1st March 2013.

Fryday S and Thompson H (2009): Literature reviews on ecotoxicology of chemicals with a special focus on plant protection products. Lot 1. Exposure of reptiles to plant protection products. EFSA (CFT/EFSA/PPR/2008/01).

Fryday S and Thompson, H (2012): Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural; Food and Environment research agency, UK.

Weltje L., Simpson P., Gross M., Crane M., Wheeler J.R. (2013): Comparative acute and chronic sensitivity of fish and amphibians: a critical review of data. Environmental Toxicology and Chemistry, Vol. 32, No. 5, pp. 984-994.

CA 8.1.5 Endocrine disrupting properties

Based on the physical, chemical and structural characteristics of the active substance dimoxystrobin as well as based on results of available long-term bird (see M-CP 10.1.1) and mammal studies (see M-CA 5.8.3), there is no indication of endocrine disrupting properties for this active substance. This is supported by several impact assessments of different organizations (see M-CA 5.8.3). Thus, no further studies are required.

An extended impact assessment study of the human health and environmental criteria for endocrine disrupting substances proposed by HSE, CRD (Chemical Regulation Directorate (CRD) and Health and Safety Executive (HSE) of the UK) was published in 2013 (Ewence et al. 2013). In this assessment, for dimoxystrobin the question "*Does the available evidence demonstrate that an endocrine disruption mode of action in fish, birds and/or mammals is reasonably linked to the adverse effects [seen in ecotoxicology studies]?*" was answered by a clear "no". This supports the conclusion that dimoxystrobin (BAS 505 F) is not an endocrine disrupting substance.

Ewence, A., P. Rumsby, and I. Johnson. 2013. Extended impact assessment study of the human health and environmental criteria for endocrine disrupting substances proposed by HSE, CRD. Report No. Defra9088.02.

CA 8.2 Effects on aquatic organisms

Since Annex I inclusion of the active substance dimoxystrobin (BAS 505 F), new toxicity studies on the active substance and its metabolite 505M96 (M505F096; Reg.No. 4091091) have been performed and as a result there are new endpoints which are now used in the aquatic risk assessment. Summaries of these new studies are provided below. For completeness this includes some older studies, which have not been submitted during the previous Annex I inclusion process (*e.g.* because there is no respective data requirement in the EU).
Details on the EU agreed studies which have been already evaluated within the Annex I inclusion of dimoxystrobin are provided in the EU Review documents of dimoxystrobin (Draft Assessment Report (DAR), Vol. 3, B.9, 2005; EFSA Scientific Report (2005) 46, 1-82).

For better transparency and traceability of the active substance history, the results of all studies are summarized in Table 8.2-1 and Table 8.2-2.

Full references used within the following chapters are given at the end M-CA 8.2. Please refer to Document N3 which contains structures and synonyms for all metabolites.

Table 8.2-1: List of studies and endpoints for aquatic organisms exposed to the active substance dimoxystrobin (BAS 505 F)

Organism	Endpoint	Value [mg a.s./L]	Reference (BASF DocID)	EU agreed
Fish (standard species)				
Oncorhynchus mykiss	96 h LC_{50}	**0.0434**	1998/10601	yes
Oncorhynchus mykiss	96 h LC_{50}	0.0444	2000/5125	yes
Oncorhynchus mykiss	28 d NOEC	0.010	1999/10311	yes
Oncorhynchus mykiss (ELS study)	97 d NOEC 97 d NOEAEC	0.000316 0.0010	1999/10521	yes
Pimephales promelas (ELS study)	36 d NOEC	0.016	2000/5156	yes
Aquatic invertebrates (standard species)				
Daphnia magna	48 h EC_{50}	**0.0394**	1999/11525	yes
Daphnia magna	21 d NOEC	0.0125	2000/1000120	yes
Sediment dwelling aquatic invertebrates				
Chironomus riparius (spiked water study)	28 d NOEC	0.010	2000/1012491	yes
Algae (standard species) #				
Pseudokirchneriella subcapitata	96 h E_rC_{50}	**0.153**	1999/11481	yes (study submitted in previous Annex I process; however, the growth rate endpoint is now used)
Macrophytes (standard species)				
Lemna gibba [1)]	14 d E_bC_{50}	0.149	2000/5099	no (new data for US registration)

Organism	Endpoint	Value [mg a.s./L]	Reference (BASF DocID)	EU agreed
Bioconcentration				
Oncorhynchus mykiss (bioconcentration study)	BCF (whole fish)	84	1999/11247	yes (but EFSA Conclusion 2005 reports transposed digits, correct value will now be considered)
Data on additional species / Higher-tier studies				
Fish				
Cyprinodon variegatus [1)]	96 h LC_{50}	0.167	2000/5062	no (new data for US registration)
Cyprinus carpio *	96 h LC_{50}	0.0503	2001/1010514	yes
Danio rerio *	96 h LC_{50}	0.0259	2001/1010513	yes
Lepomis macrochirus	96 h LC_{50}	0.0512	1998/10620	yes
Lepomis macrochirus	96 h LC_{50}	0.0519	2000/5092	yes
Lepomis macrochirus *	96 h LC_{50}	0.0752	2001/1010515	yes
Leusiscus idus melanotus *	96 h LC_{50}	0.0238	2000/1012362	yes
Oncorhynchus mykiss *	96 h LC_{50}	0.0219	1999/10954	yes
Oncorhynchus mykiss [1), 2)] **	96 h LC_{50}	0.0288	2000/1014153	no (data used for risk refinement; included for completeness)
Pimephales promelas *	96 h LC_{50}	0.0185	2001/1010512	yes
Oncorhynchus mykiss (modified ELS with variable exposure) [1), 2)]	69 d NOEC	**0.012** [3)] (based on peak conc.)	2008/1064431	no (but reviewed and in Addendum to the DAR 2009)
Leuciscus idus melanotus (ELS study in outdoor microcosms, including sediment) *	66 d NOEC	0.015	2000/1012494	yes
Aquatic invertebrates				
Asellus aquaticus [1), 2)]	48 h EC_{50} [5)]	0.437	2008/1055034	no (but reviewed and in Addendum to the DAR 2009)
Americamysis bahia (former name: *Mysidopsis bahia*) [1)]	48 h EC_{50} [5)]	0.0429	2000/5124	no (new data for US registration)
Crassostrea virginica [1)]	96 h EC_{50}	0.00842	2000/5096	no (new data for US registration)
Algae [#]				
Navicula pelliculosa [1), #]	72 h E_rC_{50} [+]	0.0078	2000/5128	no (new data for US registration)
Anabaena flos-aquae [1), #]	72 h E_rC_{50} [+]	> 2.06	2000/5129	no (new data for US registration)
Skeletonema costatum [1), #]	72 h E_rC_{50} [+]	> 4.31	2000/5127	no (new data for US registration)

Organism	Endpoint	Value [mg a.s./L]	Reference (BASF DocID)	EU agreed
Mesocosm				
Outdoor mesocosm **	approx. 5 mo NOEAEC [4)] (effect class 3A)	0.0050	2002/1004886	yes

Bold figures: Where several endpoints are available for the same species or group or where several endpoints are available for one study based on different effect parameters (*e.g.* for algae and macrophytes), only the relevant endpoint(s) for the most sensitive species is used in the risk assessment presented in chapter M-CP 10.2 of this supplemental dossier.

Abbreviations: BCF = Bioconcentration factor; ELS = Early Life Stage

* Study was conducted with the formulated product BAS 507 00 F (suspension concentrate formulation containing 133 g dimoxystrobin/L and 50 g epoxiconazole/L, nominally; former representative formulated product during Annex I inclusion process for dimoxystrobin). The results have been converted to active substance dimoxystrobin considering the analyzed content of the a.s. (*i.e.* 130.7 g a.s./L) and the formulation density (*i.e.* 1.06 g/cm^3). The recalculated values are presented here and are used for a refined risk assessment for dimoxystrobin (see chapter M-CP 10.2 of this supplemental dossier).

** Study was conducted with the solo-formulation BAS 505 01 F (containing 167 g dimoxystrobin/L, nominally). The results have been converted to active substance dimoxystrobin considering the analyzed content of the a.s. (*i.e.* 169.7 g a.s./L) and the formulation density (*i.e.* 1.05 g/cm^3). The recalculated values are presented here and are used for a refined risk assessment for dimoxystrobin (see chapter M-CP 10.2 of this supplemental dossier).

[#] In accordance with the new EFSA Aquatic Guidance Document (2013) and OECD Guidelines for aquatic primary producers (OECD 201 and 221), the endpoint growth rate (E_rC_{50}) is now used for the risk assessment for algae and macrophytes; the E_rC_{50} values obtained in the studies already submitted during Annex I inclusion process are thus included as new information.

[+] The 72 h growth rate endpoint obtained in the 120 h alga study is used for the risk assessment in accordance with recent EFSA Aquatic GD (2013)

[1)] Study has not been submitted during Annex I inclusion process of dimoxystrobin. A study summary is provided below.

[2)] Study has not been submitted during Annex I inclusion process for dimoxystrobin; however, it was considered valid in the Addendum - Confirmatory data submitted after Annex I inclusion (2009).

[3)] Relevant chronic endpoint for fish from a modified ELS study using under more realistic exposure conditions (reference is made to the Addendum - Confirmatory data submitted after Annex I inclusion, 2009).

[4)] This endpoint included recovery (please refer to the Addendum - Confirmatory data submitted after Annex I inclusion, 2009). Classification of the outdoor mesocosm study was done according to EFSA Aquatic GD (2013).

[5)] The 48-h LC_{50} obtained in the 96 h study is used as relevant endpoint in the risk assessment according to EU Regulation 283/2013 on the data requirements for active substances and the EFSA Aquatic GD (2013).

Table 8.2-2: List of studies and endpoints for aquatic organisms exposed to the major metabolites of dimoxystrobin (BAS 505 F)

Organism	Endpoint	Value [mg/L]	Reference (DocID)	EU agreed
metabolite: 505M01				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	> 100	1999/11843	yes
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	> 100	2000/1012486	yes
Algae #				
Pseudokirchneriella subcapitata	72 h E_rC_{50}	> 100	1999/10940	yes (study submitted in previous Annex I process; however, the growth rate endpoint is now used)
metabolite: 505M08				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	> 100	1999/11841	yes
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	> 100	2000/1012487	yes
Algae #				
Pseudokirchneriella subcapitata	72 h E_rC_{50}	> 100	1999/10941	yes (study submitted in previous Annex I process; however, the growth rate endpoint is now used)
metabolite: 505M09				
Fish				
Oncorhynchus mykiss	96 h LC_{50}	> 100	1999/11842	yes
Aquatic invertebrates				
Daphnia magna	48 h EC_{50}	> 100	2000/1012490	yes
Algae #				
Pseudokirchneriella subcapitata	72 h E_rC_{50}	> 100	1999/10942	yes (study submitted in previous Annex I process; however, the growth rate endpoint is now used)

Organism	Endpoint	Value [mg/L]	Reference (DocID)	EU agreed
metabolite: 505M96				
Fish				
Oncorhynchus mykiss [1)]	96 h LC_{50}	> 100	2002/1008605	no (new data for the risk assessment of the metabolite)
Aquatic invertebrates				
Daphnia magna [1)]	48 h EC_{50}	> 100	2002/1012927	no (new data for the risk assessment of the metabolite)
Algae [#]				
Pseudokirchneriella subcapitata [1)]	72 h E_rC_{50}	43.6	2002/1013884	no (new data for the risk assessment of the metabolite)

[#] In accordance with the new Aquatic Guidance Document (2013) and OECD Guidelines for aquatic primary producers (OECD 201 and 221), the endpoint growth rate (E_rC_{50}) is now used for the risk assessment for algae and macrophytes; the E_rC_{50} values obtained in the studies already submitted during Annex I inclusion process are thus included as new information.

[1)] Study has not been submitted during Annex I inclusion process of dimoxystrobin. A study summary is provided below.

CA 8.2.1 Acute toxicity to fish

An acute toxicity study with rainbow trout (*Oncorhynchus mykiss*) conducted with dimoxystrobin was already evaluated during the previous Annex I inclusion process. The following additional study with rainbow trout performed with the solo-formulation BAS 505 01 F (containing 167 g dimoxystrobin/L, nominally) has already been submitted by the applicant within the confirmatory addendum (Addendum to the DAR of dimoxystrobin, "Confirmatory data submitted after Annex I inclusion", 2009) and has been evaluated by RMS UK. However, the study has not been peer-reviewed previously on EU level. It is provided for completeness and will be used in the risk refinement for dimoxystrobin.

Report: CA 8.2.1/1
[redacted] 2000a
BAS 505 01 F - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss WALBAUM 1792) in a static system (96 hours)
2000/1014153
Guidelines: EPA 72-1, OECD 203, EPA-SEP 540/9-85-006, EEC 92/69 A V C 1
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a static acute toxicity laboratory study, juvenile rainbow trout (approx. 7 months old) were exposed to 0.100, 0.147, 0.215, 0.316, 0.464, 0.681 and 1.0 mg BAS 505 01 F/L (nominal) in groups of 10 animals in glass aquaria containing 100 L water with 2 replicates per concentration. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on nominal concentrations. After 96 hours of exposure no mortality and other toxic effects were observed in the control and at concentrations of up to and including 0.147 mg BAS 505 01 F/L, whereas 100% mortality were observed at all higher test item concentrations.

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) for BAS 505 01 F was determined to be 0.178 mg/L based on nominal concentrations. The NOEC (96 h) was 0.147 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	BAS 505 01 F, batch no. 97-1, content of a.s.: dimoxystrobin (BAS 505 F; Reg. No. 285028): 169.7 g/L (nominal: 167 g/L).

B. STUDY DESIGN

Test species:	Rainbow trout (*Oncorhynchus mykiss*), approx. 7 month old, mean body length 4.7 cm (4.4 - 5.0 cm); mean body weight 1.10 g (0.81 - 134 g); supplied by Fischzucht Worbis, Worbis, Germany.
Test design:	Static system (96 hours); 10 fish per aquarium (loading 0.1 g fish/L), 2 replicates per concentration; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control, solvent control, 0.100, 0.147, 0.215, 0.316, 0.464, 0.681 and 1.0 mg 505 01 F/L (nominal).
Test conditions:	Glass aquaria with stainless steel frames (80 x 35 x 46 cm), test volume 100 L, non-chlorinated, filtered tap water; temperature: 12 °C - 13 °C; pH 8.3 - 8.6; oxygen content: 9.3 mg/L - 11.6 mg/L; total hardness: about 250 mg $CaCO_3$/L; photoperiod 16 h light : 8 h dark; no aeration, no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics; probit analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at test initiation and at the end of the test. Measured concentrations for dimoxystrobin ranged from 91.8% to 112.6% of nominal at test initiation and 92.5% - 101.4% at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality and toxic effects were observed in the control and at concentrations of up to and including 0.147 mg BAS 505 01 F/L, whereas 100% mortality were observed at all higher test item concentrations. The results are summarized in Table 8.2.1-1.

Table 8.2.1-1 Acute toxicity (96 h) of BAS 505 01 F to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] nominal	**Control**	**0.100**	**0.147**	**0.215**	**0.316**	**0.464**	**0.681**	**1.0**
Mortality (96 h) [%]	0	0	0	100	100	100	100	100
Symptoms after 96 h	none	none	none	n.d.	n.d.	n.d.	n.d.	n.d.
Endpoints [mg BAS 505 01 F/L] (nominal)								
LC_{50} (96 h)	0.178							
NOEC (96 h)	0.147							

n.d. = not determined; all fish dead

III. CONCLUSION

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) for BAS 505 01 F was determined to be 0.178 mg/L based on nominal concentrations. The NOEC (96 h) was 0.147 mg/L (nominal).

The following acute toxicity study with sheepshead minnow (*Cyprindodon variegatus*) performed with the active substance dimoxystrobin is not required for registration in the EU and it has not been evaluated previously on EU level. However, the study was conducted due to U.S. data requirements and is provided for completeness.

Report:	CA 8.2.1/2 [redacted] et al., 2000a Flow-through acute toxicity of BAS 505 F to the sheepshead minnow, Cyprindodon variegatus 2000/5062
Guidelines:	EPA 72-3(a), EPA 850.1075
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour flow-through acute toxicity laboratory study, juvenile sheepshead minnow were exposed to a dilution water control, a solvent control and to dimoxystrobin at nominal concentrations of 0.065, 0.110, 0.180, 0.300 and 0.500 mg a.s./L (corresponding to mean measured concentrations of 0.0576, 0.113, 0.189, 0.301 and 0.512 mg a.s./L) in groups of 10 animals in glass aquaria containing 15 L water. Fish were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. After 96 hours of exposure, no mortality was observed in the dilution water control and at the lowest test item concentration of 0.0576 mg a.s./L, whereas 20%, 50%, 100% and 100% mortality was observed at test item concentrations of 0.113, 0.189, 0.301 and 0.512 mg a.s./L. In the solvent control, 5% mortality occurred. No sub-lethal effects were found in the control groups and in all test item treatments after 96 hours.

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of dimoxystrobin was 0.167 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0576 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. no.: 285028), batch no. N15, purity: 96.0%.

B. STUDY DESIGN

Test species: Sheepshead minnow (*Cyprinodon variegatus*), juveniles; mean body length of control fish: 23.7 mm; mean wet weight of control fish: 0.19 g; supplied by "Aquatic BioSystems", Fort Collins, Colorado, USA.

Test design: Flow through system (96 h); 5 test item concentrations plus a dilution water control and a solvent control; 2 replicates per treatment; 10 fish per aquarium (loading 0.13 g fish/L); assessment of mortality and sub-lethal effects directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.065, 0.110, 0.180, 0.300 and 0.500 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of < Limit of Quantification (LOQ), < LOQ, 0.0576, 0.113, 0.189, 0.301 and 0.512 mg a.s./L.

Test conditions: 20 L glass aquaria, test volume: 15 L; dilution water: filtered natural seawater mixed with deionized water, salinity: 16 ‰; flow rate: 6.3 volume additions per 24 hours on average per test vessel; temperature: 21.3°C- 22.8°C; pH 7.9 - 8.1; photoperiod 16 h light : 8 h dark; light intensity: approx. 46 foot candles; no aeration; no feeding.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; probit method for calculation of the LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of dimoxystrobin concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of dimoxystrobin ranged from 89.7% to 130.6% of nominal at test initiation and from 85.4% to 104.0% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortality was observed in the dilution water control and at the lowest test item concentration of 0.0576 mg a.s./L, whereas 20%, 50%, 100% and 100% mortality was observed at test item concentrations of 0.113, 0.189, 0.301 and 0.512 mg a.s./L. In the solvent control, 5% mortality occurred. No sub-lethal effects were found in the control groups and in all test item treatments after 96 hours. The results are summarized in Table 8.2.1-2.

Table 8.2.1-2: Acute toxicity (96 h) of dimoxystrobin to sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.065**	**0.110**	**0.180**	**0.300**	**0.500**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.0576**	**0.113**	**0.189**	**0.301**	**0.512**
Mortality [%] (96 h)	0	5	0	20	50	100	100
Symptoms (after 96 h)	none	none	none	none	none	n.d.	n.d.
Endpoints [mg dimoxystrobin/L] (mean measured)							
LC_{50} (96 h)	0.167 (95% confidence limits: 0.143 - 0.193)						
NOEC (96 h)	0.0576						

n.d. = not determined, all fish dead

III. CONCLUSION

In a flow-through acute toxicity study with sheepshead minnow the LC_{50} (96 h) of dimoxystrobin was 0.167 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.0576 mg a.s./L (mean measured).

The following acute toxicity study on rainbow trout (*Oncorhynchus mykiss*) performed with the metabolite 505M96 is provided in support of the aquatic risk assessment and has not been evaluated previously on EU level. The study is required due to new data requirements.

Report:	CA 8.2.1/3 [redacted] 2002a Reg.No. 409 1091 (metabolite of BAS 505 F) - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in a static system over 96 hours 2002/1008605
Guidelines:	EPA 72-1, EEC 92/69 A V C 1, OECD 203, EPA-SEP 540/9-85-006
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a static acute toxicity laboratory study, juvenile rainbow trout (approx. 5 month) were exposed to a dilution water control and to 505M96 (metabolite of dimoxystrobin) at a single concentration of 100 mg/L (nominal) in groups of 10 animals in glass aquaria containing 50 L water with 3 replicates. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on the nominal concentrations. After 96 h of exposure, no mortality and no sub-lethal effects were observed in the control and at the tested concentration of 100 mg 505M96/L.

In a static acute toxicity study (limit test) with rainbow trout the LC_{50} (96 h) for 505M96 (metabolite of dimoxystrobin) was determined to be > 100 mg/L based on nominal concentrations. The NOEC (96 h) was ≥ 100 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: 505M96 (Reg. No. 4091091; metabolite of dimoxystrobin); batch no. 01893-35; purity: 99.6%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792), approx. 5 month, mean body length 6.0 cm (5.2 - 6.7 cm); mean body weight 1.8 g (1.1 - 2.7 g); supplied by Forellenzucht Trostadt GbR, Trostadt, Germany.

Test design: Static system (96 hours); one test item concentrations plus a dilution water control; 2 replicates for the control and 3 replicates for the test item treatment; 10 fish per aquarium (loading 0.4 g fish/L); assessment of mortality and other symptoms of toxicity within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50}, NOEC, mortality and sub-lethal effects.

Test concentrations: Dilution water control, 100 mg 505M96/L (nominal).

Test conditions: Glass aquaria, with stainless steel frames (60 x 35 x 40 cm); test volume 50 L, natural not chlorinated tap water; temperature: 10 °C - 13 °C; pH 8.1 - 8.3; oxygen content: 8.1 -9.6 mg/L; total hardness about 2.5 mmol/L; conductivity: 550 µS/cm; photoperiod 16 h light : 8 h dark; no feeding, no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV-detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in each replicate for the control and the test item treatment at the beginning and at the end of the test. The analytically detected concentrations in the test item treatment were initially in the range of 96.7% - 99.2% and after about 96 hours between 95.2% - 96.8% of the nominal concentrations. Hence, the following biological results are based on the nominal concentrations.

Biological results: After 96 h of exposure, no mortality and no sub-lethal effects were observed in the control and at the tested concentration of 100 mg 505M96/L. The results are summarized in Table 8.2.1-3.

Table 8.2.1-3 Acute toxicity (96 h) of 505M96 (metabolite of dimoxystrobin) to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] nominal	**Control**	**100**
Mortality (96 h) [%]	0	0
Symptoms after 96 h	none	none
	Endpoints [mg 505M96/L] (nominal)	
LC_{50} (96 h)	> 100	
NOEC (96 h)	≥ 100	

III. CONCLUSION

In a static acute toxicity study (limit test) with rainbow trout the LC_{50} (96 h) for 505M96 (metabolite of dimoxystrobin) was determined to be > 100 mg/L based on nominal concentrations. The NOEC (96 h) was ≥ 100 mg/L (nominal).

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

The following early life-stage test on rainbow trout (*Oncorhynchus mykiss*) was performed with the active substance dimoxystrobin using a realistic exposure pattern. The study has already been submitted by the applicant within the confirmatory addendum (Addendum to the DAR of dimoxystrobin, "Confirmatory data submitted after Annex I inclusion", 2009) and has been evaluated by RMS UK. However, the study has not been peer-reviewed previously on EU level and is thus provided for completeness. The design of the following study had been discussed with the old RMS competent authority. It is based on the concerns raised in the last EPCO-expert meetings, the PPR-panel opinion and EFSA conclusions. In addition, it covers new developments such as recommendations of the ELink workshops.

Report:	CA 8.2.2.1/1 [redacted] 2008a BAS 505 F (Dimoxystrobin) - Modified early-life-stage test rainbow trout (Oncorhynchus mykiss) using a realistic exposure pattern 2008/1064431
Guidelines:	OECD 210, EPA 72-4, EPA 850.1400
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

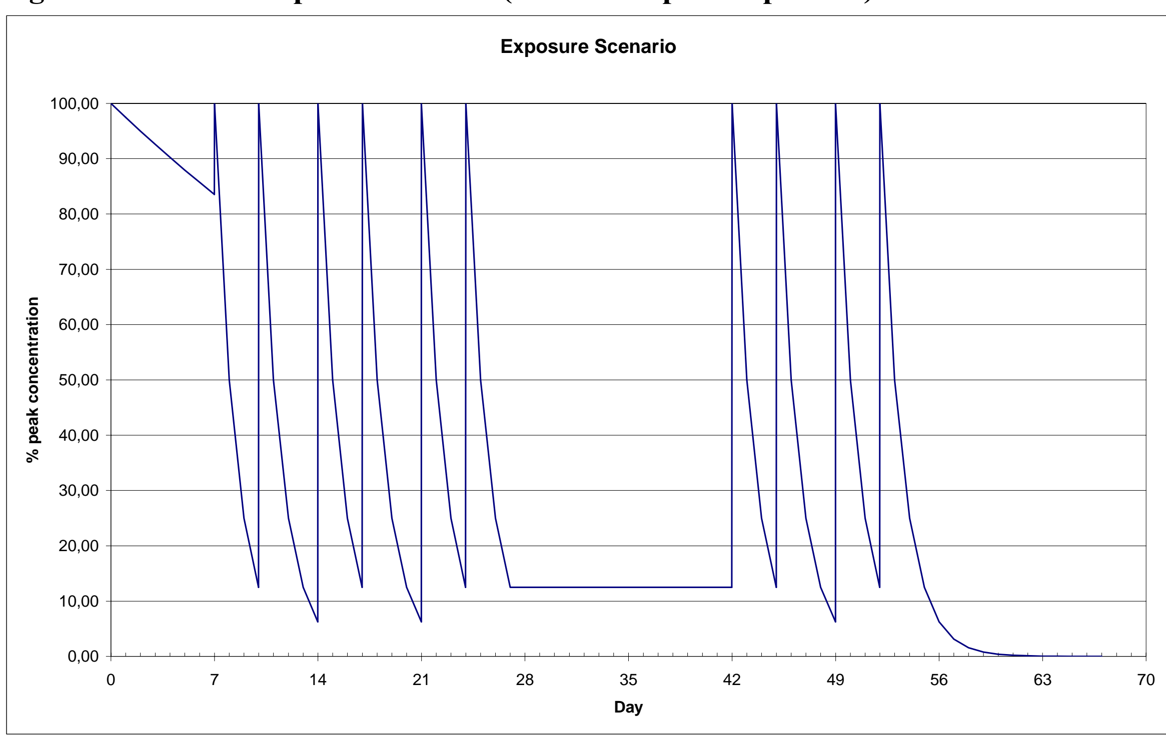
Executive Summary

The effect of dimoxystrobin on embryos, larvae and young fish of rainbow trout (*Oncorhynchus mykiss*) was investigated over 69 days under flow-through conditions in a modified early life-stage test using a realistic exposure pattern. The test was started with eyed eggs approx. one week before start of hatch. It was conducted with 4 replicates per concentration. At insertion of the eggs the maximum concentration of the test substance was applied (peak exposure of 0.0013, 0.004, 0.012 and 0.036 mg dimoxystrobin/L). The concentration declined slowly with a half life time of 27 days until day 7 (start of hatch). From day 7 onwards, 6 peak applications were made spaced by 3 - 4 days, which declined with a half life time of one day. After the 6th peak the exposure was kept constant at a (base) level of 12.5% of the peak concentration during the intermediate time period until application of the following peak series. A second peak exposure period with another 4 peaks spaced by 3 - 4 days was carried out on days 42 - 52 with the same half life time; the whole exposure scheme is shown below (see Figure 8.2.2.1-1). The peak exposure periods were selected to cover the most sensitive life stages: The first peak series covered the time period during hatch and swim-up, the second peak series was applied during a relevant growth period of the juvenile fish. Additionally one group was exposed to a constant concentration of 0.01 mg dimoxystrobin/L and one to dilution water as a control. The fish were sacrificed on day 69, 60 days after the end of hatch (post hatch study duration equivalent to the standard trout ELS study).

Biological parameters recorded were mortality of embryos until hatch, number of surviving larvae at termination of swim-up (day 30) and survival of young rainbow trout until termination of exposure (day 69), time to hatch and swim-up, signs of toxicity (symptoms), weight and total length of surviving fish at the end of exposure and total length on day 39, 30 days after hatch.

Generally the measured concentrations were in good agreement with the intended exposure pattern and confirmed that the exposure scheme could be properly established with the flow through system used. Therefore the following biological results are based on nominal concentrations. In peak exposure concentrations of 0.0013, 0.004 and 0.012 mg/L no statistically significant deviations from the survival rates of the control group were detected. At a peak concentration of 0.036 mg/L and in the test group exposed continuously to 0.01 mg/L the survival after swim-up and survival over the whole exposure period was statistically significantly decreased compared to control. No signs of toxicity or abnormalities were observed in any of the groups. No adverse effects on body weight and length in comparison to the control group were observed in any of the concentration groups.

Figure 8.2.2.1-1: Exposure scheme (variable exposure pattern)



In a modified early life-stage test, rainbow trout eggs were exposed to peak exposure patterns of dimoxystrobin over a 69-day period covering 10 peak exposure periods and baseline intervals. The overall NOEC (survival, toxic signs, growth parameters) was 0.012 mg dimoxystrobin/L based on nominal peak concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. No 285028), batch no. FF 18850, purity: 99.9%.

B. STUDY DESIGN

Test species: Rainbow trout (*Oncorhynchus mykiss*), age: 14 days old eyed eggs; fertilized at the hatchery Troutlodge Inc., USA. (An early life stage test with freshly fertilized eggs is not likely to succeed outside of the reproduction season. Once eggs of the rainbow trout are fertilized, they are extremely sensitive against handling and cannot be transported. A transport is possible after the stage of the eyed egg is reached. Thus this experiment was started with eyed eggs. However, as it has been demonstrated that the time after hatching is the most sensitive phase and since the eggs were still exposed for one week before hatching, this does not have any relevant impact the results of the study).

Test design: Flow-through system (69 d); 4 replicates per treatment group with 25 fertilized eggs each; 1 replicate/egg cup, 2 egg cups/aquarium (day 0 - 9);1 replicate/aquarium for juvenile fish (day 9 - 69); 4 test concentration groups with peak exposure plus one test group with continuous exposure and an untreated control. 4 experimental phases, phase 1, day 0 - 7 (embryo phase): initial peak concentrations decreasing with a half-life of 27 days; phase 2, day 7 - 28 (covering start of hatch and during swim-up): 6 peak applications in 3 or 4 day intervals; phase 3 (day 28 -42) constant baseline concentration at 12.5% of the peak concentration; phase 4 (day 42 - 69): 4 applications in 3 or 4 day intervals, peak concentration followed by a decrease with a half life time of 1 day. Assessment of survival until hatch, at termination of swim-up (day 30) and until termination of exposure (day 69), time to hatch and swim-up, signs of toxicity, weight and total length at the end of exposure and total length on day 39, 30 days after hatch.

Endpoints: NOEC, LOEC, based on mortality, toxic signs, body weight and body length.

Test concentrations: Control; 0.0013, 0.004, 0.012 and 0.036 mg a.s./L (peak exposure); 0.01 mg a.s./L (continuous exposure).

Test conditions:	From day 0 - 9 stainless steel egg cups (diameter: 11 cm) within aquaria, from day 9 stainless steel aquaria (water volume: 9 L); filtered drinking water; temperature 10 - 13 °C (temperature difference between replicates ≤ 1 °C); pH 7.8 - 8.4; oxygen content: 6.6 - 10.8 mg/L; conductance: 241 - 247 µS; total hardness: 1.00 - 1.08 mmol/L; acid capacity: 2.22 - 2.28 mmol/L; flow rate: 1.9 L/hour and aquarium (5 water exchanges/day); feeding at the start of swim-up with newly hatched brine shrimp larvae (*Artemia nauplii*), once or twice daily; from day 24 on fine milled „Ecostart 17“ (supplier: BioMar, Denmark) was offered once daily and from study day 35 on twice daily. Feeding was increased in quantity according to the size of the fish; from day 41 on slight aeration.
Analytics:	The test item concentrations were analyzed using HPLC with MS-detection and external calibration.
Statistics:	Standard procedures; ANOVA, Dunnett test for determination of NOEC and LOEC (body weight, body length), Fisher´s exact test for determination of NOEC (mortality), Wilcoxon-test for variability between replicates.

II. RESULTS AND DISCUSSION

Analytical measurements: Generally the measured concentrations were in good agreement with the intended exposure pattern and confirmed that the exposure scheme could be properly established with the flow-through system used. Due to a calculation mistake during the preparation of the peak concentrations on day 17 in test groups 1 - 4, too large volumes of stock solution were added to the test vessels. The actual concentrations of the peaks on day 17 were calculated to be 264% of the nominal peak concentration. Until day 18 these high concentrations declined faster than according to the intended exposure scheme. The exposure concentrations were corrected on the following day. The deviation was considered to have no influence on the study results. The concentration of group 5 with continuous exposure was within the range of ± 20% of the nominal concentration. The average measured concentrations confirmed closely the nominal ones, therefore the following biological results are based on nominal concentrations.

Biological results: In peak exposure concentrations of 0.0013, 0.004 and 0.012 mg/L no statistically significant deviations from the survival rates of the control group were detected. At a peak concentration of 0.036 mg/L the survival after swim-up and accordingly over the whole exposure period were statistically significantly decreased compared to the control; some mortality occurred (in one replicate) in the constant exposure group. Time to hatch was similar in all test groups. Hatching started on day 5 and ended on days 7 – 9 in all test groups. First swim-up was observed at days 19 - 22 in the replicates of the control group and in the peak concentration groups up to and including 0.012 mg/L. Swim-up in these test groups was completed at day 26. Swim-up in peak concentration group 4 (0.036 mg/L) was clearly delayed, starting on day 24 and completed on day 30. In test group 5 (continuous exposure) the time to swim-up was generally comparable to the control group. No signs of toxicity or abnormalities were observed in the control and concentration groups. No adverse effects on body weight and length in comparison to the control group were observed in any of the concentration groups on day 30 and on day 69. Table 8.2.2.1-1 gives a summary of the main results.

Table 8.2.2.1-1: Chronic toxicity of dimoxystrobin (BAS 505 F) on rainbow trout (*Oncorhynchus mykiss*) in a modified early life-stage test using a realistic exposure pattern

Concentration [mg a.s./L] nominal	**Control**	**0.0013 (peak)**	**0.004 (peak)**	**0.012 (peak)**	**0.036 (peak)**	**0.01 (constant)**
Survival until hatch [%]	100	100	100	100	100	100
Survival of larvae from hatch until day 30 (end of swim-up) [%]	92	95	93	93	84	95
Survival of young fish, day 30 - 69 [%]	95	93	92	91	81**	72**
Survival, day 0 - 69 [%]	88	88	83	85	68**	68**
Start of hatch [day]	5 - 6	5	5	5	5	5
End of hatch [day]	8 - 9	8 - 9	7 - 8	7 - 9	8 - 9	8 - 9
Start of swim-up [day]	19 - 22	19 - 20	19 - 21	19 - 20	24	19 - 23
End of swim-up [day]	25 - 26	24	24 - 26	24 - 25	29 - 30	24 - 27
Toxic signs	none	none	none	none	none	none
Mean body length (39 d) [cm]	3.70	3.76	3.70	3.84**	3.60	3.94*
Mean body length (69 d) [cm]	5.7	5.7	5.9	5.8	5.7	5.7
Mean body weight (69 d) [g]	1.84	1.83	1.90	1.89	1.86	1.77
	Endpoints [mg a.s./L] (nominal)					
NOEC (69 d)	0.012					
LOEC (69 d)	0.036					

* Statistically significant compared to control (Dunnett´s test (body length), $p \leq 0.05$)
** Statistically significant compared to control (Fisher´s exact test (mortality), $p \leq 0.01$; Dunnett´s test (body length), $p \leq 0.01$)

III. CONCLUSION

In a modified early life stage test, rainbow trout eggs were exposed to variable exposure patterns of dimoxystrobin over a 69-day period covering 10 peak exposure periods and baseline intervals. No negative impact on fish was observed at maximum peak concentrations of 0.012 mg a.s./L in the variable exposure group. A significant impact based on fish mortality at the time after hatching was observed in the 0.036 mg a.s./L treatment group. Thus, the overall NOEC (survival, toxic signs, growth parameters) was 0.012 mg dimoxystrobin/L, based on nominal peak concentrations. The LOEC was 0.036 mg dimoxystrobin/L (nominal).

CA 8.2.2.2 Fish full life cycle test

The chronic toxicity to fish is fully addressed by four early life stage studies (three of these studies were already evaluated during the previous Annex I inclusion process; a summary for the fourth study is provided above. No additional fish full life cycle study is required and no (new) study has been conducted.

CA 8.2.2.3 Bioconcentration in fish

A bioconcentration study performed with rainbow trout has already been evaluated during the previous Annex I inclusion process of dimoxystrobin. No additional bioconcentration studies are required and no (new) study has been conducted.

CA 8.2.3 Endocrine disrupting properties

Based on the physical, chemical and structural characteristics of the active substance dimoxystrobin as well as based on results of available long-term fish studies (and studies on terrestrial vertebrates; see chapter M-CA-8.1.5) there is no indication of endocrine disrupting properties of this active substance. This is supported by several impact assessments of different organizations (see M-CA 5.8.3). Thus, no further studies are required.
An extended impact assessment study of the human health and environmental criteria for endocrine disrupting substances proposed by HSE, CRD (Chemical Regulation Directorate (CRD) and Health and Safety Executive (HSE) of the UK) was published in 2013 (Ewence et al. 2013). In this assessment, for dimoxystrobin the question "*Does the available evidence demonstrate that an endocrine disruption mode of action in fish, birds and/or mammals is reasonably linked to the adverse effects [seen in ecotoxicology studies]?*" was answered by a clear "no". This supports the conclusion that Dimoxystrobin (BAS 505 F) is not an endocrine disrupting substance.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

The following acute toxicity study on *Daphnia magna* performed with the metabolite 505M96 is provided in support of the aquatic risk assessment and has not been evaluated previously on EU level. The study is required due to new data requirements.

Report: CA 8.2.4.1/1
Funk M., 2002a
Effect of BF 505-13 on the immobility of Daphnia magna STRAUS in a 48 hour static, acute toxicity test
2002/1012927
Guidelines: OECD 202, EEC 79/831 A V C 2, EPA 72-2, EPA 850.1010
GLP: yes
(certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to a dilution water control and to 505M96 (metabolite of dimoxystrobin, Reg. Nr. 4091091) at nominal concentrations of 10, 18, 32, 56 and 100 mg/L in 4 replicates per treatment containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.
The biological results are based on nominal concentrations of the test item. After 48 h of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 56 mg 505M96/L, whereas 5% of the daphnids were immobile at the highest test item concentration of 100 mg/L. No other effects were observed.

In a 48-hours static acute toxicity study with *Daphnia magna,* the EC_{50} (48 h) for 505M96 was > 100 mg/L based on nominal concentrations. The NOEC (48 h) was determined to be 56 mg/L (nominal).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	505M96 (Reg. No. 4091091; metabolite of dimoxystrobin); batch no. 01893-35; purity: 99.6%. The code BF 505-13 which appears in the study is wrong and was mentioned in the study by mistake due to historical changes in codes. The Reg. Nr. and the structure confirm that the correct metabolite was tested (as it was done with fish and algae). Further details are provided in Document N3.

B. STUDY DESIGN

Test species:	Water flea (*Daphnia magna* STRAUS), neonates from in-house culture; less than 24 hours old at test initiation.
Test design:	Static test (48 hours), 5 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility (and other effects) after 24 and 48 hours.
Test concentrations:	Control (dilution water), 10, 18, 32, 56 and 100 mg 505M96/L (nominal).
Test conditions:	Glass vessels; test volume 50 mL; dilution water “M4” (Elendt medium); pH 8.01 - 8.05; oxygen content 8.5 - 8.8 mg/L; total hardness 2.63 mmol/L; conductivity 695 µS/cm; temperature 20 C - 21 C; photoperiod: 16 hours light : 8 hours dark; light intensity 394-472 lux; no feeding, no ventilation.
Analytics:	Analytical verification of test item concentrations was conducted using HPLC with UV-detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of the test item concentrations was conducted in each concentration at the beginning and at the end of the test. The measured values ranged from 95.5% to 105.7% (average 101.7%) of nominal at the beginning of the test and from 97.1% to 100.4% (average 98.3%) at the end of the test, confirming the nominal data. Therefore the following biological results are based on nominal concentrations.

Biological results: After 48 h of exposure, no immobility of daphnids was observed in the control and at test item concentrations of up to and including 56 mg 505M96/L, whereas 5% of the daphnids were immobile at the highest test item concentration of 100 mg/L. No other effects were observed. The results are summarized in Table 8.2.4.1-1.

Table 8.2.4.1-1: Effect of 505M96 (metabolite of dimoxystrobin) on *Daphnia magna* mobility

Concentration [mg/L] (nominal)	**Control**	**10**	**18**	**32**	**56**	**100**
Immobility (24 h) [%]	0	0	0	0	0	0
Immobility (48 h) [%]	0	0	0	0	0	5
	Endpoints [mg 505M96/L] (nominal)					
EC_{50} (48 h)	> 100					
NOEC (48 h)	56					

III. CONCLUSION

In a 48-hours static acute toxicity study with *Daphnia magna,* the EC_{50} (48 h) for 505M96 was > 100 mg/L based on nominal concentrations. The NOEC (48 h) was determined to be 56 mg/L (nominal).

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

The following acute toxicity study on *Asellus aquaticus* performed with the active substance dimoxystrobin was conducted for the higher tier risk assessment. During the old EFSA evaluation process, one member state raised concern regarding the evaluation of the mesocosm because of "low numbers of benthic aquatic crustaceans not allowing clear statistical evaluation". Next to an argumentation (see higher tier risk assessment) an additional study has been conducted to clearly support the argumentation. The study has already been submitted by the applicant within the confirmatory addendum (Addendum of dimoxystrobin, "Confirmatory data submitted after Annex I inclusion", 2009) and has been evaluated by RMS UK. However, the study has not been peer-reviewed previously on EU level and is thus provided for completeness. The 48-h LC_{50} obtained in the 96 h study on *A. aquaticus* is used as relevant endpoint for the risk assessment according to EU Regulation 283/2013 (European Commission, 2013) which describes the data requirements for active substances. Also the recent EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013) advises to use the 48-h endpoint, which allows an easy comparison to the other standard acute invertebrate tests. Therefore only the 48-h results are shown.

Report:	CA 8.2.4.2/1 Janson G.-M.,Dohmen G.P., 2008a Acute toxicity of BAS 505 F (Reg.No. 285028) to Asellus aquaticus in a 96 hour static test 2008/1055034
Guidelines:	OECD 202
GLP:	yes (certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The acute toxicity of dimoxystrobin to *Asellus aquaticus* was investigated in a 96 h static toxicity study. 10 replicates per treatment group were treated with nominal test concentrations of 0.030, 0.060, 0.120, 0.240 and 0.480 mg dimoxystrobin/L, plus a control and a solvent control. Animals were observed for mortality and other symptoms 24, 48, 72 and 96 hours after start of exposure. The biological results are based on nominal concentrations. After 48 h of exposure, no mortality of the test organisms were observed in the controls and at up to and including the test item concentration of 0.120 mg a.s./L. At the two highest tested concentrations of 0.240 and 0.480 mg a.s./L, 30% and 50% mortality occurred, respectively. The effects in all test item treatments were not statistically significantly different compared to the controls. No sub-lethal impact of the test substance was observed.

In a 96 hours toxicity test with *Asellus aquaticus* the LC_{50} (48 h) of dimoxystrobin was determined to be 0.437 mg a.s./L, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F, Reg. No. 285028), batch FF 18850, purity 99.9%.

B. STUDY DESIGN

Test species: Freshwater isopod (*Asellus aquaticus*), the animals were collected from an uncontaminated pond (distant from industrial and agricultural sides) and maintained in-house (non-GLP) for an acclimation period of at least 19 days. The animals selected for the study were juvenile with a body length of approximately 4-7 mm.

Test design: Static system, 5 concentrations plus a control and a solvent control, 10 replicates for each treatment and controls, one animal/replicate; assessment of mortality and other symptoms 24, 48, 72 and 96 hours after start of exposure.

Endpoints: NOEC and LC_{50} (48 h), based on mortality and observations of sub-lethal symptoms.

Test concentrations: Control, solvent control, 0.030, 0.060, 0.120, 0.240 and 0.480 mg dimoxystrobin/L (nominal).

Test conditions: Glass vessels, test volume 50 mL, dilution water "M4" (Elendt medium), pH 7.94 - 8.05, oxygen content 8.6 – 9.0 mg/L, total hardness 2.28 mmol/L, conductivity 624 µS/cm (total hardness and conductivity at test initiation), temperature 20 - 21°C, light intensity 274 - 489 lux, 16 hours light : 8 hours dark, no feeding, no aeration.

Analytics: The test item concentrations were analyzed using HPLC with MS-detection and external calibration.

Statistics: Fisher's Exact Test ($p < 0.05$) for determination of NOEC, calculation of LC_{50} by probit analysis using "ToxRatPro Version 2.09".

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of dimoxystrobin was carried out in each test concentration at the beginning and at the end of the test. Measured values ranged from 98.8% to 104.9% of the nominal concentration at test initiation and from 112.0% to 115.8% at test termination. The following biological results are based on nominal test item concentrations.

Biological results: After 48 h of exposure, no mortality of the test organisms were observed in the controls and at up to and including the test item concentration of 0.120 mg a.s./L. At the two highest tested concentrations of 0.240 and 0.480 mg a.s./L, 30% and 50% mortality occurred, respectively. The measured effects were not statistically significantly different compared to the controls (Fisher`s Exact test, $p < 0.05$). No sub-lethal impact of the test substance was observed. The results are summarized in Table 8.2.4.2-1.

Table 8.2.4.2-1: Acute toxicity (48 h) of dimoxystrobin to *Asellus aquaticus*

Concentration [mg a.s./L] nominal	**Control**	**Solvent control**	**0.030**	**0.060**	**0.120**	**0.240**	**0.480**
Mortality (48 h) [%]	0	0	0	0	0	30	50
	Endpoints [mg a.s./L] (nominal)						
LC_{50} (48 h)	0.437						

III. CONCLUSION

In a 96 hours toxicity test with *Asellus aquaticus* the LC_{50} (48 h) of dimoxystrobin was determined to be 0.437 mg a.s./L, based on nominal concentrations.

The following acute toxicity study on the eastern oyster (*Crassostrea virginica*) performed with the active substance dimoxystrobin is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and it is provided for completeness.

Report:	CA 8.2.4.2/2 Wyskiel D.C. et al., 2000b Flow-through mollusc shell depostion test with BAS 505 F 2000/5096
Guidelines:	EPA 72-3(c), EPA 850.1025
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour acute toxicity laboratory study the effect of dimoxystrobin on shell deposition of eastern oysters was investigated under flow-through conditions. The eastern oysters were exposed to a dilution water control, a solvent control and to dimoxystrobin at nominal concentrations of 0.0033, 0.0055, 0.0090, 0.015 and 0.025 mg a.s./L (corresponding to mean measured concentrations of 0.00301, 0.00514, 0.00814, 0.0141 and 0.0237 mg a.s./L) in groups of 10 oysters per replicate with two replicates per treatment. Eastern oysters were observed for survival and symptoms of toxicity daily during the exposure period. Measurements of shell deposition for each oyster were made after 96 hours.

The biological results are based on mean measured concentrations of the test item. After 96 hours no mortality occurred at test item concentrations of up to and including 0.00514 mg a.s./L, whereas 5%, 10% and 30% mortality was observed at 0.00814, 0.0141 and 0.0237 mg a.s./L, respectively. After 96 hours, sub-lethal effects, observed as oysters exhibited a delayed reaction to gentle prodding (slowly closing shells) at the two highest concentrations of dimoxystrobin. Statistically significant inhibition of shell growth compared to the pooled control / solvent control was observed at the four highest tested concentrations

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for dimoxystrobin was 0.00842 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.00301 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. No. 285028), batch no. N15, purity: 96.0%.

B. STUDY DESIGN

Test species: Eastern oyster (*Crassostrea virginica*), juveniles, height: 32 - 50 mm; source: "P. Cummins Oyster Company", Baltimore, Maryland, USA.

Test design: Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates for each test item concentration and the controls with 10 oysters per replicate (20 animals per treatment); initial and daily assessment of mortality and symptoms of toxicity; measurements of shell deposition 96 hours after start of exposure.

Endpoints: EC_{50} and NOEC (96 h) for shell growth inhibition, mortality and symptoms of toxicity.

Test concentrations: Control (dilution water: unfiltered seawater), solvent control (0.1 mL dimethylformamide/L); 0.0033, 0.0055, 0.0090, 0.015 and 0.025 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of < Limit of Quantification (LOQ), < LOQ, 0.00301, 0.00514, 0.00814, 0.0141 and 0.0237 mg a.s./L.

Test conditions: 20 L glass aquaria, test volume 15 L, natural unfiltered seawater, flow rate: average of 8.5 volume additions per 24 hours in each test vessel, 0.53 L per oyster per hour; salinity: 34‰ - 35‰; temperature: 20.0°C - 20.9°C; pH 7.8 - 8.0; oxygen content: 5.7 mg/L - 7.7 mg/L; photoperiod 16 h light : 8 h dark with a 15 minute transition period between dark and light; light intensity: 54 foot candles; no aeration; live marine phytoplankton as supplement to existing food in unfiltered seawater used as dilution water.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; t-test ($\alpha = 0.05$) for comparison of shell deposition data in the control groups; standard statistical techniques for calculation of EC_{50}, ANOVA followed by Bonferroni's test ($\alpha = 0.05$) for shell deposition data of the test item treatments.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at test initiation and at test termination. Mean measured concentrations for dimoxystrobin ranged from 88.9% to 96.0% of nominal concentrations at test initiation and from 89.1% to 96.0% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours no mortality occurred at test item concentrations of up to and including 0.00514 mg dimoxystrobin/L, whereas 5%, 10% and 30% mortality was observed at 0.00814, 0.0141 and 0.0237 mg a.s./L, respectively. After 96 hours, sub-lethal effects, observed as oysters exhibited a delayed reaction to gentle prodding (slowly closing shells) at the two highest concentrations of dimoxystrobin. Control and solvent control oysters deposited an average of 2.3 and 2.5 mm of new shell during the test, respectively. No statistically significant difference in shell deposition was observed between the control groups (t-test, $\alpha = 0.05$). Subsequent statistical analyses were performed by comparing the pooled control and solvent control data to the treatment data. Statistically significant inhibition of shell growth compared to the pooled control / solvent control was observed at the four highest tested concentrations (Bonferroni's test). The results are summarized in Table 8.2.4.2-2.

Table 8.2.4.2-2: Acute toxicity (96 h) of dimoxystrobin to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0033**	**0.0055**	**0.0090**	**0.015**	**0.025**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.00301**	**0.00514**	**0.00814**	**0.0141**	**0.0237**
Mortality after 96 h [%]	0	0	0	0	5	10	30
Shell growth after 96 h [% of control]	--	109	78	70*	57*	35*	9*
Symptoms (after 96 h) #	none	none	none	none	none	A	A
Endpoints [mg dimoxystrobin/L] (mean measured)							
EC_{50} (96 h)	0.00842 (95% confidence limits: 0.00654 - 0.0108)						
$NOEC_{shell\ deposition}$ (96 h)	0.00301						

Symptoms after 96 h: A = Affected oysters exhibited a delayed reaction to gentle prodding (slowly closing shells).
* Statistically significant difference compared to the pooled control / solvent control (Bonferroni's test, $\alpha = 0.05$).

III. CONCLUSION

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96 h) for dimoxystrobin was 0.00842 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be 0.00301 mg a.s./L (mean measured).

The following acute toxicity study on the saltwater mysid *Americamysis bahia* performed with the active substance dimoxystrobin is not required for registration in the EU and it has not been evaluated previously on EU level. The study was conducted due to U.S. data requirements and it is provided for completeness.
The 48-h LC_{50} obtained in the 96 h study on *A. bahia* is used as relevant endpoint for the risk assessment according to EU Regulation 283/2013 (European Commission, 2013) which describes the data requirements for active substances. Also the recent EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013) advises to use the 48-h endpoint, which allows an easy comparison to the other standard acute invertebrate tests. Therefore only the 48-h results are shown.

Report:	CA 8.2.4.2/3 Wyskiel D.C. et al., 2000a Flow-through acute toxicity of BAS 505 F to the mysid, Americamysis bahia 2000/5124
Guidelines:	EPA 72-3(b), EPA 850.1035
GLP:	yes (certified by United States Environmental Protection Agency)

Executive Summary

In a 96-hour flow-through acute toxicity laboratory study, saltwater mysids were exposed to a dilution water control, a solvent control and to nominal concentrations of 0.0065, 0.011, 0.018, 0.030 and 0.050 mg dimoxystrobin/L (nominal) (corresponding to mean measured concentrations of 0.00767, 0.0111, 0.018, 0.0299 and 0.0497 mg a.s./L) in two replicates per treatment containing 10 mysids each. Saltwater mysids were observed for survival and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item (measured over the 96 h study period). After 48 hours of exposure, no mortality were observed in the control, the solvent control and at concentrations of up to and including 0.018 mg/L, whereas 10% and 70% mortality was observed at the two highest test item concentrations of 0.0229 and 0.0497 mg a.s./L, respectively. After 48 hours, sub-lethal effects, observed as lethargy and/or erratic swimming, were noted at the two highest tested concentrations of dimoxystrobin.

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h) for dimoxystrobin was determined to be 0.0429 mg a.s./L, based on mean measured concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. No. 285028), batch no. N15, purity: 96.0%.

B. STUDY DESIGN

Test species: Saltwater mysid (*Americamysis bahia*), juveniles, age: less than 24 hours old; average wet weight of control mysids: 0.26 mg; source: in-house cultures.

Test design: Flow-through system (96 hours); 5 test item concentrations plus a control and a solvent control, 2 replicates per treatment; 10 mysids per replicate (loading 0.00017 g mysid/L); assessment of mortality and symptoms of toxicity directly after start of exposure and 24, 48, 72 and 96 hours after start of exposure.

Endpoints: LC_{50} (48 h), mortality and sub-lethal effects.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L) and 0.0065, 0.011, 0.018, 0.030 and 0.050 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of < Limit of Quantification (LOQ), < LOQ, 0.00767, 0.0111, 0.018, 0.0299 and 0.0497 mg dimoxystrobin/L.

Test conditions: Glass aquaria (20 L), test volume 15 L; test chambers: glass cylinders (8 cm in height and 8 cm in diameter) with mesh screen attached to the bottom; dilution water: filtered, sterilized and aerated natural seawater mixed with deionized water; flow rate: 6.6 volume additions per 24 hours on average; salinity: 16 - 17‰; temperature: 21.2°C - 22.8°C; pH 7.6 - 8.0; oxygen content: 6.4 - 8.6 mg/L; photoperiod 16 h light : 8 h dark with a 15 minute transition period between dark and light; light intensity: 51 foot-candles; feeding: juvenile mysids were fed daily with live *Artemia salina* nauplii; no aeration.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics; probit method for calculation of the LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test (96 h). The analytically determined concentrations of dimoxystrobin ranged from 96.7% to 100% of nominal concentrations at test initiation and from 97.3% to 114.9% of nominal at test termination. The following biological results are based on mean measured concentrations (measured over the 96 h study period).

Biological results: After 48 hours of exposure no mortality were observed in the control, the solvent control and in concentrations of up to and including 0.018 mg/L, whereas 10% and 70% mortality was observed at the two highest test item concentrations of 0.0229 and 0.0497 mg a.s./L, respectively. After 48 hours, sub-lethal effects, observed as lethargy and/or erratic swimming were noted at the two highest tested concentrations of dimoxystrobin. The results are summarized in Table 8.2.4.2-3.

Table 8.2.4.2-3: Acute toxicity (48 h) of dimoxystrobin to saltwater mysids (*Americamysis bahia*)

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.0065**	**0.011**	**0.018**	**0.030**	**0.050**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.00767**	**0.0111**	**0.018**	**0.0299**	**0.0497**
Mortality (48 h) [%]	0	0	0	0	0	10	70
Symptoms after 48 h *	none	none	none	none	none	A*	A*
	Endpoints [mg dimoxystrobin/L] (mean measured)						
LC_{50} (48 h)	0.0429 (95% confidence limits: 0.0375 - > 0.0497)						

* Symptoms after 48 h: A = lethargy and/or erratic swimming

III. CONCLUSION

In a flow-through acute toxicity study with saltwater mysids (*Americamysis bahia*) the LC_{50} (48 h) for dimoxystrobin was determined to be 0.0429 mg a.s./L, based on mean measured concentrations.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to Daphnia magna

The reproductive and development toxicity to *Daphnia magna* has been addressed and evaluated already during the previous Annex I inclusion process. No further study is required or was conducted.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

This point is not triggered and not addressed via (new) toxicity studies.

CA 8.2.5.3 Development and emergence in Chironomus riparius

A spiked water toxicity study with *Chironomus riparius* was already evaluated during the previous Annex I inclusion process. No additional studies are required and no (new) study has been conducted.

CA 8.2.5.4 Sediment dwelling organisms

This point is not triggered and not addressed via (new) toxicity studies.

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

The following study on the freshwater green alga *Pseudokirchneriella subcapitata* performed with the metabolite 505M96 is provided in support of the aquatic risk assessment and has not been evaluated previously on EU level. The study is required due to new data requirements.

Report:	CA 8.2.6.1/1 Jatzek H.-J., 2002a Reg.No. 4091091 (metabolite of BAS 505 F) - Determination of the inhibitory effect on the cell multiplication of unicellular green algae 2002/1013884
Guidelines:	EEC 92/69 A V C 3, OECD 201, EPA 850.5400
GLP:	yes (certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of 505M96 (metabolite of dimoxystrobin) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 0 (control), 0.3, 1.0, 3.0, 10, 30 and 100 mg 505M96/L. Assessment of growth was conducted 0 h, 24 h, 48 h and 96 h after test initiation.

The biological results are based on nominal concentrations of the test item. No morphological effects on algae were observed in the control and at any of the test item concentrations tested over the 72 h study period.

In a 72 hour algal toxicity test with *Pseudokirchneriella subcapitata,* the 72 h E_rC_{50} and the E_yC_{50} of 505M96 (metabolite of dimoxystrobin) were determined to be 43.6 mg/L and 21.5 mg/L, respectively, based on nominal concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: 505M96 (Reg. No. 4091091, metabolite of dimoxystrobin), batch no. 01893-35, purity: 99.6%.

B. STUDY DESIGN

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov (syn. *Selenastrum capricornutum* Prinz), SAG 61.81; stock obtained from "Sammlung von Algenkulturen" Göttingen University, Germany.

Test design: Static system (72 hours); 6 test concentrations with three replicates plus a control with 5 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 72.

Test concentrations: Control, 0.3, 1.0, 3.0, 10, 30 and 100 mg 505M96/L (nominal).

Test conditions: 250 mL glass Erlenmeyer flasks (test volume: 100 mL); pH 7.9 – 8.1 at test initiation and pH 8.1 – 8.3 at test termination; temperature: 23 ± 2 °C; initial cell densities 1 x 10^4 cells/mL; continuous artificial light.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV-VIS detection.

Statistics: Descriptive statistics; linear regression analysis for growth rate and yield data after 72 hours.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for 505M96 ranged from 98.9% to 102.3% of nominal at test initiation and from 84.4% to 90.7% of nominal at test termination. Therefore, the following biological results are based on nominal concentrations of the test item.

Biological results: No morphological effects on algae were observed in the control and at any of the test item concentrations tested over the 72 h study period. The effects on algal growth are summarized in Table 8.2.6.1-1.

Table 8.2.6.1-1: Effect (72 h) of 505M96 (metabolite of dimoxystrobin) on the growth of green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**0.3**	**1**	**3**	**10**	**30**	**100**
Inhibition in 72 h (growth rate) [%] [1)]	--	-2.88	-4.02	-4.70	-1.80	33.1	87.5
Inhibition in 72 h (yield) [%] [1)]	--	-11.1	-15.4	-16.8	-5.39	74.1	96.6
Endpoints [mg 505M96/L] (nominal)							
E_rC_{50} (72 h)	43.6						
E_rC_{10} (72 h)	14.5						
E_YC_{50} (72 h)	21.5						
E_YC_{10} (72 h)	12.4						

1) Inhibition compared to the control; negative values indicate stimulated growth.

III. CONCLUSION

In a 72 hour algal toxicity test with *Pseudokirchneriella subcapitata,* the 72 h E_rC_{50} and the E_yC_{50} of 505M96 (metabolite of dimoxystrobin) were determined to be 43.6 mg/L and 21.5 mg/L, respectively, based on nominal concentrations.

CA 8.2.6.2 Effects on growth of an additional algal species

The following 120-hour alga study on the freshwater diatom *Navicula pelliculosa* performed with the active substance dimoxystrobin is not required for registration in the EU. The study was conducted due to U.S. data requirements and has not been evaluated previously on EU level. It is submitted for completeness. In accordance to the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 120 h study are considered as relevant endpoint for the aquatic risk assessment, therefore both the 72 h and 120 h endpoints are reported in the study summary below.

Report: CA 8.2.6.2/1
Wyskiel D.C. et al., 2000c
Growth and reproduction toxicity test with BAS 505 F and the freshwater alga, Navicula pelliculosa
2000/5128
Guidelines: EPA 123-2, EPA 850.5400
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 120-hour static toxicity laboratory study, the effect of dimoxystrobin on the growth of the freshwater diatom *Navicula pelliculosa* was investigated. The following nominal concentrations were applied: 0.00086, 0.0017, 0.0034, 0.0065 and 0.013 mg dimoxystrobin/L (corresponding to mean measured concentrations of 0.00122, 0.00177, 0.00359, 0.00607 and 0.0138 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted 24, 48, 72, 96 and 120 h after test initiation.

The biological results are based on mean measured concentrations of the test item. Statistically significant differences compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours.

In a 120 hour algae toxicity test with *Navicula pelliculosa,* the 120 h E_rC_{50} and E_bC_{50} values for dimoxystrobin were determined to be > 0.0138 mg a.s./L and 0.00264 mg a.s./L, respectively, based on mean measured concentrations. After 72 hours of exposure, the E_rC_{50} and E_bC_{50} values were determined to be 0.0078 mg a.s./L and 0.0025 mg a.s./L, respectively (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. No. 285028), batch no. N15, purity: 96.0%.

B. STUDY DESIGN

Test species: Freshwater diatom, *Navicula pelliculosa*, strain UTEX 664, stock originally obtained from the "Culture Collection of Algae", University of Texas, Austin, USA.

Test design: Static system; test duration 120 hours; 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.

Endpoints: EC_{50} with respect to biomass and growth rate after exposure over 72 and 120 hours.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.00086, 0.0017, 0.0034, 0.0065 and 0.013 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of < Limit of Quantification (LOQ), < LOQ, 0.00122, 0.00177, 0.00359, 0.00607 and 0.0138 mg a.s./L.

Test conditions: 250 mL glass flasks; test volume 50 mL; sterile enriched medium supplemented with 0.2 g/L Na_2SiO_3 x 9 H_2O; pH 7.4 - 7.5 at test initiation and pH 7.6 - 8.7 at test termination; temperature: 23.2°C - 23.6°C; initial cell densities 3 x 10^3 cells/mL; continuous light at 4200 - 4300 lux; constant shaking at 100 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.

Statistics: Descriptive statistics; t-test (α = 0.05) for comparison of cell densities and growth rate data in the control and the solvent control; weighted least squares non-linear regression analysis for determination of EC_x values. ANOVA followed by Bonferroni's test (α = 0.05) for determination of the NOEC (120 h) values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of dimoxystrobin ranged from 85.1% to 112.8% of nominal concentrations at test initiation and from 101.2% to 173.3% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data after 120 hours (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Statistically significant differences of the cell numbers and average specific growth rates compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours (Bonferroni's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.2-1.

Table 8.2.6.2-1: Effect (72 h and 120 h) of dimoxystrobin on the growth of the freshwater diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (nominal)	**Water control**	**Solvent control**	**0.00086**	**0.0017**	**0.0034**	**0.0065**	**0.013**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.00122**	**0.00177**	**0.00359**	**0.00607**	**0.0138**
Mean cell density (72 h) [% of water control] #	**--**	100	68	66	40	< 23	< 19
Growth rate (72 h) [% of water control] #	--	100	88	85	68	48	43
Mean cell density (120 h) [% of water control]	**--**	115	96	57	41	27	6
Growth rate (120 h) [% of water control]	--	102	100	91 *	87 *	80 *	57 *
Endpoints [mg dimoxystrobin/L] (mean measured)							
E_rC_{50} (72 h)	0.0078 (95% confidence limits: 0.00617 - 0.00986)						
E_bC_{50} (72 h)	0.0025 (95% confidence limits: 0.00182 - 0.00343)						
E_rC_{50} (120 h)	> 0.0138						
E_bC_{50} (120 h)	0.00264 (95% confidence limits: 0.00205 - 0.00339)						

* Statistically significantly different compared to the pooled control (ANOVA followed by Bonferroni's test, $\alpha = 0.05$).
# Statistically significant differences were only determined after 120 h of exposure.

III. CONCLUSION

In a 120 hour algae toxicity test with *Navicula pelliculosa,* the 120 h E_rC_{50} and E_bC_{50} values for dimoxystrobin were determined to be > 0.0138 mg a.s./L and 0.00264 mg a.s./L, respectively, based on mean measured concentrations. After 72 hours of exposure, the E_rC_{50} and E_bC_{50} values were determined to be 0.0078 mg a.s./L and 0.0025 mg a.s./L, respectively (mean measured).

The following toxicity study on the blue-green alga *Anabaena flos-aquae* performed with the active substance dimoxystrobin is not required for registration in the EU. The study was conducted due to U.S. data requirements and has not been evaluated previously on EU level. It is submitted for completeness. In accordance to the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 120 h study are considered as relevant endpoint for the aquatic risk assessment, therefore both the 72 h and 120 h endpoints are reported in the study summary below.

Report: CA 8.2.6.2/2
Wyskiel D.C. et al., 2000b
Growth and reproduction toxicity test with BAS 505 F and the freshwater alga, Anabaena flos-aquae
2000/5129
Guidelines: EPA 123-2, EPA 850.5400
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 120-hour static toxicity laboratory study, the effect of dimoxystrobin on growth of the blue-green alga *Anabaena flos-aquae* was investigated. The following nominal concentrations were applied: 0.13, 0.25, 0.50, 1.0 and 2.0 mg dimoxystrobin/L (corresponding to mean measured concentrations of 0.133, 0.257, 0.524, 1.07 and 2.06 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted 24 h, 48 h, 72 h, 96 h and 120 h after test initiation.

The biological results are based on mean measured concentrations of the test item. Statistically significant differences compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours.

In a 120-hour algae toxicity test with *Anabaena flos-aquae*, the 72 h and 120 h E_rC_{50} values for dimoxystrobin was determined to be both > 2.06 mg a.s./L based on mean measured concentrations. The 72 h and 120 h E_bC_{50} values were 0.960 and 1.13 mg a.s./L, respectively (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item:	Dimoxystrobin (BAS 505 F; Reg. No. 285028), batch no. N15, purity: 96.0%.

B. STUDY DESIGN

Test species:	Freshwater blue-green alga, *Anabaena flos-aquae* (UTEX 1444); stock originally obtained from the Culture Collection of Algae, University of Texas, Austin, USA; stock was maintained at test conditions for more than 14 days before the test; inocula 6 day old culture.
Test design:	Static system (120 hours); 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.
Endpoints:	EC_{50} with respect to biomass development and growth rate after exposure over 72 and 120 hours.
Test concentrations:	Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.13, 0.25, 0.50, 1.0 and 2.0 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of 0.133, 0.257, 0.524, 1.07 and 2.06 mg a.s./L.
Test conditions:	250 mL glass flasks; test volume: 50 mL; sterile enriched medium; pH 7.4 at test initiation and pH 7.5 - 7.8 at test termination; temperature: 24.5°C - 25.2°C; initial cell densities: 3 x 10^3 cells/mL; continuous light; light intensity: 2000 - 2200 lux, continuous shaking at 100 rpm.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics, weighted least squares non-linear regression for EC_{50} calculation; t-test (α = 0.05) for comparison of cell densities and growth rate in the control and solvent control; ANOVA followed by Bonferroni's test (α = 0.05) for determination of the NOEC (120 h) values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured concentrations of dimoxystrobin ranged from 102.3 to 107.0% of nominal concentrations at test initiation and from 101.6 to 106.0% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. Statistically significant differences of the cell numbers and average specific growth rates compared to the pooled control were observed at the four highest test item concentrations after exposure over 120 hours (Bonferroni's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.2-2.

Table 8.2.6.2-2: Effect (72 h and 120 h) of dimoxystrobin on the growth of the blue-green alga *Anabaena flos-aquae*

Concentration [mg a.s./L] (nominal)	**Water control**	**Solvent control**	**0.13**	**0.25**	**0.50**	**1.0**	**2.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.133**	**0.257**	**0.524**	**1.07**	**2.06**
Mean cell density (72 h) [% of water control] #	--	90	80	109	88	26	22
Growth rate (72 h) [% of water control] #	--	98	95	104	98	68	65
Mean cell density (120 h) [% of water control]	--	100	103	65*	64 *	48 *	44 *
Growth rate (120 h) [% of water control]	--	100	102	94 *	94 *	88 *	88 *
	Endpoints [mg dimoxystrobin/L] (mean measured)						
E_rC_{50} (72 h)	> 2.06						
E_bC_{50} (72 h)	0.960 (95% confidence limits: 0.665 - 1.39)						
E_rC_{50} (120 h)	> 2.06						
E_bC_{50} (120 h)	1.13 (95% confidence limits: 0.741 - 1.80)						

* Statistically significantly different compared to the pooled control (ANOVA followed by Bonferroni's test, $\alpha = 0.05$).
[#] Statistically significant differences were only determined after 120 h of exposure.

III. CONCLUSION

In a 120-hour algae toxicity test with *Anabaena flos-aquae*, the 72 h and 120 h E_rC_{50} values for dimoxystrobin was determined to be both > 2.06 mg a.s./L based on mean measured concentrations. The 72 h and 120 h E_bC_{50} values were 0.960 and 1.13 mg a.s./L, respectively (mean measured).

The following toxicity study on the marine diatom *Skeletonema costatum* performed with the active substance dimoxystrobin is not required for registration in the EU. The study was conducted due to U.S. data requirements and has not been evaluated previously on EU level. It is submitted for completeness. In accordance to the EFSA Aquatic Guidance Document (EFSA, 2013) and OECD guideline 201 (2011) the 72 h endpoints obtained in the 120 h study are considered as relevant endpoint for the aquatic risk assessment, therefore both the 72 h and 120 h endpoints are reported in the study summary below.

Report: CA 8.2.6.2/3
Wiskiel D.C. et al., 2000d
Growth and reproduction toxicity test with BAS 505 F and the marine alga, Skeletonema costatum
2000/5127
Guidelines: EPA 850.5400, FIFRA Subdivision J Series 123-2
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 120-h static toxicity laboratory study, the effect of dimoxystrobin on the growth of the marine diatom *Skeletonema costatum* was investigated. The following nominal concentrations were applied: 0.33, 0.66, 1.3, 2.5 and 5.0 mg dimoxystrobin/L, corresponding to mean measured concentrations of 0.330, 0.657, 1.29, 2.32 and 4.31 mg a.s./L. Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of growth was conducted directly after start of exposure and after 24, 48, 72, 96 and 120 h after test initiation.

The biological results are based on mean measured concentrations of the test item. After 120 hours of exposure statistically significant differences compared to the pooled control were observed for growth rate at the highest test item concentration.

In a 120 h algae test with *Skeletonema costatum*, the 72 h and 120 h E_rC_{50} were determined to be both > 4.31 mg a.s./L based on mean measured concentrations. The 72 h and 120 h E_bC_{50} values were 1.28 mg a.s./L and > 4.31 mg a.s./L, respectively (mean measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. no.: 285028), batch no. N15; purity: 96.0%.

B. STUDY DESIGN

Test species: Marine diatom, *Skeletonema costatum*, strain UTEX LB 2308, in-house culture; stock originally obtained from the "Culture Collection of Algae", University of Texas, Austin, USA.

Test design: Static system (120 hours); 5 test concentrations plus a dilution water control and a solvent control with 3 replicates for each; daily assessment of growth.

Endpoints: EC_{50} with respect to cell density and growth rate after exposure over 72 and 120 hours.

Test concentrations: Control (dilution water), solvent control (0.1 mL dimethylformamide/L), 0.33, 0.66, 1.3, 2.5 and 5.0 mg dimoxystrobin/L (nominal), corresponding to mean measured concentrations of 0.330, 0.657, 1.29, 2.32 and 4.31 mg a.s./L.

Test conditions: 250 mL Erlenmeyer flasks; test volume: 50 mL; enriched marine media; pH 8.0 at test initiation and pH 8.6 - 9.4 at test termination; temperature: 19.4°C - 19.5°C; initial cell densities: 1 x 10^4 cells/mL; photoperiod: 16 hours light : 8 hours dark, light intensity: 4100 - 4300 lux, continuous shaking at 100 rpm.

Analytics: Analytical verification of test item concentrations was conducted using an HPLC method with UV detection.

Statistics: Descriptive statistics, t-test (α = 0.05) for comparison of cell densities and growth rate data in the control and the solvent control; weighted least squares non-linear regression estimation procedure for determination of EC_{50} values, ANOVA followed by Bonferroni's test (α = 0.05) for determination of the NOEC (120 h) values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured concentrations of dimoxystrobin at test initiation ranged from 101.2% to 105.8% of nominal concentrations. At test termination measured concentrations of dimoxystrobin were between 71.0% and 94.5% of nominal. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects after 120 h of exposure. After 120 hours of exposure statistically significant differences compared to the pooled control were observed for growth rate at the highest test item concentration (ANOVA followed by Bonferroni's test; $\alpha = 0.05$). The effects on algal growth are summarized in Table 8.2.6.2-3.

Table 8.2.6.2-3: Effect (72 h and 120 h) of dimoxystrobin on the growth of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./L] (nominal)	**Water Control**	**Solvent control**	**0.33**	**0.66**	**1.3**	**2.5**	**5.0**
Concentration [mg a.s./L] (mean measured)	**--**	**--**	**0.330**	**0.657**	**1.29**	**2.32**	**4.31**
Mean cell density (72 h) [% of control] #	--	75	50	65	55	22	19
Growth rate (72 h) [% of control] #	--	93	81	89	83	61	57
Mean cell density (120 h) [% of control]	--	92	90	80	78	73	59
Growth rate (120 h) [% of control]	--	98	98	96	96	94	90 *
	Endpoints [mg dimoxystrobin/L] (mean measured)						
E_rC_{50} (72 h)	> 4.31						
E_bC_{50} (72 h)	1.28 (95% confidence limits: 0.551 - 2.96)						
E_rC_{50} & E_bC_{50} (120 h)	> 4.31						

* Statistically significantly different compared to the pooled control (ANOVA followed by Bonferroni's test, $\alpha = 0.05$).
# Statistically significant differences were only determined after 120 h of exposure.

III. CONCLUSION

In a 120 h algae test with *Skeletonema costatum*, the 72 h and 120 h E_rC_{50} were determined to be both > 4.31 mg a.s./L based on mean measured concentrations. The 72 h and 120 h E_bC_{50} values were 1.28 mg a.s./L and > 4.31 mg a.s./L, respectively (mean measured).

CA 8.2.7 Effects on aquatic macrophytes

The following toxicity study on the aquatic plant *Lemna gibba* performed with the active substance dimoxystrobin is not required for registration in the EU. The study was conducted due to U.S. data requirements and has not been evaluated previously on EU level. It is submitted for completeness.

Report: CA 8.2.7/1
Wyskiel D.C. et al., 2000c
Growth and reproduction toxicity test with BAS 505 F and the duckweed, Lemna gibba G3
2000/5099
Guidelines: EPA 123-2, EPA 850.4400
GLP: yes
(certified by United States Environmental Protection Agency)

Executive Summary

In a 14-day static toxicity laboratory study, the effect of dimoxystrobin on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0.033, 0.065, 0.13, 0.25 and 0.50 mg dimoxystrobin/L (corresponding to initial measured concentrations of 0.0335, 0.0634, 0.132, 0.247 and 0.444 mg a.s./L). Additionally, a solvent control (dimethylformamide) and a dilution water control were set up. Assessment of plant growth and other effects was conducted 2, 5, 7, 9, 12 and 14 days after test initiation. Percent growth inhibition relative to the control was calculated for each test concentration based upon biomass for the parameter frond number. Dry weight was determined at test termination.

The biological results are based on initial measured concentrations of the test item. The duckweed population in the control vessels showed sufficient growth. At the end of the test, chlorotic fronds were observed in the control, the solvent control and at the test item concentrations of 0.0335, 0.0634 and 0.444 mg a.s./L. Statistically significant effects on the number of non-chlorotic fronds and plant dry weight compared to the pooled control were observed at the four highest tested concentrations and at the three highest tested concentrations, respectively.

In a 14-day aquatic-plant test with *Lemna gibba,* the E_bC_{50} values of dimoxystrobin were determined to be 0.149 mg a.s./L based on frond number and 0.226 mg a.s./L based on dry weight (initial measured).

I. MATERIAL AND METHODS

A. MATERIALS

Test item: Dimoxystrobin (BAS 505 F; Reg. no.: 285028), batch no. N15; purity: 96.0%.

B. STUDY DESIGN

Test species: Duckweed (*Lemna gibba* G3); inoculum: 12 days old; cultures maintained in-house; stock obtained from "Climate Stress Laboratory", USDA, Beltsville, Maryland, USA.

Test design: Static system; test duration 14 days; 5 test item concentrations plus a control and a solvent control, 3 replicates for each test item concentration, the control and the solvent control; 4 plants with 3 fronds, total number of fronds at test initiation: 12 per replicate; assessment of growth and other effects on days 2, 5, 7, 9, 12 and 14; determination of dry weight at test termination.

Endpoints: EC_{50} with respect to biomass development after exposure over 14 days.

Test concentrations: Control, solvent control (0.1 mL dimethylformamide/L), 0.033, 0.065, 0.13, 0.25 and 0.50 mg dimoxystrobin/L (nominal), corresponding to initial measured concentrations of < limit of quantitation (LoQ), < LoQ, 0.0335, 0.0634, 0.132, 0.247 and 0.444 mg a.s./L.

Test conditions: 500 mL Erlenmeyer glass flasks, test volume: 200 mL, M-Hoagland's media without sucrose or EDTA, pH 5.0 at test initiation and pH 5.5 - 6.0 at test termination; temperature: 23.9°C - 24.4°C, continuous light, light intensity: about 510 - 520 foot-candles.

Analytics: Analytical verification of the test item was conducted using an HPLC-method with UV-detection.

Statistics: Descriptive statistics, t-test (α = 0.05) for comparison of control and solvent control data, weighted least squares non-linear regression for determination of EC_x values based on frond no. and dry weight, ANOVA followed by Bonferroni's test for determination of the NOEC values (α = 0.05).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of dimoxystrobin ranged from 88.8% to 101.5% of nominal at test initiation and from 59.4% to 98.4% of nominal at test termination. The following biological results are based on initial measured concentrations.

Biological results: No statistically significant differences were determined between the control and the solvent control data (t-test; $\alpha = 0.05$). Thus, the data from both control groups were pooled and the pooled data set was used for statistical evaluation of treatment related effects. The duckweed population in the control vessels showed sufficient growth, increasing from an average of 12 fronds per vessel to an average of 250 fronds per vessel, corresponding to a 21 x multiplication. At the end of the test, chlorotic fronds were observed in the control, the solvent control and at the test item concentrations of 0.0335, 0.0634 and 0.444 mg a.s./L. Statistically significant effects on the number of non-chlorotic fronds and plant dry weight compared to the pooled control were observed at the four highest tested concentrations and at the three highest tested concentrations, respectively (ANOVA followed by Bonferroni's test; $\alpha = 0.05$). Effects on biomass development are summarized in Table 8.2.7-1.

Table 8.2.7-1: Effects of dimoxystrobin on growth of duckweed *Lemna gibba*

Concentration [mg a.s./L] (nominal)	**Control**	**Solvent control**	**0.033**	**0.065**	**0.13**	**0.25**	**0.50**
Concentration [mg a.s./L] (initial measured)	**--**	**--**	**0.0335**	**0.0634**	**0.132**	**0.247**	**0.444**
Number of non-chlorotic fronds (14 d) [% of control]	--	--	92	84 *	45 *	35 *	24 *
Mean dry weight of fronds (14 d) [mg]	35.1	42.0	33.0	30.3	20.0 *	20.5 *	13.9 *
	Endpoints [mg a.s./L] (initial measured)						
E_bC_{50} (14 d) based on frond no	0.149 (95% confidence limits: 0.119 - 0.187)						
E_bC_{50} (14 d) based on dry weight	0.226 (95% confidence limits: 0.129 - 0.398)						

* Statistically significantly different compared to the pooled control (ANOVA followed by Bonferroni's test, $\alpha = 0.05$).

III. CONCLUSION

In a 14-day aquatic-plant test with *Lemna gibba,* the E_bC_{50} values of dimoxystrobin were determined to be 0.149 mg a.s./L based on frond number and 0.226 mg a.s./L based on dry weight (initial measured).

CA 8.2.8 Further testing on aquatic organisms

This point is not triggered and not addressed via (new) toxicity studies.

References

EFSA (2013) EFSA Scientific Opinion. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7): 3290.

Ewence, A., P. Rumsby, and I. Johnson. 2013. Extended impact assessment study of the human health and environmental criteria for endocrine disrupting substances proposed by HSE, CRD. Report No. Defra9088.02.

OECD (2006) OECD Guidelines for the Testing of Chemicals, Guideline 221, *Lemna* sp. Growth Inhibition Test. OECD Publishing. Adopted: 23 March 2006, pp. 22.

OECD (2011) OECD Guidelines for the Testing of Chemicals, Guideline 201, Freshwater Algae and Cyanobacteria, Growth Inhibition Test. OECD Publishing. Adopted: 23 March 2006, Annex 5 corrected: 28 July 2011. pp. 25.

CA 8.3 Effects on arthropods

CA 8.3.1 Effects on bees

Since Annex I inclusion of the active substance dimoxystrobin (BAS 505 F), new studies on honeybees have been performed with the active substance. As a result there are new endpoints, which are considered in the honey bee risk assessment. Summaries of these new studies are provided below and an overview on studies and endpoints is given in Table 8.3.1-1.

Table 8.3.1-1: List of studies and endpoints with honeybees and the active substance dimoxystrobin (BAS 505 F)

Substance	Test species	Endpoint	Value	Reference (BASF DocID)	EU agreed
dimoxystrobin	honeybee	48 h acute oral LD_{50}	> 79.4 µg a.s./bee	1999/10823	yes
		48 h acute contact LD_{50}	> 100.0 µg a.s./bee		
	honeybee	10 d chronic LD_{50} (overall)	83.3 µg a.s./bee	2013/1003175	no, new study
		10 d chronic LC_{50}	3.167 g a.s./kg food		
	honeybee larva	96 h LD_{50}	> 20 µg total a.s./larva, equivalent to > 10 µg dimoxystrobin/larva [1)]	2014/1111113	no, new study
		96 h LC_{50}	> 0.605 g total a.s./kg food, corresponding to > 0.121 g dimoxystrobin/kg food [1)]		

[1)] Based on the nominal content of both active substances in BAS 540 01 F (200 g/L dimoxystrobin + 200 g/L boscalid) and taking into account a density of 1.116 g/cm^3.

CA 8.3.1.1 Acute toxicity to bees

No new studies are available.

CA 8.3.1.1.1 Acute oral toxicity

No new studies are available.

CA 8.3.1.1.2 Acute contact toxicity

No new studies are available.

CA 8.3.1.2 Chronic toxicity to bees

Report: CA 8.3.1.2/1
Kleebaum K., 2014a
Chronic toxicity of BAS 505 F to the honeybee Apis mellifera L. under laboratory conditions
2013/1003175
Guidelines: Decourty et al. (2005), Suchail et al. (2001), CEB No. 230 (2012)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a 10-day chronic oral toxicity test, 2-3 day old worker honeybees (*Apis mellifera carnica* P.) were exposed to a daily application of BAS 505 F diluted in the bee food (50% w/v aqueous sucrose solution + 1% v/v acetone). The chronic toxicity of the test item was determined at nominal doses of 120.2, 81.7, 55.6, 37.8 and 25.7 µg a.s./bee/day (effective doses were 83.8, 52.1, 39.3, 26.0 and 18.1 µg a.s./bee/day), corresponding to concentrations of 3.087, 2.099, 1.427, 0.971 and 0.660 g a.s./kg, respectively. Additionally, honeybees were treated with Dimethoate EC 400 as reference item at nominal doses ranging from 4.3 to 20.1 ng a.s./bee/day. Untreated diet served as a control.

After 10 days of testing, the solvent control showed a mean mortality of 6.7% and the control 3.3%. In the test item group, bees consuming doses of 39.3, 52.1 and 83.8 µg a.s./bee/day showed mortalities of 35.0, 40.0 and 50.0%, respectively, which are statistically significantly increased compared to the control group.

In the course of the study only a few bees showed behavioral abnormalities occurring in all test item dosages. Most of them were described as moribund (maximum 2 out of 54 remaining bees on day 5 in the highest test item dosage).

In a 10 day chronic toxicity feeding study with BAS 505 F the LD_{50} was determined to be 83.3 µg consumed a.s./bee/day. This corresponds to a LC_{50} of 3.167 g a.s./kg food. The NOED was determined to be 26.0 µg consumed a.s./bee/day, corresponding to a NOEC of 0.971 g a.s./kg food.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 505 F (dimoxystrobin, Reg. No. 285 028), batch no. N6, purity: 97.4%.

B. STUDY DESIGN

Test species: Honeybee (*Apis mellifera carnica* P.); female worker bees (2-3 days old); obtained from a healthy and queen-right colony; source: Bienenfarm Kern GmbH, Leipzig, Germany.

Test design: 10-day chronic oral feeding test in the laboratory (dose response test). The honeybees were daily provided with 5 doses of test item treated sugar solutions (50% w/v aqueous sucrose solution + 1% (v/v) acetone) ad libitum. 4 treatment groups were set up: 5 doses of the test item, 2 untreated control groups (50% w/v aqueous sucrose solution and 50% w/v aqueous sucrose solution containing 1% (v/v) acetone) and 4 doses of the reference item with 3 replicates per dose, each consisting of 20 bees per replicate. Assessments of bee mortality and behavioral effects were done daily over the 10 days test period.

Endpoint: Mortality, behavioral impairments.

Reference item: Dimethoate 400 EC (BAS 152 11 I), 400.0 g/L dimethoate (nominal).

Test concentrations: Control: untreated diet (50% w/v aqueous sucrose solution),
solvent control: untreated diet with solvent (50% w/v aqueous sucrose solution plus 1% v/v acetone).
Test item: 0.660, 0.971, 1.427, 2.099 and 3.087 g a.s./kg food (corresponding to nominal doses of 25.7, 37.8, 55.6, 81.7 and 120.2 µg a.s./bee/day).
Reference item: 0.111, 0.186, 0.309 and 0.516 mg a.s./kg food (corresponding to nominal doses of 4.3, 7.2, 12.0 and 20.1 ng dimethoate/bee/day).

Test conditions: Temperature: 34°C – 35°C, mean relative humidity: 59% – 61%, photoperiod: darkness (except during assessments), food: 50% aqueous sucrose solution.

Statistics: Descriptive statistics; Fisher's Exact Binominal test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$). Probit analysis using maximum likelihood regression for calculation of the LC_{50}/LD_{50} values of the test and reference item.

II. RESULTS AND DISCUSSION

In the chronic toxicity test, the solvent control showed a mean mortality of 6.7% after 10 days of testing and the control 3.3%. In the test item group, bees consuming doses of 39.3, 52.1 and 83.8 µg a.s./bee/day showed mortalities of 35.0, 40.0 and 50.0%, respectively, which are statistically significantly increased compared to the control group. Based on the effective doses the LD_{50} was determined to be 83.3 µg consumed a.s./bee/day. This corresponds to a LC_{50} of 3.167 g a.s./kg food. The NOED was determined to be 26.0 µg consumed a.s./bee/day, corresponding to a NOEC of 0.971 g a.s./kg food.
The LD_{50} for the reference item was determined to be 9.3 ng consumed a.s./bee/day. This corresponds to a LC_{50} of 0.349 mg a.s./kg food. The highest reference dosage tested in the study was 20.1 ng a.s./bee/day (corresponding to 0.516 mg a.s./kg food), which caused a mean mortality of 86.7%.
In the course of the study only a few bees showed behavioral abnormalities occurring in all test item dosages. Most of them were described as moribund (maximum 2 out of 54 remaining bees on day 5 in the highest test item dosage).

The results are summarized in Table 8.3.1.2-1 and Table 8.3.1.2-2.

Table 8.3.1.2-1: Cumulative mortality of honeybees exposed to BAS 505 F in a 10-day chronic oral toxicity test

	Mean cumulative mortality [%]										
	Control groups		Test item [g a.s./kg food]					Reference item [mg a.s./kg food]			
Day	Control	Solvent control	0.660	0.971	1.427	2.099	3.087	0.111	0.186	0.309	0.516
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	3.3
3	0.0	1.7	0.0	3.3	0.0	0.0	3.3	0.0	0.0	1.7	3.3
4	0.0	1.7	0.0	3.3	0.0	5.0	8.3	0.0	0.0	1.7	13.3
5	0.0	1.7	0.0	3.3	0.0	6.7	10.0	0.0	0.0	6.7	21.7
6	0.0	1.7	5.0	6.7	5.0	13.3	26.7	0.0	1.7	8.3	41.7
7	0.0	3.3	6.7	8.3	15.0	15.0	30.0	0.0	3.3	11.7	55.0
8	1.7	3.3	8.3	8.3	16.7	16.7	35.0	0.0	3.3	21.7	65.0
9	3.3	3.3	13.3	16.7	28.3	26.7	41.7	0.0	5.0	28.3	78.3
10	3.3	6.7	13.3	18.3	35.0 *	40.0 *	50.0 *	0.0	6.7	33.3	86.7

* Statistically significant difference compared to the control.

Table 8.3.1.2-2: Endpoints of BAS 505 F to honeybees exposed in a 10-day chronic oral toxicity test

Endpoints		10 days
Test item doses *	**LD_{50}** [1)] [µg consumed a.s./bee/day]	83.3 (62.3 – 111.3)
	NOED [2)] [µg consumed a.s./bee/day]	26.0
Test item concentrations *	**LC_{50}**[1)] [g a.s./kg food]	3.167 (2.359 – 4.252)
	NOEC [2)] [g a.s./kg food]	0.971
Reference item **	**LD_{50}** [1)] [ng consumed a.s./bee/day]	9.3 (8.6 – 10.1)
	LC_{50} [1)] [mg a.s./kg food]	0.349 (0.320 – 0.380)

* Based on analyzed purity.
** Based on analyzed content of a.s.
1) Median lethal dose (and 95% confidence limits) was calculated using Probit analysis (linear maximum likelihood regression).
2) Fisher's Exact Binominal test with Bonferroni correction (one-sided greater, $\alpha = 0.05$).

The reference item dimethoate caused a mean mortality of 86.7% at day 10 at a concentration of 0.516 mg dimethoate/kg food, corresponding to a nominal dose of 20.1 ng dimethoate/bee/day.

III. CONCLUSION

In a 10 day chronic toxicity feeding study with BAS 505 F the LD_{50} was determined to be 83.3 µg consumed a.s./bee/day. This corresponds to a LC_{50} of 3.167 g a.s./kg food. The NOED was determined to be 26.0 µg consumed a.s./bee/day, corresponding to a NOEC of 0.971 g a.s./kg food.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Report: CA 8.3.1.3/1
Kleebaum K., 2015a
Acute toxicity of BAS 540 01 F to honeybee larvae Apis mellifera L. under laboratory conditions (in vitro)
2014/1111113

Guidelines: OECD 237 (2013) Honey bee (Apis mellifera) larval toxicity test single exposure

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In an acute oral larval toxicity test, four day old honeybee larvae (*Apis mellifera*) were exposed to one application of BAS 540 01 F diluted in the larvae food. Originally, it was intended to use the technical grade of the active ingredient dimoxystrobin (BAS 505 F) for the study, but preliminary tests showed that the solubility in the food solution was very low and could not be improved neither using an ultrasonic bath nor adding acetone. Thus, it was decided to use the representative formulation BAS 540 01 F.

The toxicity of the test item was determined with doses of 4.0, 8.0, 12.0, 16.0 and 20.0 µg a.s./larva (corresponding to 11.1, 22.3, 33.4, 44.6 and 55.7 µg product/larva). The concentrations of test item in the diet were 0.121, 0.242, 0.363, 0.484 and 0.605 g a.s./kg food. Additionally, honeybee larvae were treated with dimethoate the reference item. Untreated diet served as a control.

After 72 hours of exposure, a mortality of 11.1% was observed in the control and 13.9% mortality occurred in the control after 96 hours of exposure. In the test item group, mortalities ranged between 11.1% and 36.1% at both 72 h and 96 h. No statistically significant effects on survival occurred at any of the applied test item doses.

After 72 hours of exposure, deviations to the normal food consumption behavior and, correspondingly, in developing an average sized larva occurred in 22.7, 6.7, 16.0, 5.6 and 25.0% of the remaining larvae which had been treated with 4.0, 8.0, 12.0, 16.0 and 20.0 µg a.s./larva, respectively. In comparison, mean deviations to the normal food consumption behavior and correspondingly in developing an average sized larva occurred in 16.1% of the larvae in the control group after 72 hours. After 96 hours of exposure, deviations to the normal food consumption behavior and, correspondingly, in developing an average sized larva were still present in 5.6, 12.0, 5.6 and 7.5% of the remaining larvae, which were treated with 4.0, 12.0, 16.0 and 20.0 µg a.s./larva, respectively. However, since no dose-response relationship in the treatment group could be observed, and similar observations were made in the control group (6.7% after 96 h), a test item related effect can be excluded.

In an acute oral larval toxicity study with BAS 540 01 F on honeybee larvae, the LD_{50} value (96 h) was determined to be > 20.0 µg a.s./larva (equivalent to LC_{50} (96 h) > 0.605 g a.s./kg food). The NOED (96 h) was determined to be ≥ 20.0 µg a.s./larva (equivalent to NOEC (96 h) ≥ 0.605 g a.s./kg food).

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 540 01 F batch no.: FRE-000949; content of active substances: boscalid (BAS 510 F, Reg. No. 300 355): nominally: 200.0 g/L (analyzed: 202.3 g/L); dimoxystrobin (BAS 505 F, Reg. No. 285 028): nominally: 200.0 g/L (analyzed: 202.5 g/L); density: 1.116 g/cm^3.

B. STUDY DESIGN

Test species: *Apis mellifera* L. subspecies *carnica* P. (honeybee); synchronized first instar larvae (one day old); derived from three healthy and queen-right colonies; source: Bienenfarm Kern GmbH, Leipzig, Germany.

Test design: One day old honeybee larvae of *Apis mellifera* were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 3 days before start of the treatment. After this, in a 72/96 hour acute test, the 4 day old larvae were exposed to a single application of BAS 540 01 F diluted in the larvae food (aqueous sugar solution mixed with royal jelly). In total, 7 treatment groups were set up: 5 doses of the test item, one untreated control group and 1 dose of the reference item with 3 replicates per dose and 12 larvae per replicate. After the day of application, additional feeding of the larvae took place 24 and 48 hours later. Assessments of larval mortality were done after 24, 48 and 72, and 96 hours. Additionally, other observations such as small body size or large quantities of remaining food after 72 and 96 hours were noted. In an analytical phase of the study, the concentration of the active substance in the test item stock solution was determined.

Endpoints: Mortality (LD_{50}), qualitative observations: body size, remaining food.

Reference item: Dimethoate technical (analyzed purity: 99.8%).

Test doses: Control (50% aqueous sugar solution with 50% royal jelly)
Test item treatments:

Nominal dose/concentration	
Doses [µg a.s./larva]	**Concentrations [g a.s./kg food]**
4.0	0.121
8.0	0.242
12.0	0.363
16.0	0.484
20.0	0.605

Reference item treatment: 8.8 µg dimethoate/larva (0.267 g a.s./kg food).

Test conditions: Temperature: 34.2° C – 34.8° C; relative humidity: 92% - 97%, photoperiod: 24 h darkness; food: 50% aqueous sugar solution with 50% royal jelly.

Statistics: Descriptive statistics; for mortality data Fisher's Exact Binomial Test with Bonferroni Correction (one-sided greater, α = 0.05).

II. RESULTS AND DISCUSSION

After 72 hours of exposure, a mortality of 11.1% was observed in the control and 13.9% mortality occurred in the control after 96 hours of exposure. In the test item group, mortalities ranged between 11.1% and 36.1% at both 72 h and 96 h. No statistically significant effects on survival occurred at any of the applied test item doses (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater, α = 0.05).

After 72 hours of exposure, deviations to the normal food consumption behavior and, correspondingly, in developing an average sized larva occurred in 22.7, 6.7, 16.0, 5.6 and 25.0% of the remaining larvae, which were treated with 4.0, 8.0, 12.0, 16.0 and 20.0 µg a.s./larva, respectively. In comparison, mean deviations to the normal food consumption behavior and correspondingly in developing an average sized larva occurred in 16.1% of the larvae in the control group after 72 hours. After 96 hours of exposure, deviations to the normal food consumption behavior and, correspondingly, in developing an average sized larva were still present in 5.6, 12.0, 5.6 and 7.5% of the remaining larvae, which were treated with 4.0, 12.0, 16.0 and 20.0 µg a.s./larva, respectively. However, since no dose-response relationship in the treatment group could be observed, and similar observations were made in the control group (6.7% after 96 h), a test item related effect can be excluded.

The results are summarized in Table 8.3.1.3-1.

Table 8.3.1.3-1: Toxicity of BAS 540 01 F to *Apis mellifera* (honeybee) in an acute oral larval toxicity test after 72 and 96 hours

Treatment group	Dosage [µg a.s./ larva]	Concentration [g a.s./ kg food]	72 hour Mean mortality [%]		96 hour Mean mortality [%]	
			absolute	corrected [1)]	absolute	corrected [1)]
Control	--	--	11.1	--	13.9	--
Test item	4.0	0.121	11.1	0.0	11.1	0.0
	8.0	0.242	11.1	0.0	13.9	0.0
	12.0	0.363	11.1	0.0	11.1	0.0
	16.0	0.484	19.4	9.4	25.0	12.9
	20.0	0.605	36.1	28.1	36.1	25.8
Treatment	**Endpoints**		**72 h**		**96 h**	
Test item doses	LD_{50} [µg a.s./larva]		> 20.0		> 20.0	
	NOED [µg a.s./larva]		≥ 20.0		≥ 20.0	
Test item concentrations	LC_{50} [g a.s./kg food]		> 0.605		> 0.605	
	NOEC [g a.s./kg food]		≥ 0.605		≥ 0.605	

1) According to Schneider-Orelli (1947).

Larva dosed with 8.8 µg dimethoate/larva revealed on average a mortality of 83.3% after 72 h and 88.9% after 96 h (corresponding to a corrected mortality of 81.3% and 87.1%, respectively).

III. CONCLUSION

In an acute oral larval toxicity study with BAS 540 01 F on honeybee larvae, the LD_{50} value (96 h) was determined to be > 20.0 µg a.s./larva (equivalent to LC_{50} (96 h) > 0.605 g a.s./kg food). The NOED (96 h) was determined to be ≥ 20.0 µg a.s./larva (equivalent to NOEC (96 h) ≥ 0.605 g a.s./kg food).

CA 8.3.1.4 Sub-lethal effects

No new studies are available.

CA 8.3.2 Effects on non-target arthropods other than bees

No new studies are available.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

No new studies are available.

CA 8.3.2.2 Effects on Typhlodromus pyri

No new studies are available.

CA 8.4 Effects on non-target soil meso- and macrofauna

Since Annex I inclusion of the active substance dimoxystrobin (BAS 505 F), new studies on soil macro-organisms have been performed with the active substance and its relevant metabolites in soil. As a result there are new endpoints, which are considered in the respective risk assessment. Summaries of these new studies are provided below and an overview on studies and endpoints is given in Table 8.4-1.

Table 8.4-1 Toxicity to non-target soil meso- and macrofauna of dimoxystrobin and relevant metabolites

Substance (Reg.No, synonyms)	Species	Endpoint	Value [mg/kg dry soil]	Reference (BASF DocID)	EU agreed
Dimoxystrobin	*Eisenia fetida*	LC_{50}	23.65 *	1998/11186	yes
505M01 (Reg.No. 358104, BF 505-04)		LC_{50}	> 1000	2000/1003883	yes
505M08 (Reg.No. 354562, BF 505-07)		LC_{50}	> 1000	2000/1003884	yes
505M09 (Reg.No. 354563, BF 505-08)		LC_{50}	> 1000	2000/1003885	yes
Dimoxystrobin		NOEC $NOEC_{CORR}$	0.050 0.025	2014/1028577	no, new study
505M01 (Reg.No. 358104, BF 505-04)		NOEC	31.25	2011/1102405	no, new study
505M08 (Reg.No. 354562, BF 505-07)		NOEC	≥ 250	2014/1010826	no, new study
505M09 (Reg.No. 354563, BF 505-08)		NOEC	≥ 250	2014/1010827	no, new study
Dimoxystrobin	*Folsomia candida*	NOEC	≥ 1000	2014/1010825	no, new study
505M09 (Reg.No. 354563, BF 505-08)		NOEC	≥ 1000	2014/1084492	no, new study
Dimoxystrobin	*Hypoaspis aculeifer*	NOEC	≥ 1000	2014/1010824	no, new study
505M09 (Reg.No. 354563, BF 505-08)		NOEC	≥ 1000	2014/1170855	no, new study

* Toxicity endpoint is re-adjusted using a soil factor of 2 to address the organic content of the soil (peat 10%), since the log P_{ow} of the substance is > 2.

CA 8.4.1 Earthworms – sub-lethal effects

Report: CA 8.4.1/1
Friedrich S., 2014a
Sublethal toxicity of BAS 505 F (Dimoxystrobin) to the earthworm Eisenia fetida in artificial soil
2014/1028577

Guidelines: OECD 222 (2004)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of dimoxystrobin (BAS 505 F) on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations (0.0102, 0.0173, 0.0294, 0.050 and 0.085 mg a.s./kg dry soil) were incorporated into the soil (10% peat) with 4 replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. The reference item was tested in a separate study. Assessment of worm mortality, behavioral effects and biomass development was carried out after 28 days; assessment of reproduction rate (number of offspring) was carried out after another 28 days (56 days after application).

Dimoxystrobin did not show any statistically significant effects on mortality and body weight. The mortality of adult worms was 0% – 2.5% in the treated variants and 0% in the control group. The weight change of adult worms was 21.5% – 25.4% in the treated variants and 24.1% in the control group. The reproduction rate was statistically significantly different compared to the control at a concentration of 0.085 mg a.s./kg soil dry weight, the highest concentration tested. No behavioral abnormalities were observed in any of the treatment groups. No effects on feeding activity were observed in any of the treatment groups.

In a 56-day earthworm reproduction study with dimoxystrobin (BAS 505 F) on earthworms (*Eisenia fetida*), the NOEC for mortality and biomass was determined to be ≥ 0.085 mg a.s./kg dry soil. The NOEC for reproduction was determined to be 0.050 mg a.s./kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: BAS 505 F (dimoxystrobin, Reg.No. 285028), batch no. N6, purity: 97.4%.

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 371 mg – 569 mg), age: approximately 4 months old; source: W. Neudorff GmbH KG followed by in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 (10% peat); different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. The artificial soil was treated and filled into plastic vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioral effects and biomass development was carried out after 28 days; assessment of reproduction rate (number of offspring) was carried out after another 28 days (56 days after application).

Endpoints: Mortality, weight change, reproduction rate, feeding activity.

Reference item: Nutdazim 50 Flow (carbendazim, SC 500). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 0.0102, 0.0173, 0.0294, 0.050 and 0.085 mg a.s./kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with 10% peat); pH 6.07 – pH 6.12 at test initiation, pH 5.86 – pH 5.88 at test termination; maximum water holding capacity (WHC): 54.9% - 55.2% at test initiation, 54.1% - 54.7% of WHC at test termination; temperature: 19.3°C - 21.9°C; photoperiod: 16 h light : 8 h dark, light intensity: 510 lux.

Statistics: Descriptive statistics, Fisher's Exact Binominal test for mortality (one-sided greater, $\alpha = 0.05$) and Williams t-test (one-sided smaller, $\alpha = 0.05$) for weight change and reproduction data, Probit analysis.

II. RESULTS AND DISCUSSION

Dimoxystrobin (BAS 505 F) did not show any statistically significant effects on mortality and body weight. The mortality of adult worms was 0% – 2.5% in the treated variants and 0% in the control group. The weight change of adult worms was 21.5% – 25.4% in the treated variants and 24.1% in the control group.
The reproduction rate was statistically significantly different compared to the control at a concentration of 0.085 mg a.s./kg soil dry weight (Williams-t-test, $\alpha = 0.05$, one-sided smaller), the highest concentration tested. No behavioral abnormalities were observed in any of the treatment groups. No effects on feeding activity were observed in any of the treatment groups. The results are summarized below in Table 8.4.1-1.

Table 8.4.1-1: Effects of dimoxystrobin (BAS 505 F) on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Dimoxystrobin [mg a.s./kg dry soil]	Control	0.0102	0.0173	0.0294	0.050	0.085
Mortality (day 28) [%]	0.0	0.0	2.5	0.0	0.0	2.5
Weight change (day 28) [%]	24.1	25.4	23.3	21.5	22.7	22.9
Mean no. of juveniles (day 56)	133.6	128.3	144.5	134.8	125.3	99.8 *
Reproduction (day 56) [% deviation from control]	100	96.0	108.1	100.8	93.7	74.6
Endpoints [mg a.s./kg dry soil]						
$NOEC_{mortality}$ (day 28)	≥ 0.085					
$NOEC_{reproduction}$ (day 56)	0.050					
EC_{50} (day 56)	> 0.085					

* Statistically significant difference compared to the control (Williams t-test, $\alpha = 0.05$, one-sided smaller).

In a separate study the reference item had a significant effect on biomass increase and reproduction of *Eisenia fetida*. The reproduction rate was clearly inhibited by 39% and 100% compared to the control at the tested concentrations of 5 and 10 mg product/kg soil dry weight.

III. CONCLUSION

In a 56-day earthworm reproduction study with dimoxystrobin (BAS 505 F) on earthworms (*Eisenia fetida*), the NOEC for mortality and biomass was determined to be ≥ 0.085 mg a.s./kg dry soil. The NOEC for reproduction was determined to be 0.050 mg a.s./kg dry soil.

Report: CA 8.4.1/2
Schoebinger U., 2012a
Sublethal toxicity of Reg.No. 358104 (metabolite of BAS 505 F, 505M01) to the earthworm Eisenia fetida in artificial soil with 5% peat
2011/1102405
Guidelines: ISO 11268-2 (1998), OECD 222 (2004)
GLP: yes
(certified by Landesanstalt fuer Umwelt, Messungen und Naturschutz Baden-Wuerttemberg, Karlsruhe, Germany)

Executive Summary

The effects of 505M01 (Reg.No. 358104), a metabolite of dimoxystrobin, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations (15.625, 31.25, 62.5, 125 and 250 mg test item/kg dry soil) were incorporated into the soil (5% peat) with 4 replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of adult worm mortality, biomass development, and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure no mortality was observed for the control and all test item concentrations up to and including 250 mg 505M01/kg dry soil. Body weight of earthworms exposed to 505M01 was not statistically significantly different compared to the control up to 250 mg 505M01/kg dry soil, the highest concentration tested. The mean numbers of juveniles in the test item treatments were between 48.8 and 153.7, compared to 163.5 in the control group. The number of juveniles was statistically significantly different compared to the control at concentrations of 15.625, 62.5, 125 and 250 mg 505M01/kg dry soil. No effects on feeding activity were observed in any of the treatment groups.

In a 56-day earthworm reproduction study with 505M01 (Reg.No. 358104, metabolite of dimoxystrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality and biomass was determined to be ≥ 250 mg 505M01/kg dry soil. The NOEC for reproduction was determined to be 31.25 mg 505M01/kg dry soil.

I. MATERIAL AND METHODS

A. MATERIALS

Test item: 505M01 (Reg.No. 358104; metabolite of dimoxystrobin (BAS 505 F)) batch no. 01893-8; purity: 99.6%.

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 450 mg – 600 mg), age: between two and twelve months old; source: in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222 and ISO 11268-2; different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. The artificial soil was treated and filled into plastic vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioral effects and biomass development after 28 days; assessment of reproduction rate (number of offspring) after another 28 days (56 days after application).

Endpoints: Effects on mortality, weight change, reproduction rate, feeding activity.

Reference item: Twist WP (carbendazim, 602.8 g/kg). The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 15.625, 31.25, 62.5, 125 and 250 mg test item/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with reduced content of peat: 5%); pH 5.98 – pH 6.01 at test initiation, pH 6.39 – pH 6.60 at test termination; maximum water holding capacity (WHC): 56.8% - 58.0% at test initiation, 54.0% - 62.6% of WHC at test termination; temperature: 19.1 °C - 21.3 °C; photoperiod: 16 h light: 8 h dark, light intensity: 530 - 660 lux.

Statistics: Descriptive statistics, Dunnett’s t-test (two-tailed, α = 0.05) for weight change data and Williams test (one-sided, α = 0.05) for reproduction data.

II. RESULTS AND DISCUSSION

After 28 days of exposure no mortality was observed for the control and all test item concentrations up to and including 250 mg 505M01/kg dry soil.
Body weight of earthworms exposed to 505M01 was not statistically significantly different compared to the control (Dunnett's t-test, two-tailed, α = 0.05) up to 250 mg 505M01/kg dry soil, the highest concentration tested.
The mean numbers of juveniles in the test item treatments were between 48.8 and 153.7, compared to 163.5 in the control group. The number of juveniles were statistically significantly different compared to the control at concentrations of 15.625, 62.5, 125 and 250 mg 505M01/kg dry soil (Williams test, one-sided, α = 0.05).
No effects on feeding activity were observed in any of the treatment groups. The results are summarized below in Table 8.4.1-2.

Table 8.4.1-2: Effects of 505M01 on earthworms (*Eisenia fetida*) in a 56-day reproduction study

505M01 [mg/kg dry soil]	Control	15.625	31.25 [1)]	62.5	125	250
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0
Weight change (day 28) [%]	+19.1	+19.2	+13.4	+21.0	+12.2	+23.8
Mean no. of juveniles (day 56)	163.5	141.3*	153.7	121.5*	100.3*	48.8*
Reproduction (day 56) [% deviation from control]	--	-13.6	-5.99	-25.7	-38.7	-70.2
Feeding activity [g]	20	20	20	20	20	20
	Endpoints [mg 505M01/kg dry soil]					
$NOEC_{mortality}$ (day 28)	≥ 250					
$NOEC_{reproduction}$ (day 56)	31.25					

1) Value based on three replicates.
* Statistically significant differences compared to the control (Williams test, α = 0.05, one-sided).

III. CONCLUSION

In a 56-day earthworm reproduction study with 505M01 (Reg.No. 358104), a metabolite of dimoxystrobin on earthworms (*Eisenia fetida*), the NOEC for mortality and biomass was determined to be ≥ 250 mg 505M01/kg dry soil. The NOEC for reproduction was determined to be 31.25 mg 505M01/kg dry soil.

Report: CA 8.4.1/3
Friedrich S., 2014b
Sublethal toxicity of Reg.No. 354562 (metabolite of BAS 505 F, Dimoxystrobin) to the earthworm Eisenia fetida in artificial soil
2014/1010826
Guidelines: OECD 222 (2004)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of 505M08 (Reg.No. 354562), a metabolite of dimoxystrobin, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations (15.63, 31.25, 62.5, 125 and 250 mg test item/kg dry soil) were incorporated into the soil (10% peat) with 4 replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of adult worm mortality, biomass development, and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure, 505M08 did not show any statistically significant effects on mortality and body weight. The mortality of adult worms was 0% – 2.5% in the treated variants compared to 1.3% in the control group. The weight change of adult worms was about 26.6% – 29.5% in the treated variants compared to 27.9% in the control group.
The reproduction rates were not statistically significantly different compared to those in the control up to and including a concentration of 250 mg 505M08/kg dry soil. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

In a 56-day earthworm reproduction study with 505M08 (Reg.No. 354562), metabolite of dimoxystrobin, on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass and reproduction was determined to be ≥ 250 mg 505M08/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: 505M08 (Reg.No. 354562, metabolite of dimoxystrobin) batch no. 01196-241; purity: 97.8% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 401 mg – 597 mg), age: approximately 4 months old; source: in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222; different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. The artificial soil was treated and filled into plastic vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioral effects and biomass development after 28 days; assessment of reproduction rate (number of offspring) after another 28 days (56 days after application).

Endpoints: Effects on mortality, weight change, reproduction rate, feeding activity.

Reference item: Nutdazim 50 FLOW (carbendazim, SC 500). The effects of the reference item were investigated in a separate study at concentrations of 5 and 10 mg/kg dry soil.

Test concentrations: Control, 15.63, 31.25, 62.5, 125 and 250 mg test item/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with 10% peat); pH 6.01 – pH 6.03 at test initiation, pH 5.80 – pH 5.85 at test termination; maximum water holding capacity (WHC): 54.9% – 55.0% at test initiation, 53.9% –54.7% of WHC at test termination; temperature: 19.0 °C – 21.5 °C; photoperiod: 16 h light : 8 h dark, light intensity: 540 lux.

Statistics: Descriptive statistics, Fisher's Exact Binomial Test (one-sided greater, $\alpha = 0.05$) for mortality data and Williams-t-test (one-sided smaller, $\alpha = 0.05$) for reproduction and weight change data.

II. RESULTS AND DISCUSSION

After 28 days of exposure, 505M08 (Reg.No. 354562, metabolite of dimoxystrobin) did not show any statistically significant effects on mortality and body weight (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$ for mortality data and Williams-t-test, one-sided smaller, $\alpha = 0.05$ for weight change data). The mortality of adult worms was 0% – 2.5% in the treated variants compared to 1.3% in the control group. The weight change of adult worms was about 26.6% – 29.5% in the treated variants compared to 27.9% in the control group.
The reproduction rates were not statistically significantly different compared to those in the control up to and including a concentration of 250 mg 505M08/kg dry soil (Williams-t-test, one-sided smaller, $\alpha = 0.05$). No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control. The results are summarized below in Table 8.4.1-3.

Table 8.4.1-3: Effects of 505M08 (Reg.No. 354562), a metabolite of dimoxystrobin, on earthworms (*Eisenia fetida*) in a 56-day reproduction study

505M08 [mg/kg dry soil]	Control	15.63	31.25	62.5	125	250
Mortality (day 28) [%]	1.3	2.5	0.0	0.0	0.0	0.0
Weight change (day 28) [%]	27.9	26.8	29.5	26.6	27.2	27.6
Mean no. of juveniles (day 56)	123.0	118.3	129.0	123.3	111.3	104.8
Reproduction in [%] of control (day 56)	100	96.1	104.9	100.2	90.4	85.2
Feeding activity [g]	5	5	5	5	5	5
Endpoints [mg 505M08/kg dry soil]						
$NOEC_{mortality}$ (day 28)	≥ 250					
$NOEC_{reproduction}$ (day 56)	≥ 250					

III. CONCLUSION

In a 56-day earthworm reproduction study with 505M08 (Reg.No. 354562, metabolite of dimoxystrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass and reproduction was determined to be ≥ 250 mg 505M08/kg dry soil.

Report:	CA 8.4.1/4 Friedrich S., 2014c Sublethal toxicity of Reg.No. 354563 (metabolite of BAS 505 F, Dimoxystrobin) to the earthworm Eisenia fetida in artificial soil 2014/1010827
Guidelines:	OECD 222 (2004)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of 505M09 **(**Reg.No. 354563), a metabolite of dimoxystrobin, on mortality, biomass development and reproduction of the earthworm *Eisenia fetida* (Annelida: Oligochaeta) were investigated in a 56-day reproduction study. Five concentrations (15.63, 31.25, 62.5, 125 and 250 mg test item/kg dry soil) were incorporated into the soil (10% peat) with 4 replicates per treatment (each containing 10 worms). An untreated control with 8 replicates was included. Assessment of adult worm mortality, biomass development, and feeding activity was carried out after 28 days, assessment of reproduction (number of juveniles) was carried out after 56 days.

After 28 days of exposure, 505M09 did not show any statistically significant effects on mortality and body weight. The mortality of adult worms was 0% – 2.5% in the treated variants compared to 1.3% in the control group. The weight change of adult worms was about 25.7% – 28.6% in the treated variants compared to 27.9% in the control group.
The reproduction rates were not statistically significantly different compared to those in the control up to and including a concentration of 250 mg Reg. No. 354 563/kg dry soil. No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control.

In a 56-day earthworm reproduction study with 505M09 (Reg.No. 354563, metabolite of dimoxystrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass and reproduction was determined to be ≥ 250 mg 505M09/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: 505M09 (Reg.No. 354563, metabolite of dimoxystrobin) batch no. L80-82; purity: 96.3% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: Earthworm (*Eisenia fetida*), adult worms (with clitellum); weight: 400 mg – 597 mg), age: approximately 4 months old; source: in-house culture.

Test design: 56-day test in treated artificial soil according to OECD 222; different concentrations of the test item were incorporated into the soil; 6 treatment groups (5 test item concentrations, control); 4 replicates for the test item treatments, 8 replicates for the control, 10 worms each. The artificial soil was treated and filled into plastic vessels, before the earthworms were introduced on the top of the soil. Assessment of worm mortality, behavioral effects and biomass development after 28 days; assessment of reproduction rate (number of offspring) after another 28 days (56 days after application).

Endpoints: Effects on mortality, weight change, reproduction rate, feeding activity.

Reference item: Nutdazim 50 FLOW (carbendazim, SC 500). The effects of the reference item were investigated in a separate study at concentrations of 5 and 10 mg/kg dry soil.

Test concentrations: Control, 15.63, 31.25, 62.5, 125 and 250 mg test item/kg dry soil.

Test conditions: Artificial soil according to OECD 222 (with 10% peat); pH 6.03 – pH 6.12 at test initiation, pH 5.82 – pH 5.89 at test termination; maximum water holding capacity (WHC): 54.9% - 55.2% at test initiation, 53.8% - 54.9% of WHC at test termination; temperature: 19.0 °C - 21.5 °C; photoperiod: 16 h light : 8 h dark, light intensity: 540 lux.

Statistics: Descriptive statistics, Fisher's Exact Binomial Test (one-sided greater, $\alpha = 0.05$) for mortality data and Williams-t-test (one-sided smaller, $\alpha = 0.05$) for reproduction and weight change data.

II. RESULTS AND DISCUSSION

After 28 days of exposure, 505M09 (Reg.No. 354563, metabolite of dimoxystrobin) did not show any statistically significant effects on mortality and body weight (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$ for mortality data and Williams-t-test, one-sided smaller, $\alpha = 0.05$ for weight change data). The mortality of adult worms was 0% – 2.5% in the treated variants compared to 1.3% in the control group. The weight change of adult worms was about 25.7% – 28.6% in the treated variants compared to 27.9% in the control group.
The reproduction rates were not statistically significantly different compared to those in the control up to and including a concentration of 250 mg 505M09 /kg dry soil (Williams-t-test, one-sided smaller, $\alpha = 0.05$). No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control. The results are summarized below in Table 8.4.1-4.

Table 8.4.1-4: Effects of 505M09 (Reg.No. 354563), a metabolite of dimoxystrobin, on earthworms (*Eisenia fetida*) in a 56-day reproduction study

505M09 [mg/kg dry soil]	Control	15.63	31.25	62.5	125	250
Mortality (day 28) [%]	1.3	0.0	2.5	0.0	0.0	0.0
Weight change (day 28) [%]	27.9	28.6	27.7	25.7	27.5	25.9
Mean no. of juveniles (day 56)	123.0	118.0	121.5	119.5	110.5	96.0
Reproduction in [%] of control (day 56)	100	95.9	98.8	97.2	89.8	78.0
Feeding activity [g]	5	5	5	5	5	5
	Endpoints [mg 505M09/kg dry soil]					
$NOEC_{mortality}$ (day 28)	≥ 250					
$NOEC_{reproduction}$ (day 56)	≥ 250					

III. CONCLUSION

In a 56-day earthworm reproduction study with 505M09 (Reg.No. 354563, metabolite of dimoxystrobin) on earthworms (*Eisenia fetida*), the NOEC for mortality, biomass and reproduction was determined to be ≥ 250 mg 505M09/kg dry soil.

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

Report: CA 8.4.2.1/1
Friedrich S., 2014d
Effects of BAS 505 F (Dimoxystrobin) on the reproduction of the collembolan Folsomia candida
2014/1010825
Guidelines: OECD 232 (2009), ISO 11267 (1999)
GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of dimoxystrobin (BAS 505 F) on mortality and reproduction of the Collembola *Folsomia candida* were investigated in a laboratory study over 28 days. Five application rates (62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil) were incorporated into the soil with 4 replicates per test item treatment. An untreated control and a solvent control treated with acetone, each with 8 replicates, were included. All replicates contained 10 collembolans. Assessment of mortality, reproduction rate (number of juveniles) and behavior was carried out after 28 days.

No statistically significant effect on parental mortality was found for any concentration tested. Mortality rates of 0% - 2.5% were recorded in the test item treatment groups compared to 2.5% in the solvent control. No statistically significant effects on the number of juveniles compared to the solvent control were recorded at any concentration tested. The mean reproduction in the solvent control reached 577 juveniles. Reproduction rates in 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil were 569, 600, 571, 565 and 577 juveniles, respectively. Differences between the behavior of the collembolans in the solvent control group and the test item treatment groups could not be observed.

In a 28-day Collembolan reproduction study with dimoxystrobin the NOEC based on mortality and reproduction was determined to be ≥ 1000 mg a.s./kg dry soil, the highest concentration tested. The LC_{50} and the EC_{50} were determined to be > 1000 mg a.s./kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 505 F (dimoxystrobin, Reg.No. 285028), batch no. N6, purity: 97.4% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: Collembolans (*Folsomia candida)*, juveniles (9 - 12 days old); source: in-house culture.

Test design: 28-day chronic laboratory test in treated artificial soil according to OECD 232 and ISO 11267; different concentrations of the test item were mixed homogenously into artificial soil and filled into glass vessels after which collembolans were introduced on top of the soil; 6 treatment groups (5 test item concentrations, solvent control); 4 replicates for each test item treatment and 8 replicates for the control groups, each containing 10 collembolans. Assessment of adult mortality, reproduction (number of juveniles) and behavioral effects after 28 days.

Endpoints: Mortality, reproduction rate after 28 days.

Reference item: Boric acid (100% analyzed). The effects of the reference item were investigated in a separate study.

Test concentrations: Solvent control, 62.5, 125, 250, 500 and 1000 mg BAS 505 F/kg dry soil.

Test conditions: Artificial soil according to OECD 232 (peat: 5%); pH 6.15 – pH 6.19 at test initiation, pH 5.85 – pH 5.90 at test termination; water content at study initiation 56.5% - 56.7% of maximum water holding capacity and 55.8% - 56.2% of maximum WHC at test termination; temperature: 19.0 C - 21.5 C; photoperiod: 16 h light : 8 h dark, light intensity: 530 lux.

Statistics: Descriptive statistics, Fisher's Exact Binominal Test with Bonferroni Correction for mortality (one-sided greater, $\alpha = 0.05$) and Williams-t-test for reproduction data (one-sided smaller, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

No statistically significant effect on parental mortality was found for any concentration tested (Fisher's Exact Binomial Test, one-sided greater, $\alpha = 0.05$). Mortality rates of 0% - 2.5% were recorded in the test item treatment groups compared to 2.5% in the solvent control.

No statistically significant effects on the number of juveniles compared to the solvent control were recorded at any concentration tested (Williams-t-test, one-sided smaller, $\alpha = 0.05$). The mean reproduction in the solvent control reached 577 juveniles. Reproduction rates in 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil were 569, 600, 571, 565 and 577 juveniles, respectively. Differences between the behavior of the collembolans in the solvent control group and the test item treatment groups could not be observed. The results are summarized in Table 8.4.2.1-1.

Table 8.4.2.1-1: Effects of dimoxystrobin (BAS 505 F) on Collembola (*Folsomia candida*) in a 28-day reproduction study

Dimoxystrobin [mg a.s./kg dry soil]	Solvent control	62.5	125	250	500	1000
Mortality (day 28) [%]	2.5	2.5	0.0	2.5	0.0	2.5
No. of juveniles (day 28)	577	569	600	571	565	577
Reproduction in [%] of control (day 28)	100	99	104	99	98	100
	Endpoints [mg a.s./kg dry soil]					
NOEC $_{mortality + reproduction}$ (day 28)	≥ 1000					
LC_{50}	> 1000					
EC_{50}	> 1000					

III. CONCLUSION

In a 28-day Collembolan reproduction study with dimoxystrobin (BAS 505 F) the NOEC based on mortality and reproduction was determined to be ≥ 1000 mg a.s./kg dry soil, the highest concentration tested. The LC_{50} and the EC_{50} was determined to be > 1000 mg a.s./kg dry soil.

Report: CA 8.4.2.1/2
Ganssmann M., 2015b
Effects of Reg.No. 354563 (metabolite of BAS 505 F, Dimoxystrobin) on reproduction of the collembolan Folsomia candida in artificial soil with 5% peat
2014/1084492
Guidelines: OECD 232 (2009), ISO 11267 (1999)
GLP: yes
(certified by Hessisches Ministerium fuer Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Wiesbaden)

Executive Summary

The effects of 505M09 (Reg. No. 354 563, metabolite of BAS 505 F, dimoxystrobin) on mortality and reproduction of the Collembola *Folsomia candida* were investigated in a laboratory study over 28 days. Five application rates (62.5, 125, 250, 500 and 1000 mg 505M09/kg dry soil) were incorporated into artificial soil (5% peat) with 4 replicates per test item treatment. An untreated control with 8 replicates was included. All replicates contained 10 collembolans. Assessment of mortality and reproduction rate (number of juveniles) was carried out after 28 days.

Mortality of *Folsomia candida* in the test item treatments was not statistically significantly different when compared to the control up to and including 1000 mg 505M09/kg dry soil, the highest concentration tested. Mortality rates of 3% - 10% were recorded in the test item treatment groups compared to 3% in the control.
No statistically significant effects compared to the control on reproduction of *Folsomia candida* up to and including the highest concentration of 1000 mg 505M09/kg dry soil were observed. The mean reproduction in the control reached 795 juveniles. Reproduction in the 62.5, 125, 250, 500 and 1000 mg 505M09/kg dry soil treatments was 771, 792, 898, 924 and 751 juveniles, respectively. Differences between the behavior of the collembolans in the control group and the test item treatment groups could not be observed.

In a 28-day Collembolan reproduction study with 505M09 (Reg. No. 354 563, metabolite of dimoxystrobin) the NOEC based on mortality and reproduction was ≥ 1000 mg 505M09/kg dry soil, the highest concentration tested. The LC_{50} and the EC_{50} was determined to be > 1000 mg 505M09/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

Test item:	505M09 (Reg.No. 354 563, metabolite of BAS 505 F, dimoxystrobin), batch no. L80-82, purity: 96.3% (±1.0%).

B. STUDY DESIGN

Test species:	Collembolans (*Folsomia candida)*, juveniles (9 - 12 days old); source: in-house culture.
Test design:	28-day chronic laboratory test in treated artificial soil according to OECD 232 and ISO 11267; different concentrations of the test item were mixed homogenously into artificial soil and filled into glass vessels after which collembolans were introduced on top of the soil; 6 treatment groups (5 test item concentrations, 1 control); 4 replicates for each test item treatment and 8 replicates for the control group, each containing 10 collembolans. Assessment of adult mortality, behavior and reproduction (number of juveniles) after 28 days.
Endpoints:	Mortality, behavioral effects and reproduction rate after 28 days.
Reference item:	Boric acid (purity 100.3%). The effects of the reference item were investigated in a separate study.
Test concentrations:	Control (deionized water), 62.5, 125, 250, 500 and 1000 mg 505M09/kg dry soil.
Test conditions:	Artificial soil according to OECD 232 (peat: 5%); pH 5.6 – 5.9 at experimental start and pH 5.4 – 5.9 at test termination; water content 50.1% - 50.9% of the maximum water holding capacity (WHC) at experimental start and 42.4% - 46.3% at test termination; temperature: 18°C – 22°C; photoperiod: 16 h light : 8 h dark, light intensity: 400 – 800 lux; food: approximately 2 mg dry yeast at test start and on day 14.
Statistics:	Descriptive statistics, Fisher's Exact Binomial Test with Bonferroni Correction for mortality (one-sided greater, $\alpha = 0.05$) and Dunnett's-test for reproduction data (one-sided smaller, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

Mortality of *Folsomia candida* was not statistically significantly different when compared to the control up to and including 1000 mg 505M09/kg dry soil, the highest concentration tested (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). Mortality rates of 3% - 10% were recorded in the test item treatment groups compared to 3% in the control.
No statistically significant effects on reproduction of *Folsomia candida* up to and including the highest concentration of 1000 mg 505M09/kg dry soil were observed (Dunnett's-test, $\alpha = 0.05$, one-sided greater). The mean reproduction in the control reached 795 juveniles. Reproduction in the 62.5, 125, 250, 500 and 1000 mg 505M09/kg dry soil treatments reached 771, 792, 898, 924 and 751 juveniles, respectively. Differences between the behavior of the collembolans in the control group and the test item treatment groups could not be observed. The results are summarized in Table 8.4.2.1-2.

Table 8.4.2.1-2: Effects of 505M09 (Reg.No. 354 563) on Collembola (*Folsomia candida*) in a 28-day reproduction studies

505M09 [mg/kg dry soil]	**Control**	**62.5**	**125**	**250**	**500**	**1000**
Mean mortality (day 28) [%]	3	10	3	5	10	10
No. of juveniles (day 28)	795	771	792	898	924	751
Reproduction in [%] of control (day 28)	--	97	100	113	116	94
Endpoints [mg 505M09/kg dry soil]						
NOEC $_{mortality + reproduction}$ (day 28)	≥ 1000					
LOEC $_{mortality + reproduction}$ (day 28)	> 1000					
LC_{50}	> 1000					
EC_{50}	> 1000					

III. CONCLUSION

In a 28-day Collembolan reproduction study with 505M09 (Reg. No. 354 563, metabolite of dimoxystrobin) the NOEC based on mortality and reproduction was ≥ 1000 mg 505M09/kg dry soil, the highest concentration tested. The LC_{50} and the EC_{50} was determined to be > 1000 mg 505M09/kg dry soil.

Report:	CA 8.4.2.1/3 Schulz L., 2014a Effects of BAS 505 F (Dimoxystrobin) on the reproduction of the predatory mite Hypoaspis aculeifer 2014/1010824
Guidelines:	OECD 226 (2008)
GLP:	yes (certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of dimoxystrobin (BAS 505 F) on mortality and reproduction of the soil mite *Hypoaspis aculeifer* were investigated in a chronic laboratory study over 14 days. The test item was mixed into artificial soil at concentrations of 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil. Test item treatments were replicated four times each. For the control treatment, the soil was prepared with acetone (solvent control) in eight replicates. Each treatment contained 10 adult soil mites. Reproduction and mortality assessments of the mites were carried out after 14 days of exposure.

After 14 days of exposure, mortality rates of 2.5% - 7.5% were recorded in the test item treatment groups. In the solvent control the mortality rate was 2.5%. The observed mortality rates for adults in the test item treatment groups compared to the solvent control were not statistically significantly different. Reproduction rates in the 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil were 165.8, 149.8, 160.0, 180.5 and 210.8 juveniles, respectively. The mean reproduction in the solvent control reached 172.1 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations compared to the solvent control. Differences between the behavior and the morphology of the mites in the solvent control and the test item treatment groups could not be observed.

In a 14-day reproduction study with dimoxystrobin (BAS 505 F) on predatory soil mites, the NOEC based on mortality and reproduction was determined to be ≥ 1000 mg a.s./kg dry soil, the highest concentration tested. The LC_{50} and the EC_{50} was determined to be > 1000 mg a.s./kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 505 F (dimoxystrobin, Reg.No. 285028), batch no. N6, purity: 97.4% (tolerance ± 1.0%).

B. STUDY DESIGN

Test species: *Hypoaspis aculeifer*, adult females with an age difference of 2 days; source: in-house culture.

Test design: 14-day laboratory test on effects of BAS 505 F on mortality and reproduction of soil mites. Artificial soil (5% peat) was treated with different concentrations of the test item and filled in glass vessels before predatory mites were introduced on top of the soil; 6 treatment groups (5 test item concentrations, solvent control); 8 replicates for control and 4 replicates for test item, each with 10 soil mites; assessment of adult mortality and reproduction performed after 14 days.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate (analyzed purity: 99.8%, tolerance ± 1.0%). The effects of the reference item were investigated in a separate study.

Test concentrations: Solvent control, 62.5, 125, 250, 500 and 1000 mg BAS 505 F/kg dry soil.

Test conditions: Artificial soil according to OECD 226 (5% peat); pH 6.0 - 6.1 at test initiation, pH 5.8 – pH 5.9 at test termination; water content at test initiation 47.69% - 53.85% of maximum water holding capacity (WHC) and 44.53% - 47.97% of maximum WHC at test termination; temperature: 19.7°C - 21.1°C; photoperiod: 16 h light : 8 h dark; light intensity: 527 lx.

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$), Dunnett-t-test for reproduction data (one-sided smaller, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 14 days of exposure, mortality rates of 2.5% - 7.5% were recorded in the test item treatment groups. In the solvent control the mortality rate was 2.5%. The observed mortality rates for adults in the test item treatment groups compared to the solvent control were not statistically significantly different (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater $\alpha = 0.05$).
Reproduction rates in the 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil were 165.8, 149.8, 160.0, 180.5 and 210.8 juveniles, respectively. The mean reproduction in the solvent control reached 172.1 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations compared to the solvent control (Dunnett-t-test, one-sided smaller, $\alpha = 0.05$).
Differences between the behavior and the morphology of the mites in the solvent control and the test item treatment groups could not be observed. The results are summarized in Table 8.4.2.1-3.

Table 8.4.2.1-3: Effects of dimoxystrobin (BAS 505 F) on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

Dimoxystrobin [mg a.s./kg dry soil]	Solvent control	62.5	125	250	500	1000
Mortality (day 14) [%]	2.5	2.5	5.0	7.5	5.0	2.5
No. of juveniles (day 14)	172.1	165.8	149.8	160.0	180.5	210.8
Reproduction [% of control] (day 14)	100	96	87	93	105	122
	Endpoints [mg a.s./kg dry soil]					
NOEC $_{mortality + reproduction}$	≥ 1000					
LC_{50}	> 1000					
EC_{50}	> 1000					

III. CONCLUSION

In a 14-day reproduction study with dimoxystrobin (BAS 505 F) on predatory soil mites, the NOEC based on mortality and reproduction was determined to be ≥ 1000 mg a.s./kg dry soil, the highest concentration tested. The LC_{50} and the EC_{50} was determined to be > 1000 mg a.s./kg dry soil.

Report: CA 8.4.2.1/4
Schultz L., 2014a
Effects of Reg.No. 354563 (metabolite of BAS 505 F, Dimoxystrobin) on the reproduction of the predatory mite Hypoaspis aculeifer
2014/1170855

Guidelines: OECD 226 (2008)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effects of 505M09 (Reg.No. 354563, BF 505-08; metabolite of dimoxystrobin) on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* (CANESTRINI) were investigated in a chronic laboratory experiment over a time period of 14 days. The test item was mixed into artificial soil at concentrations of 62.5, 125, 250, 500 and 1000 mg a.s./kg dry soil. For the control treatment, the soil was prepared with acetone (solvent control). 8 replicates and 4 replicates were prepared for the solvent control and test item treatment groups, respectively, each containing 10 adult predatory mites (females). Assessment of mortality and reproduction was carried out after the 14-day exposure of the predatory mites.

After 14 days of exposure, mortality rates of 0.0% - 5.0 % were recorded in the test item treatment groups. In the solvent control the mortality rate was 2.5%. The observed mortality rates for adult mortality in the test item treatment groups compared to the solvent control were not statistically significant. Differences between the behaviour and the morphology of the mites in the solvent control and the test item treatment groups could not be observed.
Reproduction rates in the 62.5, 125, 250, 500 and 1000 mg a.s./kg soil dry weight were 240.8, 226.3, 231.0, 223.0 and 234.3 juveniles, respectively. The mean reproduction in the solvent control reached 246.4 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations compared to the solvent control.
Differences between the behaviour and the morphology of the mites in the solvent control and the test item treatment groups could not be observed.

In a 14-day *Hypoaspis aculeifer* reproduction study with 505M09 (Reg. No. 354563, metabolite of dimoxystrobin), the LC_{50} and the EC_{50} could not be calculated, but it can be concluded that these values are higher than 1000 mg a.s./kg dry soil. The NOEC for mortality and for reproduction was determined to be ≥ 1000 mg a.s./kg dry soil, the highest concentration tested.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: 505M09 (Reg.No. 354563, metabolite of dimoxystrobin); batch no.: L80-82; analysed purity: 96.3% (tolerance ± 1.0 %).

B. STUDY DESIGN

Test species: *Hypoaspis aculeifer (*CANESTRINI), adult mites with an age difference of 2 days; source: in-house culture.

Test design: 14-day laboratory test on effects of 505M09 on mortality and reproduction of soil mites. Artificial soil (5% peat) The different concentrations of the test item were homogeneously mixed into the artificial soil which was then used to fill glass vessels after which the predatory mites were introduced on top of the soil; 6 treatment groups (5 test item concentrations, solvent control); 8 replicates/solvent control group and 4 replicates/test item treatment group each with 10 predatory mites. Feeding of mites with *Tyrophagus putrescentiae* (SCHRANK) at the beginning and *ad libitum* during the test. Assessment of adult mortality and reproduction effects was carried out after 14 days.

Endpoints: Mortality and reproduction rate after 14 days.

Reference item: Dimethoate (analyzed purity: 99.8%, tolerance ± 1.0%). The effects of the reference item were investigated in a separate study.

Test concentrations: Solvent control, 62.5, 125, 250, 500 and 1000 mg Reg.No. 354563/kg dry soil.

Test conditions: Artificial soil according to OECD 226 (5% peat); pH 5.5 – 5.6 at test initiation, pH 5.5 – 5.7 at test termination; water content at test initiation 44.95% – 49.26% of maximum water holding capacity (WHC) and 41.86% – 46.95% of maximum WHC at test termination; temperature: 19.7°C – 21.1°C; photoperiod: 16 h light : 8 h dark; light intensity: 511 lx.

Statistics: Descriptive statistics; Fisher's Exact Binominal Test with Bonferroni Correction for mortality data (one-sided greater, $\alpha = 0.05$), Dunnett-t-test for reproduction data (one-sided smaller, $\alpha = 0.05$).

II. RESULTS AND DISCUSSION

After 14 days of exposure, mortality rates of 0.0% – 5.0 % were recorded in the test item treatment groups. In the solvent control the mortality rate was 2.5%. The observed mortality rates for adults mortality in the test item treatment groups compared to the solvent control were not statistically significant (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). Differences between the behaviour and the morphology of the mites in the solvent control and the test item treatment groups could not be observed.
Reproduction rates in the 62.5, 125, 250, 500 and 1000 mg a.s./kg soil dry weight were 240.8, 226.3, 231.0, 223.0 and 234.3 juveniles, respectively. The mean reproduction in the solvent control reached 246.4 juveniles. The test item showed no statistically significantly adverse effects on reproduction at all tested concentrations compared to the solvent control (Dunnett-t-test, $\alpha = 0.05$, one-sided smaller).
Differences between the behaviour and the morphology of the mites in the solvent control and the test item treatment groups could not be observed. The results are summarized in Table 8.4.2.1-4.

Table 8.4.2.1-4: Effects of 505M09 (Reg.No. 354563), a metabolite of dimoxystrobin on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproduction study

505M09 [mg a.s./kg dry soil]	Solvent control	62.5	125	250	500	1000
Mortality (day 14) [%]	2.5	2.5	2.5	0.0	5.0	2.5
No. of juveniles (day 14)	246.4	240.8	226.3	231.0	223.0	234.3
Reproduction [% of control] (day 14)	100	98	92	94	91	95
	Endpoints [mg a.s./kg dry soil]					
$NOEC_{mortality + reproduction}$	≥ 1000					
LC_{50}	> 1000					
EC_{50}	> 1000					

III. CONCLUSION

In a 14-day *Hypoaspis aculeifer* reproduction study with 505M09 (Reg.No. 354563, metabolite of dimoxystrobin), the LC_{50} and the EC_{50} could not be calculated, but it can be concluded that these values are higher than 1000 mg a.s./kg dry soil. The NOEC for mortality and for reproduction was determined to be ≥ 1000 mg a.s./kg dry soil, the highest concentration tested.

CA 8.5 Effects on nitrogen transformation

Since Annex I inclusion of the active substance dimoxystrobin (BAS 505 F) a new study on nitrogen transformation has been performed. As a result, there is a new endpoint which is considered in the risk assessment. A summary of this new study is provided under CA 8.5/1 below. Endpoints are listed in Table 8.5-1 below.

Table 8.5-1 Toxicity to nitrogen transformation of dimoxystrobin and metabolites

Test substance	Endpoint	Endpoint (< 25% effect) [mg/kg dry soil]	Reference (BASF DocID)	Study EU agreed?
dimoxystrobin	Effects on nitrogen transformation	0.72	2014/1083453	No, new study
dimoxystrobin [1)]	Effects on nitrogen transformation	2.67	1999/10118	Yes
505M01 = BF 505-04 = Reg. no. 358 104	Effects on nitrogen transformation	0.133	2000/1003888	Yes
505M08 = BF 505-07 = Reg. no. 354 562	Effects on nitrogen transformation	0.266	2000/1011459	Yes
505M09 = BF 505-08 = Reg. no. 354 563	Effects on nitrogen transformation	0.266	2000/1011473	Yes

[1)] Test carried out with a dimoxystrobin solo-formulation BAS 505 01 F, containing 167 g a.s./L.

Report: CA 8.5/1
Schulz L., 2014b
Effects of BAS 505 F (Dimoxystrobin, Reg.No. 285028) on the activity of soil microflora (Nitrogen transformation test)
2014/1083453

Guidelines: OECD 216 (2000)

GLP: yes
(certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effect of BAS 505 F (dimoxystrobin, Reg. No. 285 028) on nitrogen transformation was tested in a lucerne-enriched loamy sand soil. BAS 505 F was applied to samples of the soil, in a laboratory, at nominal test concentrations of 0.07 and 0.72 mg/kg dry soil. The treated soils and untreated controls were incubated at 20 °C ± 2 °C in the dark for 28 days. Triplicate samples of each treatment were removed for analysis of NH_4-nitrogen and NO_3-nitrogen 0, 7, 14, and 28 and days after application.

After 28 days, the NO_3-nitrogen production deviated by -0.7% in the 0.07 mg/kg dry soil and by +1.0% in the 0.72 mg/kg dry soil treatment from the control. There were no unacceptable adverse effects (> 25%) on the rate of conversion of NH_4-N to NO_3-N at any application rate at the end of the 28-day incubation period.

Based on the results of this study, BAS 505 F caused no unacceptable adverse effects (< 25% deviation from control; OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in a field soil tested up to a concentration of 0.72 mg BAS 505 F/kg dry soil.

I. MATERIALS AND METHODS

A. MATERIALS

Test item: BAS 505 F (dimoxystrobin, Reg. No. 285 028); batch no. N-6; content of a.s.: BAS 505 F): 97.4% purity (± 1.0%).

B. STUDY DESIGN

Test soil: Biologically active agricultural soil: loamy sand (DIN 4220) / sandy loam (USDA), pH 6.2, 1.40% C_{org}, water holding capacity: 36.15 g/100 g dry soil.

Test design: Determination of the N-transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil with 3 replicates per treatment and concentration. NH_4-nitrogen formed from organically bound nitrogen and NO_3-nitrogen from the nitrification process was determined by using a calibrated Autoanalyzer. Sampling scheme: 0, 7, 14, and 28, days after treatment; subsamples were withdrawn from the bulk batches and subjected to the measurement.

Endpoints: Effects on the NO_3-nitrogen production after 28 days of exposure.

Test rates: Control, 0.07 mg BAS 505 F/kg dry soil and 0.72 mg BAS 505 F/kg soil.

Reference item: Dinoterb (purity: 98% ± 0.5% analyzed). The reference item was applied at rates of 6.80, 16.00 and 27.00 mg dinoterb/kg dry soil in a separate study.

Test conditions: Soil moisture: 45% of its maximum water holding capacity; measured water content: 46.30 – 49.25 % of WHC; pH: 5.9 – 6.0. Soil samples were incubated at 20.1 °C – 21.0 °C while stored in glass bottles in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

28 days after treatment, the NO_3-nitrogen production deviated by -0.7% in the 0.07 mg/kg dry soil and by +1.0% in the 0.72 mg/kg dry soil treatment from the control. There were no unacceptable adverse effects (> 25%) on the rate of nitrogen transformation at any application rate at the end of the 28-day incubation period.

Table 8.5-2: Effects of BAS 505 F on soil micro-organisms (nitrogen transformation) on days 7, 14, and 28 days of incubation

Soil (days)	Control	0.07 mg BAS 505 F/kg dry soil		0.72 mg BAS 505 F/kg dry soil	
	NO_3-N [mg/100 g soil d.w.]	NO_3-N [mg/100 g soil d.w.]	% Deviation from control [1)]	NO_3-N [mg/100 g soil d.w.]	% Deviation from control [1)]
Loamy sand (0-7 d)	29.63	26.70	-9.9	29.90	+0.9
Loamy sand (0-14 d)	44.07	38.63	-12.3	44.00	-0.2
Loamy sand (0-28 d)	58.43	58.03	-0.7	59.00	+1.0

[1)] Based on NO_3-nitrogen production; - = inhibition, + = stimulation

In a separate study, the reference item dinoterb produced a stimulation of nitrogen transformation of 47.3%, 67.7% and 35.1% at 6.80, 16.00 and 27.00 mg dinoterb/kg dry soil, respectively, determined 28 days after application.

III. CONCLUSION

Based on the results of this study, BAS 505 F caused no unacceptable adverse effects (< 25% deviation from control; OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) in a field soil tested up to a concentration of 0.72 mg BAS 505 F/kg dry soil.

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

Non-target plant GLP studies with the representative formulation are available. Further screening data are not required.

CA 8.6.2 Testing on non-target plants

No new studies are available.

Studies have been conducted during the previous Annex I inclusion with the representative formulation and are described in M-CP 10.6.2.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

Studies with the active substance are not required. Studies conducted with the representative formulation are described in M-CP 10.7 (including studies which are not required under 1107/2009).

CA 8.8 Effects on biological methods for sewage treatment

The results of the already peer-reviewed and accepted study are still valid and they are summarized in Table 8.8-1. No new study has been performed.

Table 8.8-1: Effects on biological methods for sewage treatment

Test item	Study type	Endpoint [mg a.s./L]	Reference (BASF DocID)	EU agreed
BAS 505 F (dimoxystrobin)	Respiration inhibition test (inhibition of oxygen consumption activated sludge from wastewater plant)	EC_{50} (30 min) <1000	1999/10268	yes

CA 8.9 Monitoring data

According to the knowledge of the applicant, there are currently no monitoring studies available, which are assessing ecotoxicological effects of dimoxystrobin (BAS 505 F).

Dimoxystrobin

Document M-CA, Section 9

LITERATURE DATA

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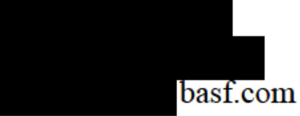

Version history[1]

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

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

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CA 9 LITERATURE DATA

A literature search on dimoxystrobin was performed by the BASF Group Information Center. The Literature Search Report on dimoxystrobin describes the general search and evaluation process as well as details on search profiles, search histories and summary tables.

The complete search report is provided in K-CA 9 (BASF DocID 2015/1134718).

The first step of the search result processing based on summary records was done by the Information Center and involved the separation into "hits" and "ballast" (obviously irrelevant records). The "ballast" was not further processed.

The "hits" were further evaluated by the scientific experts and categorized into "not relevant", "not reliable", and "used for dossier". This is documented in EXCEL files which are attached to the search report in K-CA 9 with the file names as listed below (alphabetical order):

Analytics:	Dimoxystrobin Literature Analytics
Ecotoxicology:	Dimoxystrobin Literature Ecotox Aquatic
	Dimoxystrobin Literature Ecotox General
	Dimoxystrobin Literature Ecotox Terrestric
	Dimoxystrobin Literature Ecotox Wildlife
Environmental Fate:	Dimoxystrobin Literature Environmental Fate
Consumer Safety:	Dimoxystrobin Literature Metabolism and Residues in Animals
	Dimoxystrobin Literature Metabolism and Residues in Plants
Product Chemistry:	Dimoxystrobin Literature Product Chemistry
Toxicology:	Dimoxystrobin Literature Toxicology

The hits in Analytics, Ecotox, Environmental Fate, Metabolism and Residues in Animals as well as Product Chemistry did not contribute to the risk assessment and were therefore not further discussed in the dossier.

Dimoxystrobin

Document M-CA, Section 10

CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

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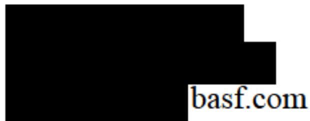

Version history[1]

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

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

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CA 10 CLASSIFICATION AND LABELLING OF THE ACTIVE SUBSTANCE

The following harmonized classification and labelling was adopted for Dimoxystrobin:

Table 10-1: Harmonised classification of dimoxystrobin according to (EC) No 1272/2008

Legislation	Classification	Labelling	Concentration limits
Regulation (EC) No 1272/2008	Hazard class and category code: Carc. 2 Repr. 2 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1 Hazard statement code: H332, H351, H361d, H400, H410	Pictogram signal word code: GHS08 GHS07 GHS09 Wng Hazard statement code: H351, H361d, H332, H410	M = 10
Directive 67/548/EEC	Carc. Cat. 3; R40 Repr. Cat. 3; R63 Xn; R20 N; R50-53	Xn; N R: 20-40-63-50/53 S: (2-)36/37-46-60-61	N; R50-53: C ≥ 2,5 % N; R51-53: 0,25 % ≤ C < 2,5 % R52-53: 0,025 % ≤ C < 0,25 %

Since the last evaluation of dimoxystrobin new mechanistic data on reproductive toxicity and the assumed susceptibility of young vs. older rats were generated. The new data have been presented in the context of the results of the generation toxicity studies and the mechanistic investigations showing that the classification with Repr. Cat. 2 (H361d) is not justified. A justification document ("Proposal for the re-classification of Dimoxystrobin") has been prepared and is submitted with this dossier (BASF DocID 2015/1152529). All relevant (new) data and assessments are also included in the respective chapters of this dossier (M-CA 5.6, 5.8 and Doc N1).

The following changes to classification and labelling of the active substance dimoxystrobin are proposed. Thus, the submission of a classification and labelling dossier to the European Chemicals Agency (ECHA) is required.

Table 10-2: Proposed changes in Hazard and Precautionary Statements

Legislation	Classification	Labelling	Concentration limits
Regulation (EC) No 1272/2008	Hazard class and category code: Carc. 2 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1 Hazard statement code: H332, H351, H400, H410	Pictogram signal word code: GHS08 GHS07 GHS09 Wng Hazard statement code: H351, H332, H410	